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Efficient Access to Peptidyl Ketones and Peptidyl Diketones via C-Alkylations and C-Acylations of Polymer-Supported Phosphorus Ylides Followed by Hydrolytic and/or Oxidative Cleavage

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ABSTRACT:

Novel syntheses of peptidyl ketones and peptidyl diketones on polymer support are described. Peptidyl phosphoranylidene acetates were prepared via C-acylation of polymer-supported phosphorus ylides. Selective alkylation of the ylide carbon with various alkyl halides, such as methyl iodide and benzyl bromide was established. Peptidyl diketones were obtained by oxidative cleavage. Peptidyl ketones were furnished by hydrolysis of the peptidyl phosphorus ylides under either basic or acidic conditions. © 2010 Wiley Periodicals, Inc. Biopolymers (Pept Sci) 94: 220–228, 2010.

Keywords: peptidyl ketones; C-acylation; phosphorus ylides; protease inhibitors

INTRODUCTION

C-terminal peptide electrophiles have found broad use in synthetic chemistry, biochemistry, and chemical biology.[1] For example, many cysteine and serine proteases are inhibited efficiently by peptide electrophiles, such as aldehydes or α-haloketones, which act via reversible or irreversible reactions with the nucleophilic residue in the active site of the protein.[2] Recently, peptide electrophiles have proven potential for the detection of protein-binding fragments.[3] Even low affinity fragments, reversibly ligated to peptide electrophiles, can be detected with high sensitivity by dynamic ligation screening. For fragment ligation, hitherto C-terminal peptide aldehydes[3a] and peptidyl-α-ketoaldehydes[3b] have been used successfully.

Moreover, peptide electrophiles are valuable synthetic intermediates for the preparation of peptide heterocycles.[4] For example, peptidyl phosphoranes can undergo 1,3-dipolar cycloaddition reactions yielding 1,5-disubstituted triazolyl-peptides.[5] Such reactions were found to deliver the ligation product regioselectively without the need of a metal catalyst, and thus, can be useful for biocompatible ligation.

Peptidyl ketones are chemically and metabolically more stable and thus can display superior bioactivity compared with their respective aldehydes. Whereas C-terminal peptide aldehydes occupy only the substrate binding pockets in the N-terminal direction of the cleaved substrate (the S1, S2, S3-subsites etc.), peptidyl ketones can bind to the pockets in the C-terminal direction (S1′, S2′-subsites etc.) as well. This can be exploited to develop protease inhibitors with significantly increased activity as demonstrated recently for a picomolar ketone inhibitor of caspase-3.[3b] In addition, α-haloketones have found broad use as irreversible inhibitors of cystein proteases.[6]

For these reasons, several approaches for the synthesis of peptidyl ketones (Scheme 1, compound **I**) have been reported.[7,8] Classically, activated amino acids or peptides were employed as electrophiles (Scheme 1, compound **II**), which were converted by addition of various C-nucleophiles to peptidyl ketones **I**. In many examples diazomethane has been used as a nucleophile.[7] By reaction with hydrogen halogenide the obtained diazoketones furnished α-haloketones which reacted smoothly with various nucleophiles to deliver α-substituted peptidyl ketones.[7a–c] Alternatively, peptidyl

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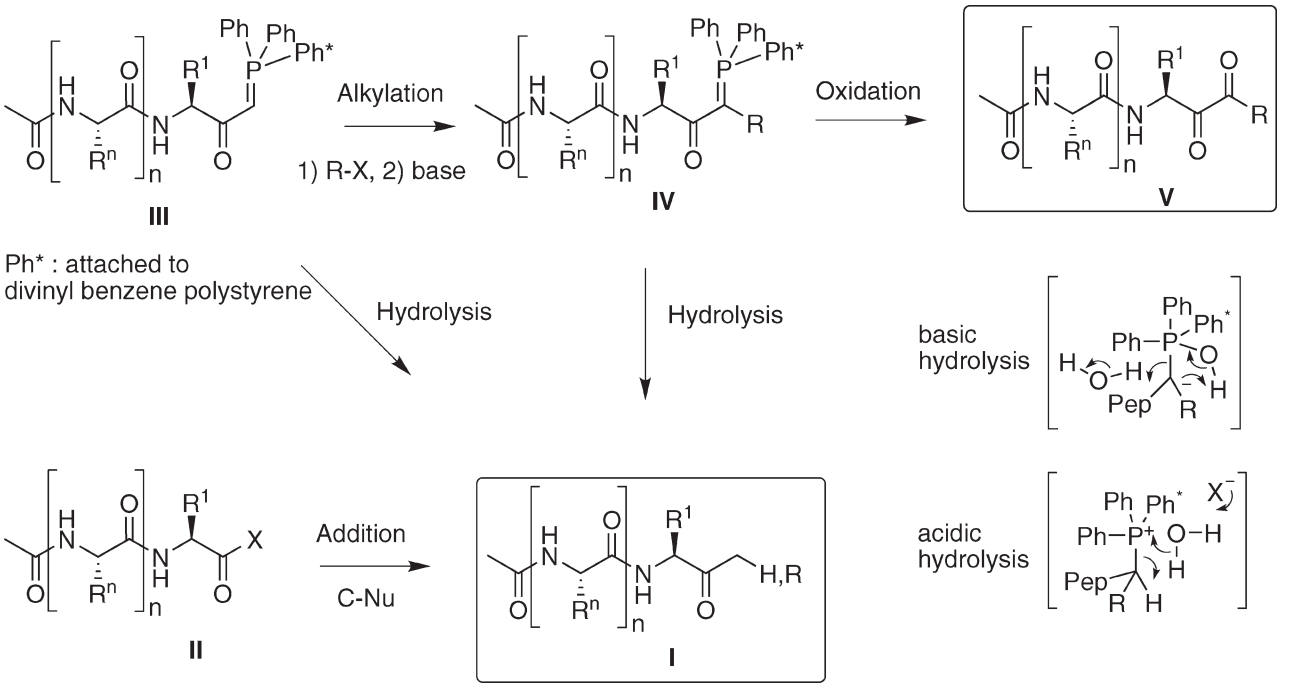


SCHEME 1 Synthesis of peptidyl ketones **I** and peptidyl diketones **V**. Published methods mostly employed a C-terminally activated, electrophilic peptide derivative **II** to which a C-nucleophile was added. In the approach introduced here, a peptidyl phosphorus ylide **III** is used as starting material for a C-alkylation yielding alkylated ylide **IV**. Oxidative cleavage of **IV** provides the peptidyl diketone **V**. Alternatively, hydrolytic cleavage of phosphorus ylides **III** or **IV** yields peptidyl ketones **I** under either basic or acidic conditions.

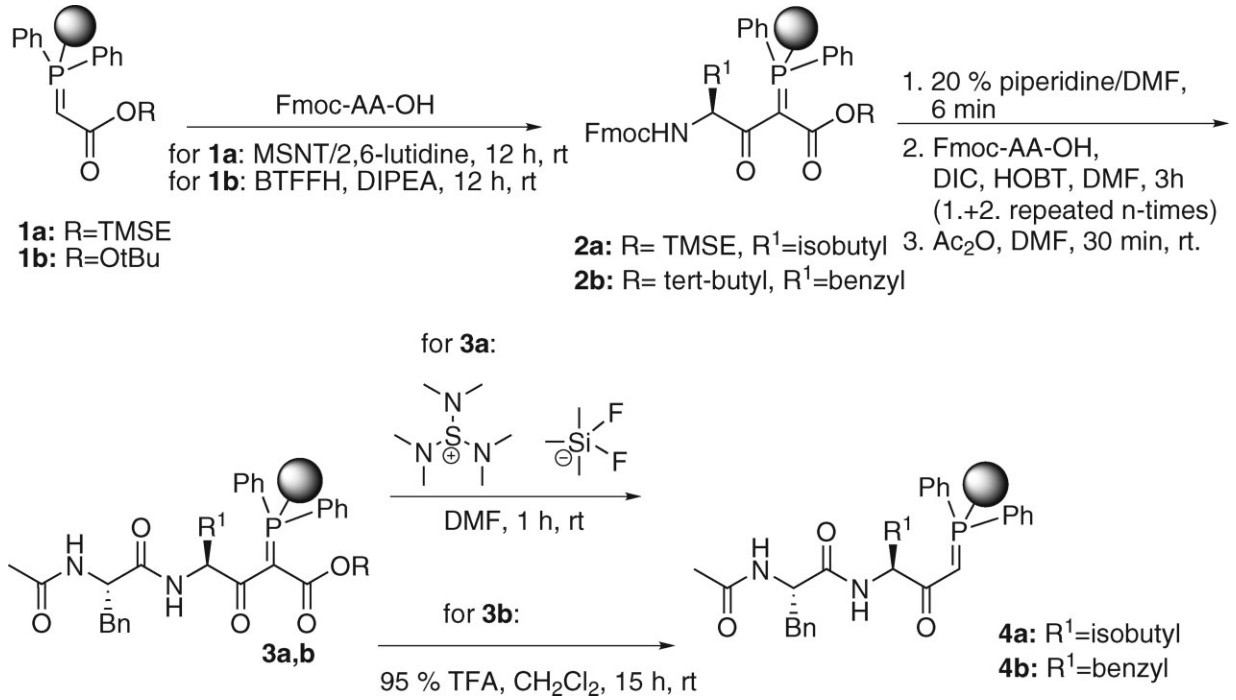
diazoketones have been converted via carbene insertion into various heteroatom-hydrogen bond.[7d] In other cases, amino acid esters or other activated amino acid building blocks have been used for the preparation of ketones employing carbanion nucleophiles such as Grignard reagents.[8a,b] Because of the high basicity of the carbanion reagents, this approach is limited to simple amino acid building blocks and therefore lacks flexibility. For milder conditions, copper-activated aryl boronic acids can be used instead.[8c] Another approach broadly used employs the coupling of amino alcohols followed by oxidation of a secondary alcohol to a ketone.[8d]

Herein, we report the first solid-phase synthesis of peptidyl ketones **I** from a peptidyl phosphorus ylide resin (Scheme 1, compound **III**). The phosphorus ylide **III** was either hydrolyzed directly to a peptidyl ketone **I** with R = Me, or it was alkylated at the ylide carbon first, yielding alkylated phosphorus ylide **IV**. Subsequently, **IV** could be either cleaved off the resin by oxidative cleavage furnishing peptidyl diketone **V** or by hydrolysis yielding the alkylated peptidyl ketones **I** with R ≠ Me.

Recently, the C-acylation of polymer-supported phosphorus ylides has been reported and have been employed in the synthesis of various peptidyl electrophiles.[9,10] Ketoacids, their esters and amides have been made accessible by a solid-phase synthesis on polymer-bound triphenylphosphoranylidene acetonitrile without racemization.[9] Phosphoranylidene acetate esters have been discovered as starting points for 2,3-diketoesters, 2-ketoaldehydes, and vinylketones.[10]

RESULTS AND DISCUSSION

Our earlier findings on the synthesis of peptidyl electrophiles prompted us to develop an efficient access to peptidyl ketones. We reasoned that the alkylation of polymer-bound phosphorus ylides should allow for the synthesis of peptidyl diketones via oxidative cleavage. Peptidyl ketones should be available directly from peptidyl-phosphoranes via hydrolysis of phosphoranes either under basic or acidic conditions. Such cleavage protocols have been reported for other phosphoranes.[11] Mechanistically, they proceed via a hydrolytic redox mechanism and thus can be considered as "oxidative hydrolyses," in which the nucleophilic attack of a water molecule or hydroxide anion at the ylide phosphorus leads to cleavage of the C—P bond accompanied by reduction of the ylide carbon and oxidation of the phosphorus to a phosphine



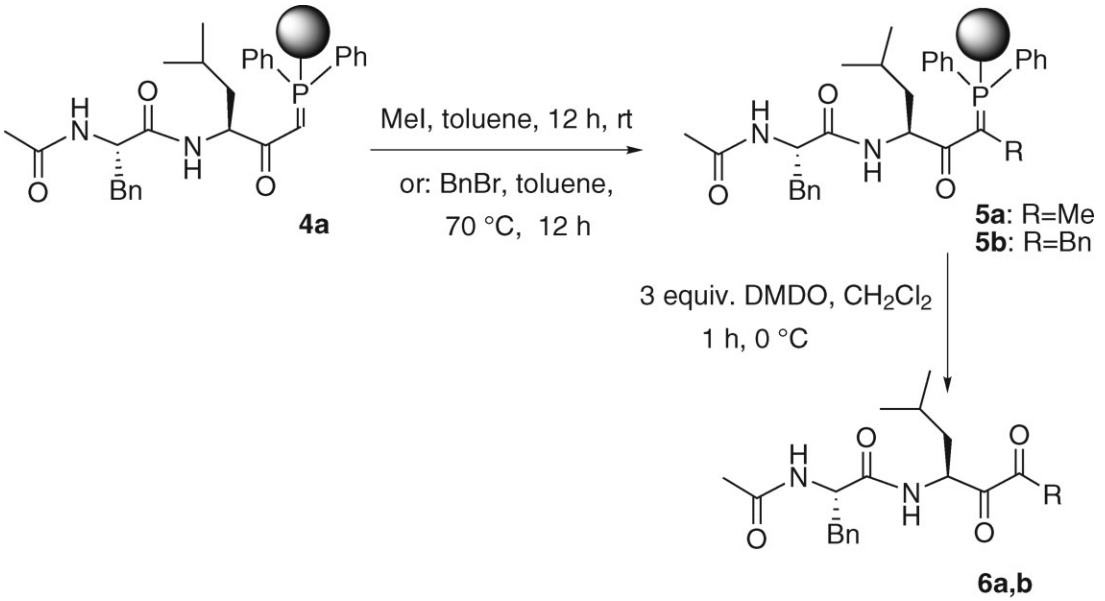
SCHEME 2 Synthesis of peptidyl phosphoranes **4a,b**. C-acylation of the polymer-supported phosphoranyliden acetate **1** followed by Fmoc solid-phase peptide synthesis yielded peptidyl phosphoranylidene acetate **3**. Saponification of **3** was effected by TAS-F (for R = TMSE) or by 95% TFA/CH_2Cl_2 (for R = *tert*-Bu), leading to the decarboxylated peptidyl phosphoranes **4a,b**.

oxide. In theory this oxidative hydrolysis should proceed under acidic as well as basic conditions (Scheme 1).

The starting point for the synthesis of peptidyl ketones was the polymer-supported triphenylphosphoranylidene acetate as trimethylsilylethyl ester **1a** or as *tert*-butyl ester **1b** (Scheme 2). The ylide was first acylated with Fmoc-protected amino acid employing 1-(2-mesitylenesulfonyl)-3-nitro-1H-1,2,4-triazole (MSNT) with lutidine as base (for **1a**) or with fluoro-*N*,*N*,*N*′,*N*′-bis(tetramethylene)formamidinium hexafluorophosphate (BTFFH) with diisopropylethylamine (DIPEA) as base for **1b**. Fmoc-cleavage and peptide elongation was conducted under standard conditions with 20% piperidine/DMF (v/v) and DIC/HOBt. The N-terminus of the obtained peptidyl-phosphoranylidene acetate ester was acetylated to produce the N-acetyl-protected resins **3a,b**. Finally the ester groups of **3a,b** were deprotected with either tris(dimethylamino)sulfonium difluoro-trimethylsilicate (TAS-F) (for **1a**, R = TMSE) or with 95% TFA/CH_2Cl_2 (v/v) (for R = *tert*-butyl) leading to instantaneous decarboxylation resulting in peptidyl-phosphoranylidene propanone resin **4a,b**.

Next, the alkylation of **4a** was investigated using methyl iodide, benzyl bromide, allyl bromide, and *tert*-butyl 2-iodoacetate as alkylating agents. Progress of the alkylation reaction was studied following oxidative cleavage of the alkylation product with dimethyldioxirane (DMDO) (Scheme 3, Table I). Methylation of resin **4a** proceeded smoothly at room temperature (rt) with two equivalents of methyl iodide, preferably in toluene as solvent. Oxidative cleavage furnished the peptidyl diketone **6a** in very good purity and yield. No amide alkylation was detected by LC-MS or NMR spectroscopy. Microwave irradiation succeeded to accelerate the alkylation reaction, however, with reduced purity. Benzylation with benzyl bromide worked similarly well in toluene at 70°C. Following oxidation, the peptidyl diketone **6b** was obtained. Oxidatively cleaved alkylation products were characterized by HR-MS and NMR spectroscopy displaying a single set of signals and no signs of epimerization. Alkylation with allyl bromide required the same conditions, oxidative cleavage, however, delivered a mixture of the diketone product together with its epoxidation product. Alkylations with *tert*-butyl 2-iodoacetate were not successful.

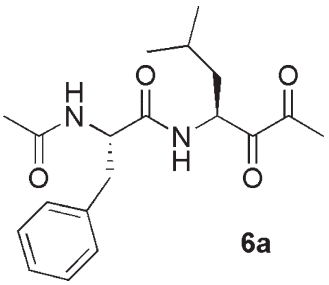
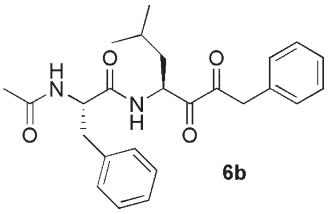
For the preparation of peptidyl ketones, hydrolytic cleavage protocols were investigated. In the first place, basic hydrolyses of the peptidyl phosphoranylidene propane resin **4b** and the alkylation product **5c** derived thereof were investigated (Scheme 4, Table II). Basic hydrolysis required THF/water mixtures and gentle heating (50°C). Sodium and potassium bicarbonate at pH 9 proved to be superior compared



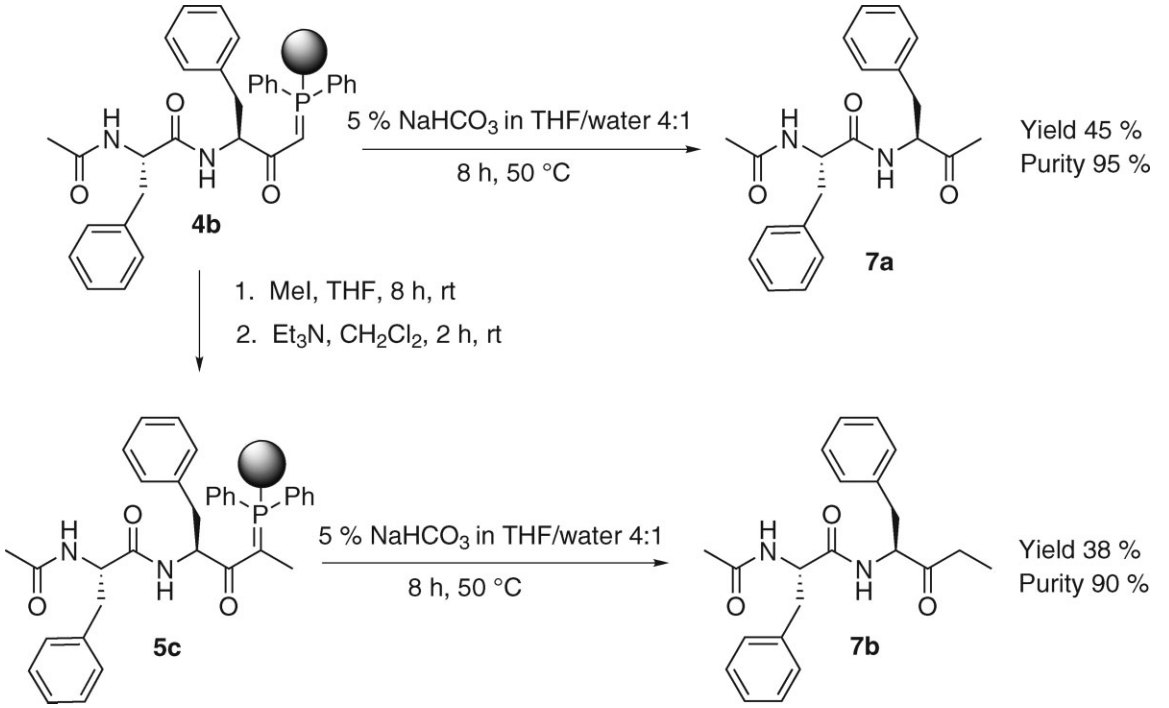
SCHEME 3 Alkylation of peptidyl phosphorane **4a** with either methyl iodide or benzyl bromide yielded the alkylated phosphoranes **5a,b**. Following to oxidative cleavage peptidyl diketones **6a,b** were obtained.

with tertiary amine bases; sodium as counter ion was superior to potassium. The water-free reaction with tetrabutylammonium hydroxide in MeOH and the cleavage with potassium trimethylsilanoxide or sodium hydroxide (both at pH 12) were not successful. Alkylated and nonalkylated peptidyl phosphoranes furnished the peptidyl ketones **7a,b** in

Table I Peptidyl diketones 6a-b Obtained by Oxidative Cleavage Using DMDO

Product	Reaction Conditions	Purity (%)	Yield (%)[a]
 **6a**	MeI (2 equiv), THF, 12 h, rt	90[b,c]	75
	MeI (2 equiv), toluene, 12 h, rt	95[b,c]	73
	MeI (2 equiv), THF, 30 min, 80°C, MW	80[b,c]	69
 **6b**	benzyl bromide (2 equiv), toluene, 12 h, 70°C	93[d]	63

[a] Purities (at 220 nm) and yields of crude products.
[b] pH 9.
[c] Corresponding to a 4:1 mixture of THF and a saturated aqueous solution of the bicarbonate.
[d] pH 12 on wet pH indicator paper.



SCHEME 4 Basic hydrolysis of resin **4b** furnished the peptidyl ketone **7a**. Alkylation of **4b** followed by basic hydrolysis delivered the peptidyl ketone **7b**.

moderate yields and good purity. In the NMR spectrum, compound **7a** displayed two sets of signals in a 4:1 ratio, whereas **7b** contained a single set of signals (Scheme 4).

Acidic hydrolysis was found to proceed well with a 1:1:4 mixture of acetic acid, water, and THF at 50°C for 8 h delivering the peptidyl methylketone **7c** (Scheme 5) displaying two sets of signals in the NMR spectrum. Remarkably, acidic cleavage of **4b** proceeded with high purity and significantly higher yield than the basic cleavages. Use of trifluoroacetic acid (TFA) and water under the same conditions or at rt led to efficient resin cleavage, however, complex product mixtures were formed which could not be characterized. Acidic hydrolyses were also studied for the peptidyl-phosphoranylidene acetate **8** (Scheme 5, bottom) employing acetic as well as trifluoroacetic acid in water. Both conditions effected cleavage from the resin, however, complex product mixtures were obtained which could not be assigned.

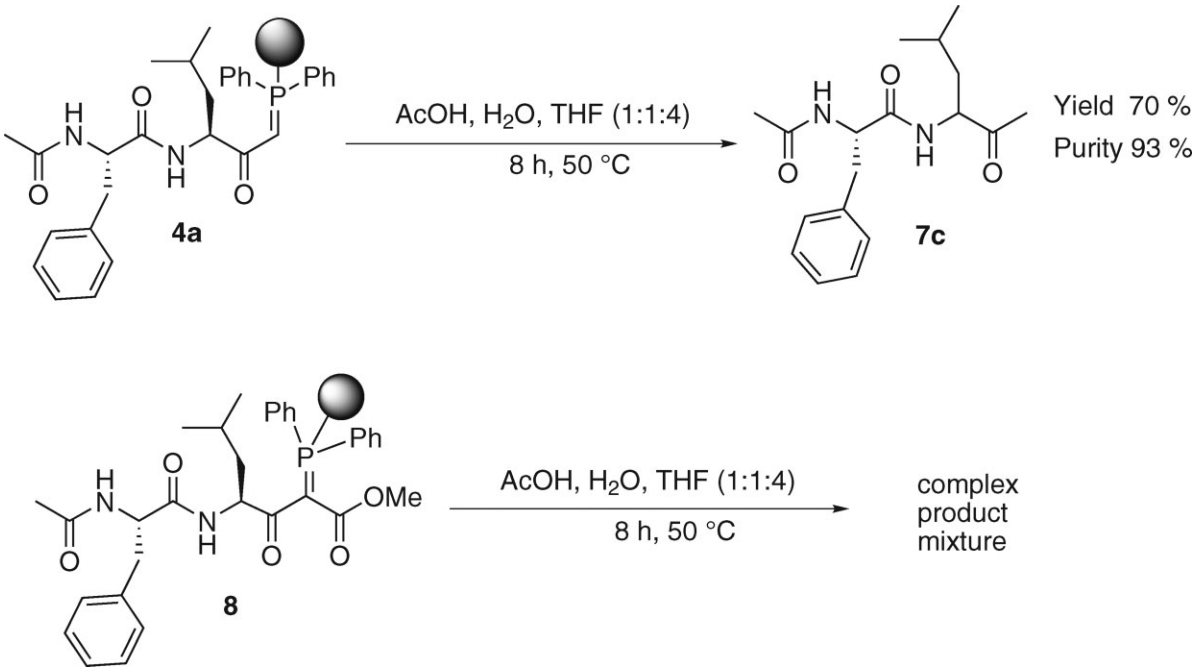
CONCLUSIONS

In summary, we have demonstrated the use of polymer-supported phosphoranes in a novel synthesis of peptidyl ketones

Table II Basic Hydrolysis of Peptidylphosphoranes 4b and 5c (Scheme 4) Using Different Conditions

Reaction Conditions	R	Purity (%)[a]	Yield (%)[a]
5% $NaHCO_3$ in THF/water 4:1, 50°C, 8 h[b,a]	Me	90	38
5% $NaHCO_3$ in THF/water 4:1, 50°C, 8 h[b]	H	95	45
5.4% $KHCO_3$ in THF/water 4:1, 50°C, 8 h[b]	Me	50	<5
5.4% $KHCO_3$ in THF/water 4:1, 50°C, 8 h[b]	H	60	10
DIPEA/water/THF 1:1:4 (v/v), 50°C, 8 h[b]	H	<5	0
0.12% NaOH in THF/water 4:1, (0.1 equiv), 50°C, 8 h[b]	H	<5	<5
THF/H_2O (1:4, v:v), 50°C, 8 h	H	0	0
3.5% $KOSi(Me)_3$ in THF/water 4:1, (1 equiv), rt, 8 h[c]	H	0	0
5.6% $N(Bu)_4OH$ in MeOH, (1 equiv), rt, 8 h[c]	H	0	0

[a] Purities (at 220 nm) and yields of crude products; corresponding to a 4:1 mixture of THF and a saturated aqueous solution of the bicarbonate.
[b] pH 9.
[c] pH 12 on wet pH indicator paper.



SCHEME 5 Synthesis of peptidyl methylketone **7c** using acidic hydrolysis of resin **4a**. Conditions optimized for the cleavage from resin **4a** were applied to resin **8** and yielded a complex mixture of products.

and peptidyl diketones. Polymer-bound phosphoranylidene acetates were employed as C-nucleophiles, which can be C-acylated with standard Fmoc-protected amino acid building blocks under mild conditions. Saponification of the phosphoranylidene acetate ester led to decarboxylation yielding peptidyl phosporane resins. Introduction of the ketone functionality was realized without using the strongly basic C-nucleophiles conventionally employed such as Grignard reagents. Likewise, diazoalkanes such as diazomethane were not required. Instead, polymer-bound peptidyl phosphoranes were alkylated by aliphatic halogenides, such as methyl iodide and benzyl bromide.

Peptidyl ketones were released from solid support in high purity by either basic or acidic hydrolytic cleavage of the peptidyl phosphoranes. While both methods delivered relatively pure products, acidic cleavage was superior with respect to the yields. In addition, peptidyl diketones were obtained from the alkylated peptidyl phosphoranes by oxidative cleavage. To the best of our knowledge peptidyl diketones have not been described before.

Both product classes (peptidyl ketones and peptidyl diketones) can be modified using this approach by variation of the peptide sequence and by alkylation of the peptidyl phosphorane on solid support. In addition, further variations might be accessible by exchanging the starting bromoacetate employed in the initial phosphine alkylation step by α-haloketones or other electrophiles.

Flexible access to peptidyl ketones as well as peptidyl diketones will enable investigation of the biological potential of these classes of compounds, their application in fragment-based high throughput screening as in dynamic ligation screening. Broader applications of the chemistry described here and the use of products as synthetic intermediates, e.g. for the construction of novel peptide-heterocycle chimera, are currently under investigation in our laboratory.

MATERIALS AND METHODS

Unless otherwise stated, all reagents were obtained from commercial suppliers and used without further purification. Dry solvents were purchased and stored over molecular sieves. Solid-phase chemistry was performed in plastic syringes equipped with teflon filters. Triphenylphosphine polystyrene resin was purchased from Fluka. Microwave-assisted solid-phase reactions were performed with a Biotage Initiator monomodal microwave reactor. Resins were washed with DMF, THF, toluene, and CH_2Cl_2 unless otherwise stated. Resin loadings were determined by photometric determination after Fmoc cleavage. Solid-phase reactions were monitored by FT-ATR-IR spectra of the resins recorded with a Bruker Tensor 27 FTIR spectrometer. Absorptions are reported in wave numbers (cm^{-1}). Purification of products was performed on a semipreparative HPLC column (10 μm, 250 × 20 mm, Grom-SIL 300 ODS-5 ST RP-C18) employing individual gradients derived from analytical

runs. LC-MS was conducted on an Agilent 1100Series HPLC system equipped with an ESI-MS with single quadrupol detector. HRMS measurements were conducted on an Agilent 6220 accurate mass ESI-ToF-MS.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 300 MHz instrument and chemical shifts were measured in parts per million (ppm).

Synthesis of Polymer-Supported 2-(trimethyl)-silylethyl 2-phosphoranylidene-acetate 1a and *Tert*-butyl 2-phosphoranylidene-acetate 1b

Triphenylphosphine polystyrene resin (0.5 g, 1.6 mmol/g, 0.8 mmol, 1% divinyl benzene, 100–200 mesh) was weighed into a microwave vial and suspended in dry toluene (4 mL). After addition of 2-(trimethylsilyl)ethyl 2-bromoacetate (990 mg, 4 mmol, 5 equiv) or *tert*-butyl 2-bromoacetate (770 mg, 4mmol, 5 equiv), the vial was sealed and heated at 100°C for 15 min in a microwave synthesizer. The vial was cooled to rt before opening, the resin was filtered and washed with dry toluene (3 × 8 mL) and CH_2Cl_2 (3 × 8 mL). The obtained polymer phosphonium salt was suspended in dry CH_2Cl_2 (5 mL) and triethylamine (Et_3N) (557 μL, 4 mmol, 5 equiv) was added. After shaking for 2 h at rt, the yellow-colored resin **1a,b** was filtered, washed with dry CH_2Cl_2 (3 × 8 mL), DMF (3 × 8 mL), THF (3 × 8 mL) and dried in vacuo.

IR (ATR) of resin **1a**: cm^{-1} = 3056, 3025, 2923, 2852, 1725, 1600, 1493, 1452, 1437, 1380, 1310, 1249, 1183, 1116, 835, 751, 698, 542.

Synthesis of Polymer-Supported 2-Acyl-2-phosphoranylidene acetates 2a,b

R* = *Trimethylsilylethyl. Triphenylphosphoranylidene acetate resin **1a** (200 mg, 1.43 mmol/g, 0.28 mmol) was preswollen in dry CH_2Cl_2. The Fmoc-AA-OH (1.43 mmol, 5 equiv) was suspended in dry CH_2Cl_2 (4 mL) and dissolved under addition of 2,6-lutidine (162,7 μL, 1.40 mmol, 4.9 equiv). The clear solution obtained after addition of 1-(2-mesitylenesulfonyl)-3-nitro-1H-1,2,4-triazole (MSNT) (414.8 mg, 1.43 mmol, 5 equiv) was mixed with the resin suspension and shaken overnight at rt. After the coupling step the product resin **2a** was washed with CH_2Cl_2 (3 × 5 mL), DMF (3 × 5 mL), THF (3 × 5 mL) and dried in vacuo. Acylation yields, determined by photometric determination after Fmoc cleavage of small samples, were 85% for phenylalanine and 95% for leucine.

IR (ATR) of resin **2a** after acylation with Fmoc-Phe-OH: cm^{-1} 3058, 3025, 2923, 2853, 1942, 1718, 1654, 1601, 1559, 1521, 1507, 1492, 1451, 1437, 1384, 1351, 1248, 1195, 1117, 1080, 1029, 835, 742, 698, 613, 541.

R* = tert-*Bu. Resin **1b** (300 mg, 1.32 mmol/g, 0.396 mmol) was preswollen in dry CH_2Cl_2. The Fmoc-AA-OH (1.98 mmol, 5 equiv) was dissolved in dry DMF (6 mL) after addition of diisopropylethylamine (DIPEA) (687 μL, 3.96 mmol, 10 equiv) and fluoro-*N,N,N′,N′*-bis(tetramethylene)formamidinium hexafluorophosphate (BTFFH) (625 mg, 1.98 mmol, 5 equiv). The clear solution obtained was mixed with the resin suspension and shaken overnight at rt. After the coupling step the resin was washed thoroughly with CH_2Cl_2 and dried in vacuo to yield product **2b**. Based on Fmoc determination, the yield for the acylation with leucine was 86%.

Synthesis of Polymer-Supported *N*-acetyl-2-peptidyl-2-phosphoranylidene acetates 3a and 3b

Piperidine/DMF (20% v/v, 2 × 6 min 5 mL) was added to resin **2a** (200 mg, 1.43 mmol/g, 0.28 mmol) for deprotection of the Fmoc group. The deprotected resin was washed with DMF (3 × 0.5 min 5 mL) and suspended in dry DMF (2 mL). Fmoc-amino acid (1.43 mmol, 5 equiv) and HOBt H_2O (214.3 mg, 1.43 mmol, 5 equiv) were dissolved in dry DMF (4 mL), preactivated (3 min) under addition of *N,N′*-diisopropylcarbodiimide (DIC) (221.4 μL, 1.43 mmol, 5 equiv) and added to the resin following standard Fmoc solid-phase peptide synthesis. The mixture was shaken for 3 h at rt, filtered and the resin was washed with DMF (3 × 5 mL), CH_2Cl_2 (3 × 5 mL), THF (3 × 5 mL). The procedure (cleavage and coupling step) was repeated with further amino acids. In the final step, the free amino resin was treated with acetic anhydride (142.8 μL, 1.43 mmol, 5 equiv) in DMF (5 mL) and the mixture was shaken for 30 min at rt. The resin was filtered, washed with DMF (3 x 5 mL), CH_2Cl_2 (3 × 5 mL), THF (3 × 5 mL) and dried in vacuo. Quantitative couplings were confirmed employing the Kaiser test.

Representative IR (ATR) after the acylation with Fmoc-Phe-OH and the peptide elongation with further Fmoc-Phe-OH **3a**: IR (ATR): cm^{-1} 3057, 3025, 2973, 2924, 2854, 1954, 1743, 1665, 1600, 1546, 1493, 1452, 1437, 1381, 1275, 1249, 1195, 1116, 1071, 1029, 834, 750, 698, 621, 541, 522.

Synthesis of Polymer-Supported *N*-acetyl-peptidyl-3-amino-2-oxo-1-phosphoranylidene propane 4

From 3a. Tris(dimethylamino)sulphur (trimethylsilyl)difluoride (TAS-F, 231.3 mg, 0.84 mmol, 3 equiv) in dry DMF was added to a suspension of peptidyl-α,β-keto(trimethylsilylethylester)methylene-triphenylphosphorane resin **3a** (200 mg, 1.43 mmol/g, 0.28 mmol) in DMF and the mixture was stirred for 1 h at rt. The resin was filtered, washed with DMF (3 × 5 mL), CH_2Cl_2 (3 × 5 mL), THF (3 × 5 mL) and dried in vacuo. The deprotection was quantitative.

IR (ATR) **4**: cm^{-1} 3059, 3025, 2926, 2851, 2149, 1731, 1599, 1492, 1451, 1437, 1256, 1185, 1111, 751, 698, 569, 517, 512.

From 3b. The resin **3b** (200 mg, 1.50 mmol/g, 0.3 mmol) was treated with TFA: CH_2Cl_2 (95:5, 8 mL) for 15 h to remove the *tert*-butyl group. After washing with CH_2Cl_2 (5 times), the resin was deprotonated with Et_3N (42 μL, 0.9 mmol, 3 equiv) in CH_2Cl_2 (5 mL) for 1 h. Then it was washed with CH_2Cl_2 and dried. The deprotection was quantitative.

Synthesis of Peptidyl Diketones 6a,b

Alkyl halide (0.76 mmol, 3 equiv) was added to a suspension of peptidyl-3-amino-2-oxo-1-phosphoranylidene propane resin **4** (200 mg, 1.43 mmol/g, 0.28 mmol) in dry THF. The mixture was stirred for 12 h at rt for methyl iodide (119.2 mg, 0.84 mmol, 3 equiv) and heated at 70°C in the case of benzyl bromide (141.9 mg, 0.84 mmol, 3 equiv), filtered and washed with THF (3 × 5 mL), DMF (3 × 5 mL), and CH_2Cl_2 (3 × 5 mL). The resin was preswollen in CH_2Cl_2 and deprotonated with Et_3N (3 equiv) for 3 h at rt, washed with

CH_2Cl_2 (3 × 0.5 min, 5 mL) and dried in vacuo yielding alkylated resins **5a-c**. For oxidative cleavage the resin was pre-swollen in dry CH_2Cl_2. Cold DMDO solution in acetone (12 mL each, approx. 0.072 mmol/mL, 0.864 mmol, 3–4 equiv, −20 °C) was carefully added and the mixture was stirred for 1 h at 0°C with the yellow colored resins turning pale. The resin was filtered off and washed with CH_2Cl_2 (2 × 0.5 min 3mL). Solvents from combined filtrate were removed under vacuo to get crude products **6a,b**. Whereas **6a** was pure in the crude material **6b** was purified by preparative HPLC. Both **6a** and **6b** were analyzed by NMR spectroscopy contained a single diastereomer.

N-acetyl-L-phenylalanyl-4-(S)-amino-4-isobutyl-butan-2,3-dione 6a. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.16–7.29 (m, 5H, Phe), 6.61 (d, 1H, $J = 6.1$ Hz, NH_{II}, Phe), 6.42 (d, 1H, $J = 7.5$ Hz, NH_I, Phe), 4.86–4.93 (dd, 1H, $J = 3.4$ Hz, $C^{\alpha}H$, Phe), 4.67–4.74 (m, 1H, $C^{\alpha}H$, isobutyl), 2.98–3.05 (m, 2H, $C^{\beta1,\beta2}H$, Phe), 2.30 (s, 3H, CH_3-diketone), 1.95 (s, 3H, CH_3-CO), 1.24–1.50 (m, 3H, $C^{\beta1}H_2$, $C^{\gamma}H$, isobutyl), 0.84–0.86 (d, 6H, $J = 7.2$ Hz, CH_3, CH_3, isobutyl); HRMS (ESI): Calculated for $C_{19}H_{26}N_2O_4[M+H]^+$: 347.1965 Da. Found: 347.1972 m/z. (Yield: 65.8 mg, 73%; Purity of isolated product 95%).

N-acetyl-L-phenylalanyl-4-(S)-amino-4-isobutyl-1-phenyl-butan-2,3-dione 6b. ^{1}H-NMR: (300 MHz, $CDCl_3$): δ 7.86 (d, 1H, $J = 7.3$ Hz, NH_{II}), 7.16–7.42 (m, 10 H, arom.), 6.38 (d, 1H, $J = 7.9$ Hz, NH_I), 4.65–4.75 (m, 1H, $C^{\alpha}H$, isobutyl), 4.04 (d, 2H, $J = 15.8$ Hz, CH_2, benzyl), 2.92–3.07 (m, 2H, CH_2, Phe), 1.94 (s, 3H, CH_3-CO), 1.18–1.50 (m, 3H, CH_2, CH, isobutyl), 0.79, 0.83 (d, 6H, $J = 6.1$Hz, CH_3, CH_3, isobutyl). ^{13}C-NMR: (75.5 MHz, $CDCl_3$): δ 196.4, 195.6, 170.8, 170.4, 144.9, 135.9, 130.6, 129.9, 128.6, 127.3, 126.9, 115.7, 54.6, 52.3, 49.3, 42.8, 38.6, 24.8, 23.0, 21.8, 21.4; HRMS (ESI): Calculated for $C_{25}H_{30}N_2O_4$ $[M+H]^+$: 423.22838 Da. Found: 423.2288 m/z. (Yield: 67 mg, 63%; Purity of isolated product 93%).

Synthesis of Peptidyl Ketones 7a,b via Basic Hydrolysis

A 5% solution of $NaHCO_3$ in THF/water 4:1 (v/v) (1 mL, 50 mg $NaHCO_3$ were dissolved in 200 μL H_2O and mixed with 800 μL THF) was added to the peptidyl-3-amino-2-oxo-1-phosphoranylidene propane resin **4b** (200 mg, 1.43 mmol/g, 0.28 mmol) or the alkylated resin **5c** and agitated for 8 h at 50°C. The resin was filtered off and washed with THF (2 × 0.5 min shaking). The combined filtrates were evaporated in vacuo and crude product was purified by preparative HPLC. The obtained products **7a,b** were characterized by LC-MS and NMR. The NMR of **7a** displayed two sets of signals in a 4:1 ratio whereas the spectrum of **7b** showed a single set of signals.

N-acetyl-L-phenylalanyl-3-(S)-amino-3-benzyl-propan-2-one 7a. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.96–7.27 (m, 10H, aromatic H, Phe), 6.31–6.33 (d, 1H, $J = 6.1$ Hz, NH_{II}, Phe), 5.97–5.99 (d, 1H, $J = 7.3$ Hz, NH_I, benzyl), 4.60–4.72 (m, 2H, $C^{\alpha}H$, benzyl, $C^{\alpha}H$, Phe), 2.86–3.07 (m, 4H, $C^{\beta1,\beta2}H_2$, benzyl, $C^{\beta1,\beta2}H_2$, Phe), 2.02 (s, 3H, CH_3-CO, ketone), 1.94 (s, 3H, CH_3-CO, amide); HRMS (ESI): Calculated for $C_{21}H_{24}N_2O_3$ $[M+H]^+$: 353.1860 Da. Found: 353.1869 m/z. (Yield: 40.2 mg, 45%; Purity of crude product 95%).

N-acetyl-L-phenylalanyl-4-(S)-amino-4-benzyl-butan-3-one 7b. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.97–7.22 (m, 10H, aromatic H, Phe), 6.25–6.30 (d, 1H, $J = 7.3$ Hz, NH_{II} Phe), 5.94–6.96 (d, 1H, $J = 7.2$ Hz, NH_I benzyl), 4.58–4.68 (m, 2H, $C^{\alpha}H$, Phe, C^{α}H, benzyl), 2.80–3.06 (m, 4H, $C^{\beta1,\beta2}H_2$, Phe, $C^{\beta1,\beta2}H_2$, benzyl), 2.23–2.29 (m, 2H, CH_2CH_3), 1.97 (s, 3H, CH_3CO), 0.95–0.99 (t, 3H, $J = 6.7$ Hz, CH_3CH_2); HRMS (ESI): Calculated for $C_{22}H_{26}N_2O_3$ $[M+H]^+$: 367.2016 Da. Found: 367.2018 m/z. (Yield: 35.3 mg, 49%; Purity of crude product 90%).

Synthesis of Peptidyl Ketone *N*-acetyl-L-phenylalanyl-3-amino-3-isobutyl-propan-2-one 7c via Acidic Hydrolysis

Glacial acetic acid, water, and THF were mixed in the ratio 1:1:4 (v/v), the mixture (1 mL) was added to peptidyl-3-amino-2-oxo-1-phosphoranylidene propane resin **4b** (200 mg, 1.43 mmol/g, 0.28 mmol) and was agitated for 8 h at 50°C. The resin was filtered off and washed with THF (2 × 0.5 min). The combined filtrates were evaporated in vacuo and crude product was purified by preparative HPLC. The obtained product **7c** was characterized by LC-MS and NMR.

^{1}H-NMR (300 MHz, DMSO-d_6): δ 8.33–8.35 (d, 1H, $J = 8.5$ Hz, NH_{II}, isobutyl); 8.12–8.15 (d, 1H, $J = 7.3$ Hz, NH_I, Phe), 7.18–7.26 (m, 5H, aromatic H, Phe), 4.50–4.58 (m, 1H, $C^{\alpha}H$, Phe), 4.06–4.16 (m, 1H, $C^{\alpha}H$, isobutyl), 2.71–2.78, 2.86–2.99 (m, 2H, $C^{\beta1,\beta2}H_2$, Phe), 2.03 (s, 3H, CH_3CO ketone), 1.90 (s, 3H, CH_3CO), 1.23–1.35, and 1.73–1.77 (m, 3H, $C^{\beta1,\beta2}H_2$, isobutyl, $C^{\gamma}H$, isobutyl), 0.82–0.84 (d., 3H, $J = 6.7$ Hz, $C^{\delta2}H_3$, isobutyl), 0.72–0.74 (d, 3H, $J = 6.7$ Hz, $C^{\delta1}H_3$, isobutyl). ^{13}C NMR (100 MHz, DMSO-d_6) δ 208.63, 171.71, 169.33, 138.08, 129.36, 128.28, 127.35, 126.48, 57.18, 54.07, 40.61, 40.33, 40.05, 39.78, 39.22, 38.95, 26.32, 24.16, 23.33, 22.63, 21.39; HRMS (ESI): Calculated for $C_{18}H_{26}N_2O_3$ $[M+H]^+$: 319.2016 Da. Found: 319.2019 m/z. (Yield: 56.5 mg, 70%; Purity of crude product 93%).

REFERENCES

1. (a) Wolkenberg, S. E.; Wisnoski, D. D.; Leister, W. H.; Wang, Y.; Zhao, Z.; Lindsley, C. W. Org Lett 2004, 6, 1453–1456; (b) Martin, J. L.; Begun, J.; Schindeler, A.; Wickramasinghe, W. A.; Alewood, D.; Alewood P. F.; Bergman, D. A.; Brinkworth, R. I.; Abbenante, G.; March, D. R.; Reid, R. C.; Fairlie, D. P. Biochemistry 1999, 38, 7978–7988; (c) Angliker, H. J Med Chem 1995, 38, 4014–4018; (d) Smith, A. E.; Helenius, A. Science 2004, 304, 237–242; (e) Han, B. H.; Xu, D.; Choi, J.; Han, Y.; Xanthoudakis, S.; Roy, S.; Tam, J.; Vaillancourt, J.; Colucci, J.; Siman, R.; Giroux, A.; Robertson, G. S.; Zamboni, R.; Nicholson, D. W.; Holtzman, D. M. J Biol Chem 2002, 277, 30128–30136.
2. Lynas, J.; Martin, S.; Walker, B. J Pharm Pharmacol 2001, 53, 473–480.
3. (a) Schmidt, F. M.; Isidro, A.; El-Dahshan, A; Lisurek, M.; Hilgenfeld, R.; Rademann, J. Angew Chem 2008, 120, 3319–3323; (b) Schmidt, F. M.; Isidro, A.; El-Dahshan, A; Lisurek, M.; Hilgenfeld, R.; Rademann, J. Angew Chem Int Ed 2008, 47, 3275–3278; (c) Schmidt, F. M.; El-Dahshan, A.; Keller, S.; Rademann,

J. Angew Chem 2009, 121, 6464–6467, Angew Chem Int Ed 2009, 48, 6346–6349.

4. (a) Costanzo, M. J. J Med Chem 2005, 48, 1984–2008; (b) Akamatsu, H.; Fukase, K.; Kusumoto, S. J Comb Chem 2002, 4, 475–483; (c) Groth, T.; Meldal, M. J Comb Chem 2001, 3, 45–63; (d) Aldington, R. M.; Baldwin, J. E.; Catterick, D.; Pritchhard, G. J. J Chem Soc Perkin Trans 2000, 1, 299–302.
5. Ahsanullah; Schmieder, P.; Kühne, R.; Rademann, J. Angew Chem 2009, 121, 5143–5147.
6. Giordano, C.; Gallina, C.; Consalvi, V.; Scandurra, R. Eur J Med Chem 1989, 24, 357–362.
7. (a) Tripathi, R.; Ator, M. A.; Mallamo, J. P. Bioorg Med Chem Lett 2000, 10, 2315–2319; (b) Wagner, B. M.; Smith, R. A.; Coles, P. J.; Copp, L. J.; Ernest, M. J.; Krantz, A. J Med Chem 1994, 37, 1833–1840; (c) Marquis, R. W.; Ru, Y.; Yamashita, D. S.; Oh, H.; Yen, J.; Thompson, S. K.; Carr, T. J.; Levy, M. A.; Tomaszek, T. A.; Ijames, C. F.; Smith, W. W.; Zhao, B.; Janson, C. A.; Abdel-Meguid, S. S.; D'Alessio, K. J.; McQueney, M. S.; Veber, D. F. Bioorg Med Chem 1999, 7, 581–588; (d) O'Leary, P.; Maguire, A. R. Arkivok 2009, 130–151.
8. (a) Conrad, K.; Hsiao, Y.; Miller R. Tetrahedron Lett 2005, 46, 8587–8589; (b) Ooi, T.; Takeuchi, M.; Kato, D.; Uematsu, Y.; Tayama, E.; Sakai, D.; Maruoka, K. J Am Chem Soc 2005, 127, 5073–5083; (c) Liebeskind, L. S.; Yang, H; Li, H. Angew Chem Int Ed 2009, 121, 1445–1449, Angew Chem Int Ed 2009, 48, 1417–1421; (d) Calabretta, R.; Giordano, C.; Gallina, C.; Morea, V.; Consalvi, V.; Scandurra, R. Eur J Med Chem 1995, 30, 930–941.
9. (a) Weik, S.; Rademann, J. Angew Chem Int Ed 2003, 115, 2595–2598, Angew Chem Int Ed 2003, 42, 2491–2494; (b) Weik, S.; Luksch, T.; Evers, A.; Böttcher, J.; Sotriffer, C. A.; Hasilik, A.; Löffler, H. G.; Klebe, G.; Rademann, J. ChemMedChem. 2006, 1, 445–57.
10. El-Dahshan, A.; Weik, S.; Rademann, J. Org Lett 2007, 129, 12670–12671.
11. (a) Araya-Maturana, R; Castaneda, F. Phosphorus Sulfur Silicon 1993, 81, 165–172; (b) Baldoldi, C.; Licandro, E.; Maiorana, S.; Menta, E.; Papagni, A. Synthesis 1987, 288–291; (c) Cooke, M. P.; Burman, D. L. J Org Chem 1982, 47, 4955–4963; (d) Gouterman, M.; Connell, C. R.; Sayer, P. J Am Chem Soc 1977, 19, 642–644.