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C-Acylations of Polymeric Phosphoranylidene Acetates for C-Terminal Variation of Peptide Carboxylic Acids

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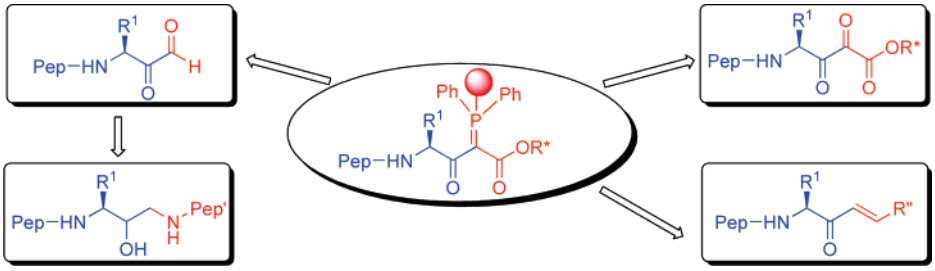
ABSTRACT



C-Acylations of polymer-supported 2-phosphoranylidene acetates ("linker reagents") with protected amino acids yielded 2-acyl-2-phosphoranylidene acetates as flexible intermediates for the C-terminal variation of carboxylic acids: peptidyl-2,3-diketoesters, peptidyl vinyl ketones, peptidyl-2-ketoaldehydes, and 1,3-diamino-2-hydroxy-propanes were obtained as products.

In the biosynthesis of many natural products including fatty acids, polyketides, terpenes, and steroids, C-acylations are the initiating steps for the construction of carbon chains and complex scaffolds.[1] While O-, N-, and S-acylations can be operated routinely in solution and on solid phase, C-acylations have not been investigated for solid-phase synthesis and peptide preparation to a larger extent so far.[2] Integration of C-acylation steps into the standard protocols of peptide synthesis would be especially attractive for the synthesis of peptide mimetics and in the attempts to reduce the peptide character of inhibitors, a common challenge in drug development programs.

As a first step toward polymer-supported C-acylation, linker reagents have been introduced as a tool in solid-phase synthesis combining the functions of a polymer reagent and those of an anchoring group allowing for subsequent derivatization of the immobilized product.[3] Polymeric 2-phosphoranylidene acetonitrile was demonstrated in efficient C-acylation reactions and was applied for the synthesis of a library of norstatines as transition-state isosteres being active as aspartic protease inhibitors.[4]

In these works, the reagent linkers proved to be especially useful for the variation of all the positions around the isosteric core as standard Fmoc-protected amino acids could be employed for the CC coupling on the polymer support. In this contribution, we investigate the use of 2-phosphoranylidene acetates **2a**,**b** as linker reagents. C-Acylation of polymer-supported 2-phosphoranylidene acetates **2a**,**b** could

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open novel opportunities for the on-resin synthesis of peptidomimetics and heterocyclic products. The significantly reduced nucleophilicity of resin **2**, however, posed a major challenge to find efficient acylation conditions. Resin **2** could be obtained from methyl α-bromoacetate[5−7] and the commercially available triphenylphosphane resin **1** under gentle microwave heating, yielding the intermediary phosphonium salt which was deprotonated with triethylamine as a base (Scheme 1). Complete alkylation of the phosphane was

Scheme 1. Phosphoranes **2a**,**b** Acylated Efficiently with Aliphatic and Aromatic Carboxylic Acids

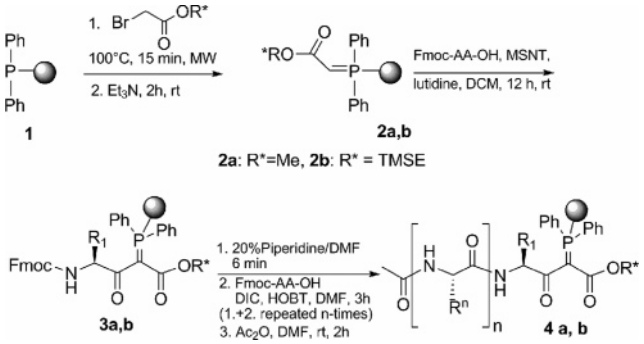

Table 1. Acylation of Amino Acids on Linker Reagents Using Various Acylation Methods

entry	Fmoc−amino acid	activation methods	yield **3a** [%][a]
1	Fmoc−F-OH	EDC (5 equiv)/ DMAP (0.74 equiv)	46
2	Fmoc−F-OH	DIC (5 equiv)/ DMAP (0.75 equiv)	33
3	Fmoc−F-OH	HATU (5 equiv)/ DIPEA (0.75 equiv)	29
4	Fmoc−F-OH	TFFH (5 equiv)/ DIPEA (10 equiv)	86
5	Fmoc−F-OH	MSNT (5 equiv)/ 2,6-lutidin (4, 9 equiv)	85
6	Fmoc−L-OH	MSNT (5 equiv)/ 2,6-lutidin (4, 9 equiv)	84
7	Fmoc−G-OH	MSNT (5 equiv)/ 2,6-lutidin (4, 9 equiv)	79
8	Fmoc−T(^{t}Bu)-OH	MSNT (5 equiv)/ 2,6-lutidin (4, 9 equiv)	82
9	Fmoc−D(O^{t}Bu)-OH	MSNT (5 equiv)/ 2,6-lutidin (4, 9 equiv)	84

[a] Yields are determined by spectrophotometric quantification of Fmoc groups cleaved off a weighed and dried resin sample.

accomplished within 15 min. The acylation of **2a**,**b** was investigated employing various Fmoc-protected amino acids under a variety of coupling conditions. Standard conditions used for peptide couplings failed completely, including the use of DIC/HOBt, TBTU, and PyBOP.[8−13] Stronger activation such as the use of *N*-ethyl-*N*′-(3-dimethylaminopropyl)-carbodiimide (EDC) with catalytic DMAP that succeeded in acylations of the 2-phosphoranylidene acetonitrile[3,4] furnished only low yields of products (Table 1, entries 1−3).

On the contrary, efficient acylations could be performed with fluoro-*N*,*N*,*N*′,*N*′-tetramethylformamidinium hexafluorophosphate (TFFH) or with 1-(2-mesitylenesulfonyl)-3-nitro-1*H*-1,2,4-triazole (MSNT).[14,15]

Coupling yields of acylated products **3a** were determined by spectrophotometric quantification of Fmoc groups cleaved off a dried resin sample. Acylations of amino acids activated with MSNT and lutidine as base proceeded without racemization as proven by total hydrolysis and chiral separation of the products.[4] C-Acylation of phosphorane **2a** could be performed with a broad choice of carboxylic acids and tolerated the standard side-chain protecting groups used in peptide synthesis such as *tert*-butyl, Boc, and trityl. The method works well with Fmoc-protecting groups. The basicity of the phosphorane reagents **2** did not cleave detectable amounts of the Fmoc-protecting group as verified by a negative Kaiser test subsequent to all acylations.

Following the C-acylation reaction, the Fmoc group could be removed under standard conditions (20% piperidine in DMF) and the liberated N-terminus could be used for peptide synthesis yielding peptidyl 4-amino-3-oxo-2-phosphoranylidene butanoates **4a**,**b** (Scheme 1).

Oxidative cleavage[16−18] of **4a** employing dimethyldioxirane in acetone afforded the peptidyl 4-amino-2,3-dioxobutanoates ("peptidyl diketoesters") **5** (Scheme 2). To our knowledge, this novel class of compounds has not been described in the literature so far. In aqueous solution, compounds **5** were present as the 2-hydrates as observed in the ES mass and the NMR spectra. HPLC analysis displayed a narrow peak unlike the broad signals observed for classical peptide aldehydes (Figure 1).[19] If MSNT/lutidine was employed for C-acylation, no epimerization products were detected, an observation which is in accordance with the

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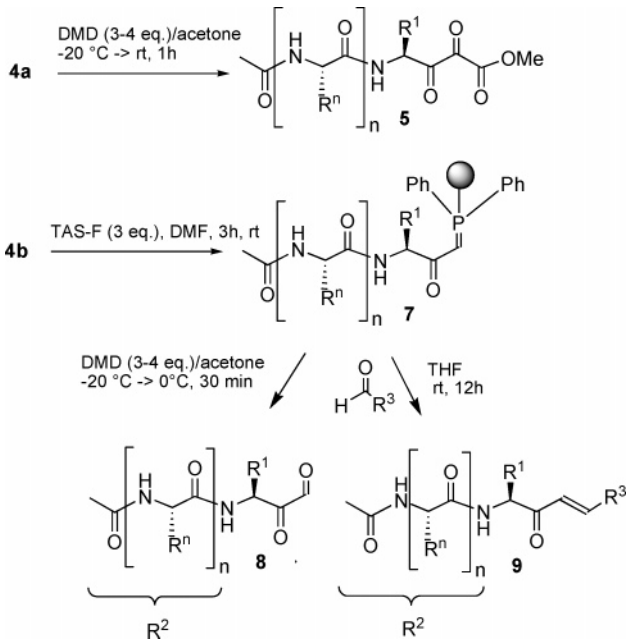
Scheme 2. Peptidyl-2,3-diketoesters **5** Synthesized by Oxidative Cleavage with Dimethyldioxirane (DMD)[a]



[a] Peptidyl-2-ketoaldehydes **8** were prepared by saponification of the ester followed by decarboxylation and oxidation with DMD. Wittig reaction with aldehydes delivered the vinylketones **9**.

results obtained by product hydrolysis and chiral separation of the building blocks.[4]

Peptidyl-2,3-ketoesters, being highly reactive 1,2,3-tris electrophiles, could be employed as versatile starting materials for the C-terminal modification of peptides via conversion into heterocycles.[20]

To realize an even broader product variation starting from a linker reagent, the saponification and decarboxylation of the polymer-bound peptidyl 4-amino-3-oxo-2-phosphoranylidene butanoates **4** were investigated.[21] As cleavage of the methyl ester required harsh basic treatment of **4a**, the trimethylsilylethyl (TMSE) ester **4b** was prepared. Starting from trimethylsilyl bromoacetate,[5,6] the orthogonally protected phosphorane **2b** was synthesized from the triphenyl phosphine resin. **2b** was acylated employing MSNT as described before yielding the 2′-trimethylsilylethyl *N*-peptidyl-4-amino-3-oxo-2-phosphoranylidene butanoate **3b**. It should be noted that the TFFH[22] reagent could not be used for the acylation of **2b** as it led to instant saponification of the TMSE ester group.

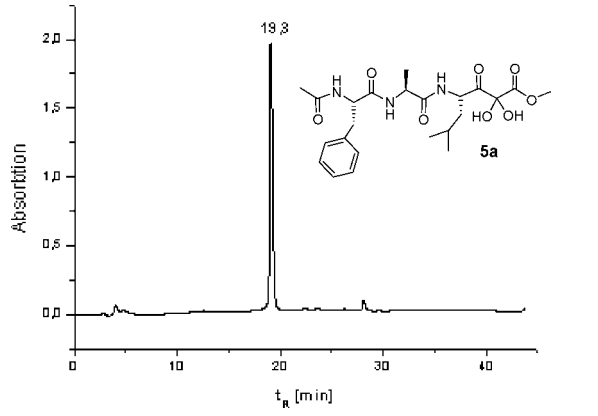


Figure 1. HPLC chromatogram of the crude peptidyl-2,3-diketoester **5a** (220 nm).

Following peptide elongation yielding **4b**, the deprotection of the TMSE group was first carried out with tetrabutylammonium fluoride (TBAF).[23]

As under basic reaction conditions the molecule was destroyed, the reaction was buffered with the addition of acetic acid leading to the properly deprotected resin **7**. Removal of tetrabutylammonium ions from the resin, however, failed even with prolonged washings as indicated in the LC-MS. However, when tris(dimethylamino)sulfonium difluoro-trimethylsilicate (TAS-F)[24] was employed as the desilylation reagent, the TMSE removal succeeded smoothly without washing problems. The intermediary carboxylic acid decarboxylated spontaneously to the *N*-peptidyl 3-amino-2-oxo-1-phosphoranylidene propane ("acylphosphorane") **7**. Oxidative cleavage of **7** with DMD yielded *N*-peptidyl 3-amino-2-oxo-propanals ("peptide ketoaldehydes") **8**. Very recently, this class of compounds was first prepared in solution from activated peptides via the use of diazomethane followed by ozonolysis of diazomethyl ketones.[25,26] Our novel approach employing the phosphorane linker reagent **2b** now allows for the first time a flexible solid-phase synthesis of the ketoaldehydes **8** and avoids the use of diazomethane.

8 could be characterized by fully assigned NMR and MS. The product was isolated with an epimerized C-3 position. Accordingly, it displayed a broad peak in the HPLC trace at 220 and 280 nm, comparable to the classical peptide aldehydes.[19] The hydrated product was observed as the strongest signal in the LC-MS.

Peptidyl ketoaldehydes have been reported as powerful inhibitors of cysteine proteases.[27] In addition, they are useful biselectrophiles for the C-terminal variation of peptides and for the preparation of peptidomimetics.

For an alternative cleavage, the acylphosphorane **7** was reacted with aldehydes yielding very pure peptidyl-1-amino-but-3-en-4-ones **9** ("peptidyl vinyl ketones") with complete

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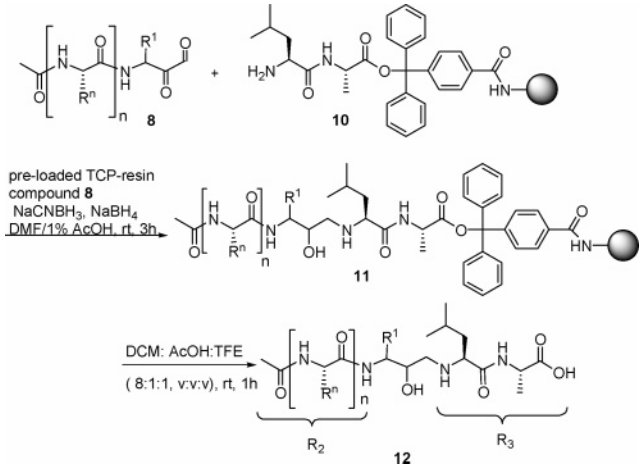
stereospecificity for the *E*-diastereomer of the α,β-unsaturated ketones with no epimerization at the aliphatic stereocenter adjacent to the ketone functionality (Scheme 2). Again, this is the first solid-phase synthesis approach for this class of compounds that has been prepared and proven active in protease inhibition and with only a single report earlier.[28]

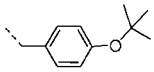
To highlight the potential of the presented peptidyl variants for the synthesis of peptidomimetics, we decided to demonstrate an on-bead synthesis of the hydroxyethylene transition state isostere **12** starting from peptidyl ketoaldehydes **8** (see Scheme 3).[29]

Scheme 3. Preparation of 1,3-Diamino Propanol **12** via Reductive Amination of Resin-Bound Peptides **10**



The P′-side of the desired peptidomimetic which is located in the C-terminal direction from the transition state isostere (and which binds to the S′-site within the target protease) was first synthesized on a trityl chloride resin and deprotected at the amino terminus to furnish **10**. Peptidyl 3-amino-2-oxo-propanal **8** was added to **10** and coupled via reductive amination employing $NaCNBH_3$ as a reducing agent yielding the intermediary ketone product (not shown). For reduction of the ketone, $NaBH_4$ was employed yielding the diaminopropanol **11** on the polymer. Subsequently, the product **12** was cleaved off the resin with hexafluoro isopropanol as a mild acid. Product **12** was obtained in reasonable yield (Table 2). The diastereomeric ratio (syn/anti) was ca. 9:1 according to high-resolution NMR.

Table 2. Synthesis of Peptidyl-2,3-diketoesters **5**, Peptidyl-α-ketoaldehyde **8**, Peptidyl Vinyl Ketones **9**, and 1,3-Diamino Propanol (Hydroxyethylamino Isoster) **12**[a]

Prod.	R^1	R^2	R^3	Purity [%]	Yield [%]
5a		Ac-F-A-	---	93	77
5b	-H	Ac-F-A-	---	83	97
5c		Ac-F-A-	---	89	84
8		Ac-F-A-	---	95	68
9a		Ac-F-A-		68	94
9b		Ac-F-A-		65	80
12		Ac-F-A-	-L-A-OH	95	40

[a] Purities of crude products (at 220 nm) and isolated yields (>95% pure).

In summary, 2-phosphoranylidene acetates **2** are powerful tools for diversity-oriented synthesis. Polymer-supported C-acylation provides an access to a variety of products. Bis- and triselectrophiles derived of peptide carboxylic acids can be directly released from the resin; multiple opportunities have been demonstrated to transfer these valuable intermediates into diversely modified peptides. Currently, the application of this work to the synthesis of various heterocycles is under investigation and will be reported in due course.

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Supporting Information Available: All experimental procedures and analytical data of the novel compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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