ChemPubSoc Europe

DOI: 10.1002/chem.201500838

CHEMISTRY A European Journal Full Paper

Nucleotides | *Very Important Paper* |

VIP A Modular Synthesis of Modified Phosphoanhydrides

Alexandre Hofer,[a] Gregor S. Cremosnik,[b] André C. Müller,[c] Roberto Giambruno,[c] Claudia Trefzer,[c] Giulio Superti-Furga,[c] Keiryn L. Bennett,[c] and Henning J. Jessen*[a]

Abstract: Phosphoanhydrides (P-anhydrides) are ubiquitously occurring modifications in nature. Nucleotides and their conjugates, for example, are among the most important building blocks and signaling molecules in cell biology. To study and manipulate their biological functions, a diverse range of analogues have been developed. Phosphate-modified analogues have been successfully applied to study proteins that depend on these abundant cellular building blocks, but very often both the preparation and purification of these molecules are challenging. This study discloses a general access to P-anhydrides, including different nucleotide probes, that greatly facilitates their preparation and isolation. The convenient and scalable synthesis of, for example, ^{18}O labeled nucleoside triphosphates holds promise for future applications in phosphoproteomics.

Introduction

Numerous important roles of nucleotides in biology are well-established and it is difficult to identify areas in which nucleotides and their conjugates are not critically involved.[1] As a consequence, a great deal of research has been dedicated to the development of nucleoside-derived drugs and nucleotide probes as tools to study biological processes.[2] In addition to modifications of the nucleobase and the sugar moiety, variations in the phosphate esters and anhydrides have been introduced by chemical synthesis.[1a,3] Although there have been promising recent developments in the field of phosphoanhydride (P-anhydride) synthesis,[4] a flexible, straightforward, and rapid synthetic approach to target different modified nucleotides remains desirable. This unmet need motivated the development of a phosphoramidite (P-amidite)[5]-based coupling strategy that facilitates the rapid synthesis of a range of different P-anhydride conjugates. Based on the idea of iterative couplings to obtain nucleotide oligophosphates,[6] this study unveils the true power of the concept. The broad applicability in the synthesis of different established and novel nucleotide conjugates and probes is showcased.

Nucleotide probes that have been widely applied in chemical biology studies comprise such diverse structures as, for example, phosphorothioate analogues of ATP and GTP in different positions (α-S, β-S, γ-S),[3b] and isotopically labeled probes, such as the important radiolabeled nucleotides. Recently, non-radioactive heavy isotope labels such as ^{18}O have been proposed as alternative tools for mass spectrometric profiling of kinases.[7] In the future, and once this promising technology finds more widespread application, further developments in this direction will most likely lead to increased demand for stable heavy isotope labeled nucleotides. In addition to the terminally monosubstituted oligophosphates that have been described so far, conjugates also exist in which the oligophosphate bridges two terminal organic residues. These structures can be found, for example, in naturally occurring nucleoside diphosphate sugars,[8] dinucleoside polyphosphates,[9] prenylated diphosphate saccharides,[10] and adenylated thiamine phosphates.[11] These can also exist as artificial structures, such as the recently developed dye conjugates for single molecule DNA sequencing,[12] nucleoside diphosphate prodrugs[13] or photocaged nucleotides.[14] During the introduction and modification of the oligophosphate moiety, problems often arise from low coupling efficiencies and extended reaction times, hydrolysis and the requirement for excess reagents that complicate purification of the polar products. These syntheses have therefore been referred to as "arduous"[3k] and "notoriously difficult to reproduce".[4n] Such difficulties have significantly hampered progress in many different areas of research that require access to the abundant phosphoanhydride conjugates. In many instances, the preparation of the compounds is simply restricted to specialized laboratories.

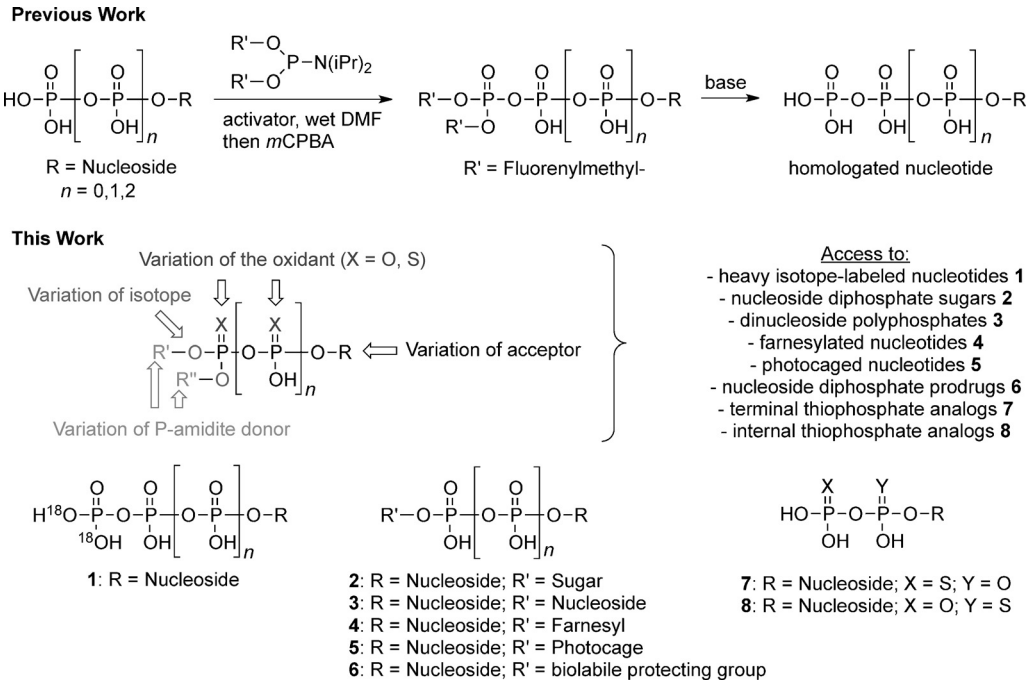
[a] *A. Hofer, Prof. Dr. H. J. Jessen*
Department of Chemistry, University of Zürich
Winterthurerstrasse 190
8057 Zürich (Switzerland)
E-mail: henningjacob.jessen@chem.uzh.ch

[b] *G. S. Cremosnik*
Chemistry Research Laboratory
University of Oxford, 12 Mansfield Road
Oxford OX1 3TA (UK)

[c] *A. C. Müller, Dr. R. Giambruno, Dr. C. Trefzer, Prof. Dr. G. Superti-Furga, Dr. K. L. Bennett*
CeMM Research Center for Molecular Medicine
of the Austrian Academy of Sciences
Lazarettgasse 14, AKH BT 25.3
1090 Vienna (Austria)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201500838.

© 2015 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim



Scheme 1. Anhydride bond formation using P-amidites under ambient coupling conditions provides access to a variety of interesting metabolites and probes in a rapid, modular, and reproducible way.

In a recent study, it was shown that P-amidites can be chemoselectively coupled to phosphate groups; thereby releasing a P^V-P^V anhydride after oxidation and cleavage of fluorenylmethyl (Fm) groups that block the terminal phosphate (Scheme 1).[6] Repetition of this procedure enables an iterative elongation. The method is easy to implement because the reactions occur under ambient conditions without any drying of solvents and reagents. Moreover, no protecting groups on the nucleotides are necessary, the yields are usually high, and the coupling reactions occur in less than 10 min. Purification of the final products is often possible by simple precipitation. Consequently, the method is well-suited to scale-up. An extension of this method would greatly facilitate flexible and chemoselective P-anhydride-forming reactions to obtain such diverse molecules as heavy isotope labeled nucleotides, nucleoside diphosphate sugars, dinucleoside polyphosphates, internal and terminal thiophosphate analogues, caged and membrane-permeable nucleotides, farnesyl oligophosphate conjugates, and many others (**1**–**8**; Scheme 1).

Results and Discussion

Synthesis

The strategy described in this publication relied on a P-anhydride bond-forming reaction in which a P-amidite was coupled as an electrophile (donor) to a nucleophilic phosphate (acceptor). The terms are chosen in analogy to glycosylation chemistry. Subsequently, the intermediate mixed P^{III}-P^V anhydride was oxidized or sulfurized to a P^V-P^V anhydride. Finally, the protecting groups were removed if present on the anhydride or donor, whereas the acceptor did not require protection. All reactions were performed under ambient conditions (open flask, wet solvents); under these conditions the acceptor phosphate was completely consumed followed by hydrolysis of excess donor. The comparatively fast hydrolysis of excess donor thus enabled the use of unprotected acceptors, thereby substantially streamlining the synthesis. Rather than covering only a set of different acceptors in combination with a single P-amidite donor, a variety of P-amidite donors were combined with different acceptor phosphates to showcase the scope of this straightforward protocol. It is important to note that residues R in P-amidites can be easily introduced by well-established chemistry from commercially available building blocks. With these protocols, symmetric and asymmetric modifications of the P-amidite can be introduced in a reliable way. Complementarily, many different acceptors such as nucleotides and sugar-phosphates are either commercially available or can be readily accessed by established protocols.

A set of P-amidite donors was chosen (Figure 1, upper section). Amidite **9** is commercially available, but alternatively can be prepared on a large scale in one step.[15] Amidite **10** was obtained in one step from ^{18}O labeled benzyl alcohol, which is accessible on gram scale by hydrolysis of benzyl bromide with $H_2{}^{18}O$ (see the Supporting Information). Amidite **11** has been reported previously.[16] The preparation of the asymmetric P-amidites was possible in two steps (see the Supporting Information). The phosphate acceptors (Figure 1, lower section) are commercially available.

Table 1 gives an overview of the products that were synthesized. As summarized in the experimental section, these syntheses are easy to conduct. Briefly, a soluble salt of the phosphate (tetrabutylammonium) in wet *N*,*N*-dimethylformamide (DMF) was coupled with 1–2 equivalents of the P-amidite

© 2015 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

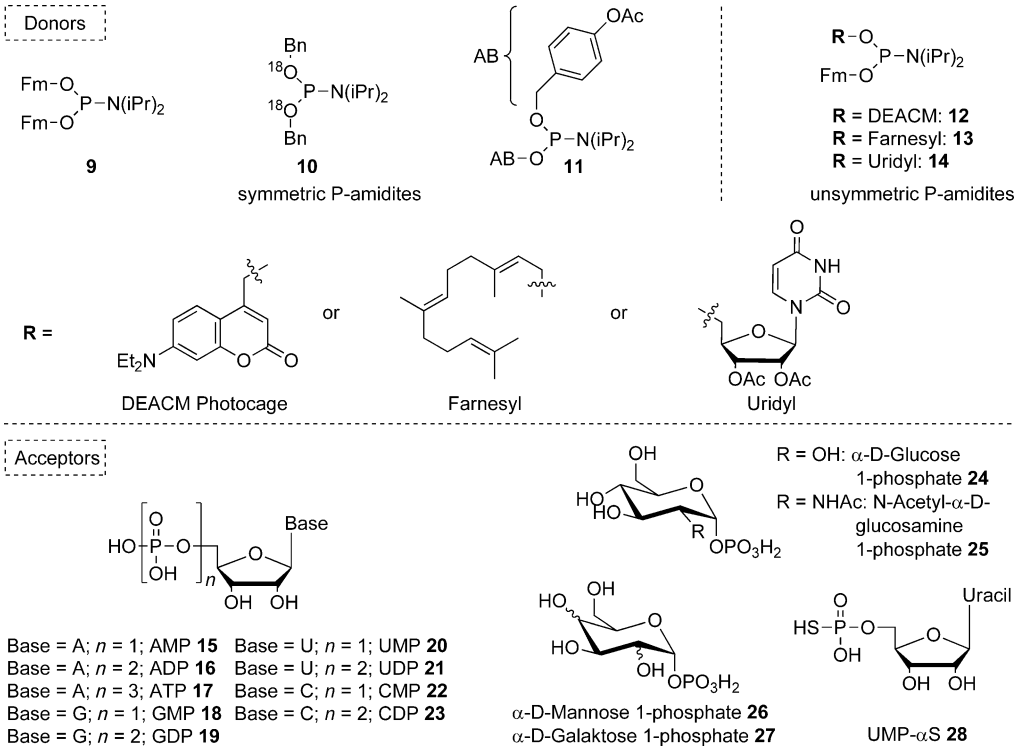


Figure 1. Overview of the acceptors and donors used in this study.

donor in the presence of an activator (4,5-dicyanomidiazole, DCI; or ethylthiotetrazole, ETT)[17] under ambient conditions. Progress of the reaction was monitored by ^{31}P NMR spectroscopic analysis. After 1–30 min, the intermediate P^{III}-P^{V} anhydride was oxidized (2 min) and, if present, the protecting groups were removed under basic conditions (usually 5 min). The products were precipitated by addition of diethyl ether. In cases where significant impurities were present, for example, H-phosphonates or phosphates as a result of hydrolysis (see the Supporting Information), further purification was possible by chromatography (strong anion exchange or reverse phase).

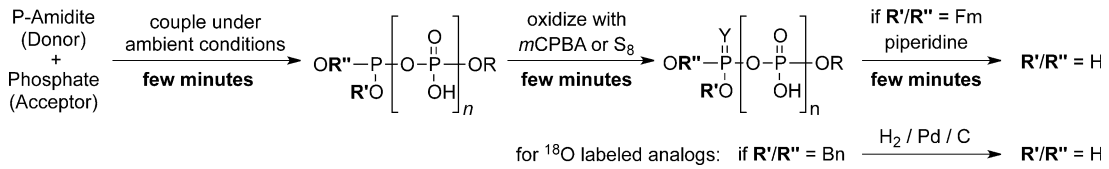
Different conjugates of groups **1**–**8** were accessible in highly competitive yields, purity, and time (Table 1; see Figure 2 and the spectra section in the Supporting Information for a comprehensive ^{31}P NMR spectroscopic analysis of all reactions). Nucleoside thiophosphate conjugates were prepared from amidite **9** in combination with S_8-mediated oxidation.[18] The products (Table 1, entries 1–5) did not require further purification, as judged by ^{31}P NMR spectroscopic analysis, and ADP-βS was readily synthesized on a 0.8 g scale. To synthesize UDP-α-S (Table 1, entry 6), UMP-α-S **28** was coupled with amidite **9**. Selective functionalization of the oxygen rather than sulfur was observed and oxidation required the use of *t*BuOOH; otherwise the sulfur atom was additionally oxidized leading to decomposition. In contrast to the terminally modified nucleotides (Table 1, entries 1–5), UDP-α-S (entry 6) required purification by strong anion exchange chromatography.

Table 1, entries 7–9 summarize the preparation of heavy isotope (^{18}O)-labeled nucleoside triphosphates with P-amidite **10**. The benzyl (Bn) protecting groups were chosen because facile incorporation of ^{18}O atoms from heavy water is enabled; this was not possible with fluorenylmethyl (Fm) groups. After coupling, deprotection by hydrogenation under aqueous conditions was therefore necessary. These conditions led to partial hydrolysis of the protected P-anhydride. Whereas the overall deprotection was very clean, hydrolysis led to the presence of 10–20% of the corresponding nucleoside diphosphate; this was not considered problematic in assays that eventually release the NDP. The method summarized in entries 7–9 has been scaled to produce up to 200 mg of ^{18}O labeled nucleoside triphosphates with a purity of 80%. If required, the nucleoside diphosphate (20%) can be removed by strong anion exchange chromatography, but at the cost of reduced yield. Cytosine was converted quantitatively into 5,6-dihydro uracil under the hydrogenation conditions (Table 1, entry 9).

Table 1, entries 10 and 11 summarize the facile preparation of nucleoside diphosphate prodrugs $(AB)_2$-GDP and $(AB)_2$-CDP. The acetoxybenzyl group (AB) is the only known group that can be conjugated to nucleoside diphosphates to render the prodrugs cell permeable.[13] Once cells have taken up the molecule, enzymes cleave the AB groups and release the bioactive nucleoside diphosphate. Both $(AB)_2$ prodrugs described herein have been obtained in essentially quantitative yield (>95%) within 15 min (coupling and oxidation) and in high purity upon simple precipitation (see the Supporting Information). Table 1, entries 12–14 show an easy preparation of photocaged nucleotides. The [7-(diethylamino)coumarin-4-yl]methyl (DEACM) photocage has been used extensively to exert control over the spatiotemporal release of biologically important phosphates (e.g., inositol polyphosphates, and nucleotides).[16,19] By using a mixed P-amidite **12**, this group can now be introduced within a few minutes to biologically relevant nucleoside di-

© 2015 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

Table 1. Scope of P-amidite-based P-anhydride forming reactions.



Entry	Acceptor	Donor	Oxidant	Product	Yield [%]	Purification
1	**9**	**15**	S_8	ADP-βS	71	precipitation
2	**9**	**16**	S_8	ATP-γS	57	precipitation
3	**9**	**19**	S_8	GTP-γS	53	precipitation
4	**9**	**20**	S_8	UDP-βS	74	precipitation
5	**9**	**22**	S_8	CDP-βS	91	precipitation
6	**9**	**28**	*t*BuOOH	UDP-αS	33	SAX
7	**10**	**16**	*m*CPBA	ATP-γ(^{18}O)$_2$	95 44	precipitation[b] SAX
8	**10**	**21**	*m*CPBA	UTP-γ(^{18}O)$_2$	95 52	precipitation[b] SAX
9	**10**	**23**	*m*CPBA	5,6-dihydro-UTP-γ(^{18}O)$_2$[c]	95 44	precipitation[b] SAX
10	**11**	**18**	*m*CPBA	(AB)$_2$-GDP	95	precipitation
11	**11**	**22**	*m*CPBA	(AB)$_2$-CDP	95	precipitation
12	**12**	**15**	*m*CPBA	ADP-β-DEACM	44	RP-18 FC
13	**12**	**16**	*m*CPBA	ATP-γ-DEACM	30	RP-18 FC
14	**12**	**18**	*m*CPBA	GDP-β-DEACM	63	RP-18 FC
15	**13**	**22**	*m*CPBA	CDP-β-farnesyl	95	precipitation
16	**13**	**23**	*m*CPBA	CTP-γ-farnesyl	95	precipitation
17	**14**[a]	**24**	*m*CPBA	UDP-α-D-Glc	quant.	precipitation
18	**14**[a]	**25**	*m*CPBA	UDP-α-D-GlcNAc	92	precipitation
19	**14**[a]	**26**	*m*CPBA	UDP-α-D-Man	88	precipitation
20	**14**[a]	**27**	*m*CPBA	UDP-α-D-Gal	75	precipitation
21	**14**[a]	**17**	*m*CPBA	AppppU	36	SAX
22	**14**[a]	**20**	*m*CPBA	UppU	83	precipitation

[a] Acetyl groups were removed by treatment with aqueous ammonia or aqueous methanol/NEt_3 after cleavage of the Fm protecting groups (piperidine). [b] Precipitated material contained approximately 10–20% hydrolyzed XDP. Removal of XDP by SAX yielded highly enriched material, although with diminished yield (second line). [c] Cytidine was reduced under these conditions to 5,6-dihydrouridine.

and triphosphates such as adenosine and guanosine. After removal of the Fm group in a few minutes under basic conditions, the products were purified by RP-18 chromatography because precipitation was difficult to achieve and was incomplete.

Similar to the AB nucleotides, lipidated nucleoside di- and triphosphates have been proposed as potentially cell-permeable analogues.[2b] P-amidite coupling can be used to obtain such conjugates (Table 1, entries 15 and 16). As judged by ^{31}P NMR spectroscopic analysis, β-farnesylated CDP (entry 15) was obtained in essentially quantitative yield and high purity. γ-Farnesylated CTP, however, contained more impurities (15% according to ^{31}P NMR analysis, see the Supporting Information) arising from partial hydrolysis. Again, coupling and oxidation occurred within a few minutes and verification by mass spectrometry revealed that epoxidation of the double bonds in the farnesyl residue had not occurred during oxidation of the P^{III}-P^V intermediate with *m*CPBA.

Application of the P-amidite protocol to obtain non-natural analogues (Table 1, entries 1–16) was further extended to naturally occurring conjugates. Most importantly, nucleoside diphosphate sugars were readily prepared by using P-amidite **14** (Table 1, entries 17–20). Here, one residue is acetylated uridine, which enables facile conjugation to sugar phosphates. Acetylation of uridine is not strictly required; however, amidite **14** with acetylated uridine can be purified and this is not the case for the unprotected derivative. After coupling, the Fm and acetyl groups were removed by treatment with piperidine and either ammonia or triethylamine.

The high selectivity of the coupling reaction is showcased in Figure 2 for the coupling of P-amidite **14** with *N*-acetyl glucosamine 1-phosphate to obtain uridine diphosphate *N*-acetyl glucosamine (Table 1, entry 18). The proton-decoupled ^{31}P NMR spectrum of the reaction mixture after completed P^{III}-P^V anhydride coupling (Figure 2, spectrum **II**) shows that the only significant side reaction is the hydrolysis of P-amidite **14**. Complete consumption of the unprotected sugar phosphate in wet solvent can thus be achieved by using the reported conditions. Precipitation after oxidation of the P^{III} intermediate enables the removal of the majority of excess hydrolyzed **14** and yields almost pure Fm-protected diphosphate intermediate (**29**; Figure 2, spectrum **III**, mixture of diastereomers). After Fm-deprotection, precipitation gives the free diphosphate **30** in essentially pure form (Figure 2, spectrum **IV**). Product **31** is finally obtained in a similarly high purity after removal of the acetyl groups on the uridyl moiety followed by lyophilization of the reaction mixture (Figure 2, spectrum **V**). This procedure does not require any chromatography.

© 2015 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

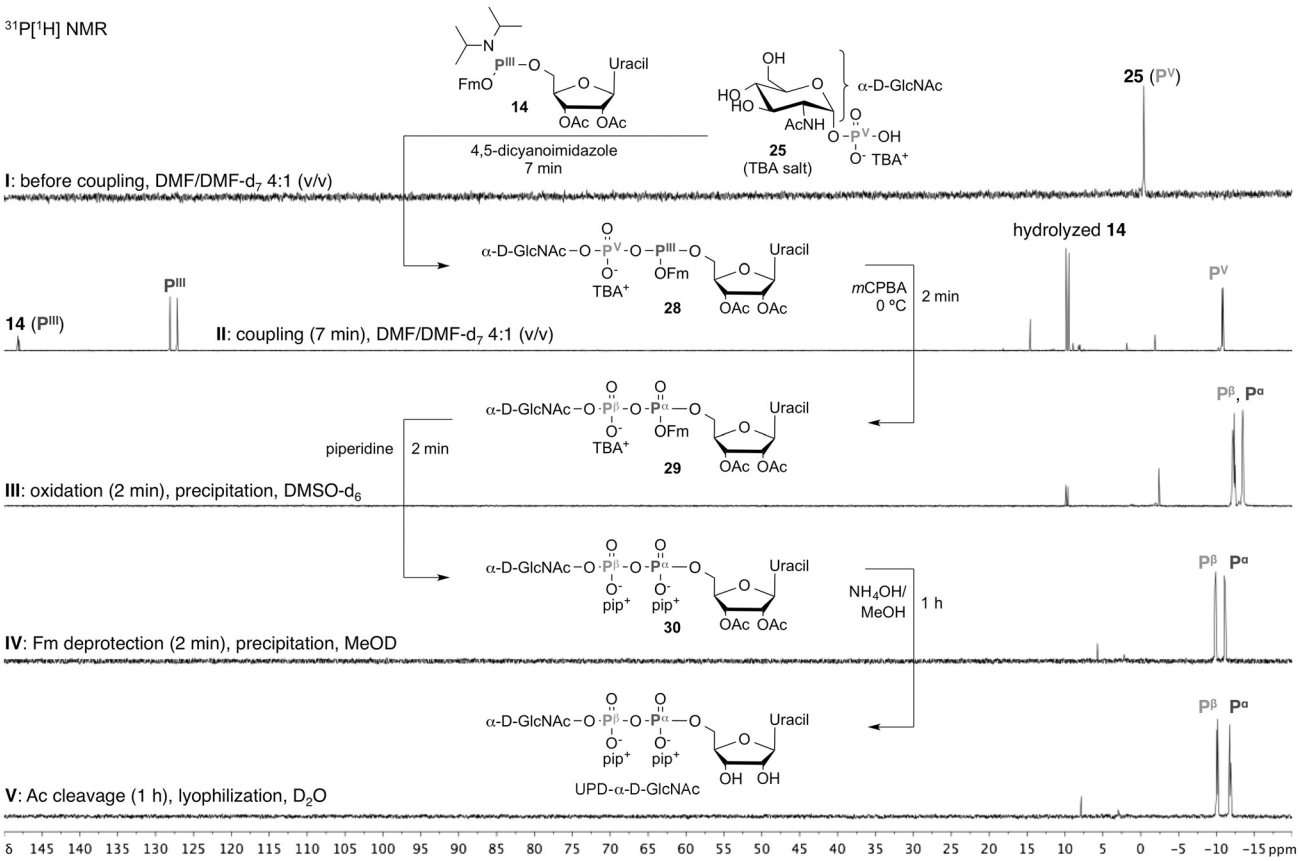


Figure 2. Reaction monitoring by proton-decoupled ^{31}P NMR spectroscopy (recorded in 20% [D_7]DMF under ambient conditions) for the coupling of P-amidite **14** to *N*-acetyl glucosamine 1-phosphate (**25**) to obtain UDP-α-D-GlcNAc (Table 1, entry 18). I) *N*-acetyl glucosamine 1-phosphate (**25**); II) addition of P-amidite **14** and activator (7 min); III) oxidation with *m*CPBA (2 min); IV) Removal of fluorenylmethyl (Fm) protecting group and precipitation; V) acetyl group cleavage and precipitation. Abbreviations: Pip^+ = piperidinium; TBA = tetra-*n*-butyl ammonium; Ac = acetyl; Fm = fluorenylmethyl; *m*CPBA = *meta*-chloroperbenzoic acid.

Four different UDP sugars (glucose, *N*-acetyl glucosamine, mannose, and galactose) were obtained in high yields (75% to quantitative) and purity (60 to 86%, by HPLC and ^{31}P NMR analyses) by simple precipitation from the reaction mixture. Although further purification by chromatography can be achieved, Hindsgaul and co-workers[4n] showed that nucleotide sugars can be used in enzymatic glycosylations even in the presence of byproducts and thus do not strictly require high purity.

Finally, C_2-symmetric (UppU) and nonsymmetric (AppppU) dinucleoside polyphosphates with different P-anhydride chain lengths can be easily accessed (Table 1, entries 21 and 22). Again, it is evident that longer anhydride chains were more challenging to prepare and thus the product required purification by strong anion exchange chromatography. It is conceivable that this method could also be used to introduce thiophosphates as non-natural analogues.

Application to Phosphoproteomics

To demonstrate the utility of the prepared analogues, γ-$(^{18}O)_2$-labeled 'heavy' ATP of high purity (>95%) was prepared on a 30 mg scale. It has been previously shown that $(^{18}O)_4$-labeled ATP can be used in quantitative phosphoproteomics to study phosphorylation in vitro.[7,20] By using a 1:1 mixture of ATP and heavy ATP, combined with a kinase of interest and a cellular extract, phosphorylation sites can be identified by proteolytic digestion of proteins, phosphopeptide enrichment, and subsequent analysis by liquid chromatography mass spectrometry. This method relies on the generation of doublets showing a separation in mass of 6 Da. The next step was to study whether γ-$(^{18}O)_2$-ATP could be used in such assays to generate a mass shift of 4 Da. A synthetic peptide (biotin-GGEAIYAAPFKK-NH_2) with the preferred ABL1 consensus motif YxxP was phosphorylated in vitro by the tyrosine kinase Abelson murine leukemia viral oncogene homolog 1 (ABL1) using ATP and γ-$(^{18}O)_2$-ATP. Briefly, a 1:1 mixture of ATP and $(^{18}O)_2$-ATP was added at a final concentration of 1 mM to a vial containing immunopurified, constitutively active ABL1-PP. The reaction was initiated by adding 5 mM of the synthetic ABL1 peptide at room temperature on a rotary shaker (see the Supporting Information, Figure S1). After one hour and centrifugation, the supernatant was enriched for phosphopeptides by immobilized metal affinity chromatography.[21] Samples were analyzed with a Q-Exactive mass spectrometer coupled to a Dionex Ultimate 3000 HPLC nanoflow system via a nanoelectrospray ion source using a liquid junction. The LC-MSMS analysis clearly identified the expected mass shift of 4 Da

© 2015 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

(Figure 3). Moreover, the reporter immonium ion, indicative of a phosphotyrosine-containing peptide, was observed, also with the expected mass shift of 4 Da (see the Supporting Information, Figures S2 and S3).

Conclusions

The described protocol, in combination with iterative oligophosphate synthesis, provides a powerful toolbox that is suitable for the generation of a multitude of oligophosphate conjugates (e.g., **1**–**8**) and their analogues in a straightforward manner. It is important to note that these couplings typically occur within a few minutes under ambient conditions with very little byproduct formation. This is in contrast to the majority of other protocols; for example, the reaction times for nucleotide sugar synthesis usually require multiple days to ensure significant product formation.[8] Recent notable exceptions to this, however, do exist.[4n,q,22] Nonetheless, the simple, reproducible, and modular coupling under ambient conditions within a few minutes in approximately equimolar amounts of donor and acceptor as described in this publication has been a long-standing synthetic challenge. The sensitivity of the P-anhydride forming reaction to a 30-fold scale-up has been analyzed in the case of ADP-βS (0.6 g of starting material **9** instead of 20 mg; see the Supporting Information) and no loss of efficiency regarding yield or purity of ADP-βS were observed. The protocols described herein will thus make available a variety of nucleotide probes on large scale that have been difficult to obtain by other methods.

Along these lines, it is shown that synthetic γ-($^{18}O)_2$-labeled ATP can be used in mass spectrometry-based proteomics to identify and assign the phosphorylation sites introduced by an exogenous kinase. Given the ease of preparation and thus the ability to obtain large amounts (200 mg have been prepared so far), this "heavy" ATP will be developed as an alternative to expensive γ-($^{18}O)_4$-labeled ATP.

Experimental Section

General P-Anhydride Synthesis Procedure

Monophosphate TBA salt was dissolved in DMF/[D_7]DMF. P-Amidite (1.3–2.7 equiv) and activator (ETT or DCI; 2 equiv) were added and the progress of the coupling was monitored by ^{31}P NMR spectroscopic analysis. Subsequently, *m*CPBA (77%, 1.5–2.8 equiv) was added in small portions at 0 °C. The product was obtained by precipitation with Et_2O/hexane (5:1 v/v), centrifugation, washing of the pellets with Et_2O, and drying under high vacuum. Protecting groups were removed under basic conditions (e.g., piperidine) and

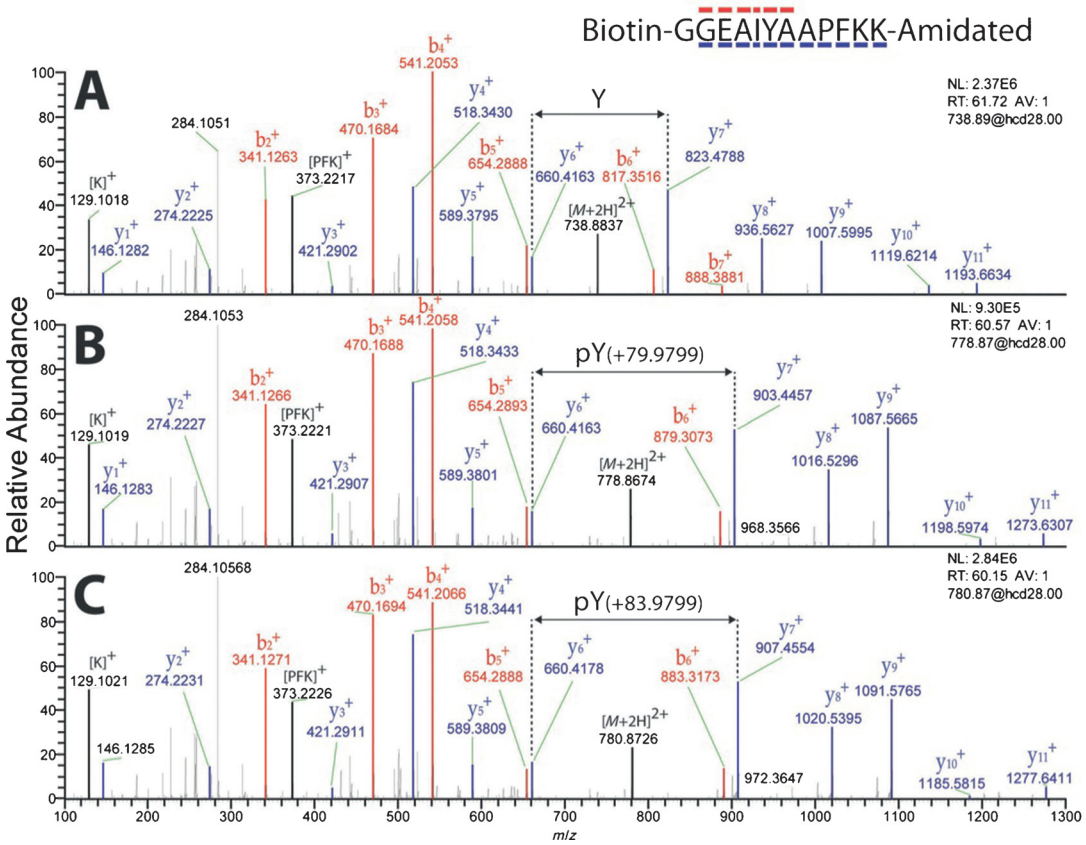


Figure 3. MS^2 spectra of the respective isolated and fragmented precursor ions A) non-phosphorylated synthetic peptide (*m/z* 738.87); B) normal phosphorylated synthetic peptide (*m/z* 778.87), and C) ($^{18}O)_2$-'heavy' phosphorylated synthetic peptide (*m/z* 780.87). For amino acid sequence identification, b-fragment and y-fragment ions are annotated in red and blue, respectively. Sequence coverage of the synthetic peptide is indicated by the lower blue or upper red lines next to the letter of the respective amino acid at the top right. The distance between the y_6 and y_7 ions corresponds to the mass of the modified or unmodified tyrosine residue showing the expected additional mass shift of 4 Da for the ($^{18}O)_2$-'heavy' phosphorylated synthetic peptide (C).

© 2015 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

the product was precipitated by addition of Et_2O and isolated by centrifugation.

Acknowledgements

The authors would like to thank Jay S. Siegel and John Robinson for their continuous support. The work was funded by the University of Zürich (Forschungskredit to H. J. J.) and the Swiss National Science Foundation (Professorship PP00P2_157607 to H. J. J.). Research in the Bennett laboratory at CeMM is supported by the Austrian Academy of Sciences and the Austrian Science Fund FWF.

Keywords: chemoselectivity · isotopic labeling · nucleotides · phosphorylation · polyanions

[1] a) G. K. Surya Prakash, M. Zibinsky, T. G. Upton, B. A. Kashemirov, C. E. McKenna, K. Oertell, M. F. Goodman, V. K. Batra, L. C. Pedersen, W. A. Beard, D. D. Shock, S. H. Wilson, G. A. Olah, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 15693 – 15698; b) F. H. Westheimer, *Science* **1987**, *235*, 1173 – 1178.

[2] a) L. P. Jordheim, D. Durantel, F. Zoulim, C. Dumontet, *Nat. Rev. Drug Discovery* **2013**, *12*, 447 – 464; b) U. Pradere, E. C. Garnier-Amblard, S. J. Coats, F. Amblard, R. F. Schinazi, *Chem. Rev.* **2014**, *114*, 9154 – 9218.

[3] a) D. L. M. Verheyden, J. G. Moffatt, W. E. Wehrli, *J. Am. Chem. Soc.* **1964**, *86*, 1253; b) F. Eckstein, *Angew. Chem. Int. Ed.* **1983**, *22*, 423 – 439; *Angew. Chem.* **1983**, *95*, 431 – 447; c) V. M. Dixit, C. D. Poulter, *Tetrahedron Lett.* **1984**, *25*, 4055 – 4058; d) Y. Ahmadibeni, K. Parang, *Org Lett.* **2005**, *7*, 5589 – 5592; e) J. Kowalska, M. Lewdorowicz, E. Darzynkiewicz, J. Jemielity, *Tetrahedron Lett.* **2007**, *48*, 5475 – 5479; f) Y. Ahmadibeni, R. K. Tiwari, G. Q. Sun, K. Parang, *Org Lett.* **2009**, *11*, 2157 – 2160; g) A. M. Rydzik, M. Lukaszewicz, J. Zuberek, J. Kowalska, Z. M. Darzynkiewicz, E. Darzynkiewicz, J. Jemielity, *Org. Biomol. Chem.* **2009**, *7*, 4763 – 4776; h) I. Zlatev, T. Lavergne, F. Debart, J. J. Vasseur, M. Manoharan, F. Morvan, *Org. Lett.* **2010**, *12*, 2190 – 2193; i) L. N. Lin, J. Caton-Williams, M. Kaur, A. M. Patino, J. Sheng, J. Punetha, Z. Huang, *Rna* **2011**, *17*, 1932 – 1938; j) S. M. Hacker, M. Mex, A. Marx, *J. Org. Chem.* **2012**, *77*, 10450 – 10454; k) M. A. Martinez Farias, V. A. Kincaid, V. R. Annamalai, L. L. Kiessling, *J. Am. Chem. Soc.* **2014**, *136*, 8492 – 8495.

[4] a) K. Burgess, D. Cook, *Chem. Rev.* **2000**, *100*, 2047 – 2059; b) A. L. Marlow, L. L. Kiessling, *Org Lett.* **2001**, *3*, 2517 – 2519; c) W. D. Wu, C. L. F. Meyers, R. F. Borch, *Org. Lett.* **2004**, *6*, 2257 – 2260; d) S. Mohamady, D. L. Jakeman, *J. Org. Chem.* **2005**, *70*, 10588 – 10591; e) S. Warnecke, C. Meier, *J. Org. Chem.* **2009**, *74*, 3024 – 3030; f) G. J. van der Heden van Noort, M. G. van der Horst, H. S. Overkleeft, G. A. van der Marel, D. V. Filippov, *J. Am. Chem. Soc.* **2010**, *132*, 5236 – 5240; g) H. A. Kistemaker, L. N. Lameijer, N. J. Meeuwenoord, H. S. Overkleeft, G. A. van der Marel, D. V. Filippov, *Angew Chem. Int. Ed.* **2015**, *54*, 4915 – 4918; *Angew. Chem.* **2015**, *127*, 4997 – 5000; h) J. Caton-Williams, L. N. Lin, M. Smith, Z. Huang, *Chem. Commun.* **2011**, *47*, 8142 – 8144; i) J. Caton-Williams, M. Smith, N. Carrasco, Z. Huang, *Org. Lett.* **2011**, *13*, 4156 – 4159; j) S. Mohamady, S. D. Taylor, *J. Org. Chem.* **2011**, *76*, 6344 – 6349; k) V. C. Tonn, C. Meier, *Chem. Eur. J.* **2011**, *17*, 9832 – 9842; l) H. Tsukamoto, D. Kahne, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5050 – 5053; m) M. Strenkowska, P. Wanat, M. Ziemniak, J. Jemielity, J. Kowalska, *Org. Lett.* **2012**, *14*, 4782 – 4785; n) H. Tanaka, Y. Yoshimura, M. R. Jorgensen, J. A. Cuesta-Seijo, O. Hindsgaul, *Angew. Chem. Int. Ed.* **2012**, *51*, 11531 – 11534; *Angew. Chem.* **2012**, *124*, 11699 – 11702; o) S. Mohamady, S. D. Taylor, *Org. Lett.* **2013**, *15*, 2612 – 2615; p) B. Steigenberger, S. Schiesser, B. Hackner, C. Brandmayr, S. K. Laube, J. Steinbacher, T. Pfaffeneder, T. Carell, *Org. Lett.* **2013**, *15*, 366 – 369; q) P. Dabrowski-Tumanski, J. Kowalska, J. Jemielity, *Eur. J. Org. Chem.* **2013**, 2147 – 2154; r) Q. Sun, S. S. Gong, J. Sun, S. Liu, Q. Xiao, S. Z. Pu, *J. Org. Chem.* **2013**, *78*, 8417 – 8426; s) S. Capolicchio, D. T. Thakor, A. Linden, H. J. Jessen, *Angew. Chem. Int. Ed.* **2013**, *52*, 6912 – 6916; *Angew. Chem.* **2013**, *125*, 7050 – 7054; t) S. Capolicchio, H. C. Wang, D. T. Thakor, S. B. Shears, H. J. Jessen, *Angew. Chem. Int. Ed.* **2014**, *53*, 9508 – 9511; u) H. J. Jessen, N. Ahmed, A. Hofer, *Org. Biomol. Chem.* **2014**, *12*, 3526 – 3530; v) A. M. Marmelstein, L. M. Yates, J. H. Conway, D. Fiedler, *J. Am. Chem. Soc.* **2014**, *136*, 108 – 111; w) I. Pavlovic, D. T. Thakor, L. Bigler, M. S. C. Wilson, D. Laha, G. Schaaf, A. Saiardi, H. J. Jessen, *Angew. Chem. Int. Ed.* **2015**, *54*, DOI: 10.1002/anie.201503094.

[5] M. H. Caruthers, *Science* **1985**, *230*, 281 – 285.

[6] G. S. Cremosnik, A. Hofer, H. J. Jessen, *Angew. Chem. Int. Ed.* **2014**, *53*, 286 – 289; *Angew. Chem.* **2014**, *126*, 290 – 294.

[7] a) M. Zhou, Z. Meng, A. G. Jobson, Y. Pommier, T. D. Veenstra, *Anal. Chem.* **2007**, *79*, 7603 – 7610; b) C. Fu, X. Zheng, Y. Jiang, Y. Liu, P. Xu, Z. Zeng, R. Liu, Y. Zhao, *Chem. Commun.* **2013**, *49*, 2795 – 2797.

[8] G. K. Wagner, T. Pesnot, R. A. Field, *Nat. Prod. Rep.* **2009**, *26*, 1172 – 1194.

[9] A. G. McLennan, *Pharmacol. Ther.* **2000**, *87*, 73 – 89.

[10] B. Schwartz, J. A. Markwalder, Y. Wang, *J. Am. Chem. Soc.* **2001**, *123*, 11638 – 11643.

[11] a) L. Bettendorff, B. Wirtzfeld, A. F. Makarchikov, G. Mazzucchelli, M. Frederich, T. Gigliobianco, M. Gangolf, E. De Pauw, L. Angenot, P. Wins, *Nat. Chem. Biol.* **2007**, *3*, 211 – 212; b) M. Frédérich, D. Delvaux, T. Gigliobianco, M. Gangolf, G. Dive, G. Mazzucchelli, B. Elias, E. De Pauw, L. Angenot, P. Wins, L. Bettendorff, *FEBS J.* **2009**, *276*, 3256 – 3268.

[12] a) A. Sood, S. Kumar, S. Nampalli, J. R. Nelson, J. Macklin, C. W. Fuller, *J Am Chem. Soc.* **2005**, *127*, 2394 – 2395; b) J. Korlach, A. Bibillo, J. Wegener, P. Peluso, T. T. Pham, I. Park, S. Clark, G. A. Otto, S. W. Turner, *Nucleosides Nucleotides Nucleic Acids* **2008**, *27*, 1072 – 1083; c) J. Eid, A. Fehr, J. Gray, K. Luong, J. Lyle, G. Otto, P. Peluso, D. Rank, P. Baybayan, B. Bettman, A. Bibillo, K. Bjornson, B. Chaudhuri, F. Christians, R. Cicero, S. Clark, R. Dalal, A. Dewinter, J. Dixon, M. Foquet, A. Gaertner, P. Hardenbol, C. Heiner, K. Hester, D. Holden, G. Kearns, X. X. Kong, R. Kuse, Y. Lacroix, S. Lin, P. Lundquist, C. C. Ma, P. Marks, M. Maxham, D. Murphy, I. Park, T. Pham, M. Phillips, J. Roy, R. Sebra, G. Shen, J. Sorenson, A. Tomaney, K. Travers, M. Trulson, J. Vieceli, J. Wegener, D. Wu, A. Yang, D. Zaccarin, P. Zhao, F. Zhong, J. Korlach, S. Turner, *Science* **2009**, *323*, 133 – 138; d) J. Ibach, S. Brakmann, *Angew. Chem. Int. Ed.* **2009**, *48*, 4683 – 4685; *Angew. Chem.* **2009**, *121*, 4777 – 4779; e) S. Serdjukow, F. Kink, B. Steigenberger, M. Tomas-Gamasa, T. Carell, *Chem. Commun.* **2014**, *50*, 1861 – 1863.

[13] H. J. Jessen, T. Schulz, J. Balzarini, C. Meier, *Angew. Chem. Int. Ed.* **2008**, *47*, 8719 – 8722; *Angew. Chem.* **2008**, *120*, 8847 – 8850.

[14] G. Mayer, A. Heckel, *Angew. Chem. Int. Ed.* **2006**, *45*, 4900 – 4921; *Angew. Chem.* **2006**, *118*, 5020 – 5042.

[15] Y. Watanabe, T. Nakamura, H. Mitsumoto, *Tetrahedron Lett.* **1997**, *38*, 7407 – 7410.

[16] D. Subramanian, V. Laketa, R. Muller, C. Tischer, S. Zarbakhsh, R. Pepperkok, C. Schultz, *Nat. Chem. Biol.* **2010**, *6*, 324 – 326.

[17] DCI leads to higher reaction rates compared with ETT and in cases where it was difficult to follow the reactions by ^{31}P NMR spectroscopic analysis, ETT was used instead of DCI. ETT-mediated reactions are slower but also occur with high efficiency.

[18] It is interesting to note that other more expensive reagents, such as the Beaucage reagent and sulfurizing reagent **2**, gave less clean results in the oxidation step (e.g., partial introduction of O instead of S).

[19] R. O. Schönleber, J. Bendig, V. Hagen, B. Giese, *Bioorg. Med. Chem.* **2002**, *10*, 97 – 101.

[20] L. Xue, P. C. Wang, P. P. Cao, J. K. Zhu, W. A. Tao, *Mol. Cell. Proteomics* **2014**, *13*, 3199 – 3210.

[21] S. B. Ficarro, G. Adelmant, M. N. Tomar, Y. Zhang, V. J. Cheng, J. A. Marto, *Anal. Chem.* **2009**, *81*, 4566 – 4575.

[22] S. Wendicke, S. Warnecke, C. Meier, *Angew. Chem. Int. Ed.* **2008**, *47*, 1500 – 1502; *Angew. Chem.* **2008**, *120*, 1523 – 1525.

Received: March 2, 2015
Published online on June 1, 2015

© 2015 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim