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Soluble Peptidyl Phosphoranes for Metal-Free, Stereoselective Ligations in Organic and Aqueous Solution

Ahsanullah,[‡] Samer I. Al-Gharabli,[§,‡] and Jörg Rademann*[,†,‡]

Institute of Pharmacy, University of Leipzig, Brüderstrasse 34, 04103 Leipzig, Germany, Leibniz Institute of Molecular Pharmacology (FMP), Robert-Rössle-Strasse 10, 13125 Berlin, Germany, and Chemical-Pharmaceutical Engineering, German-Jordanian University, 35247 Amman, Jordan

rademann@uni-leipzig.de

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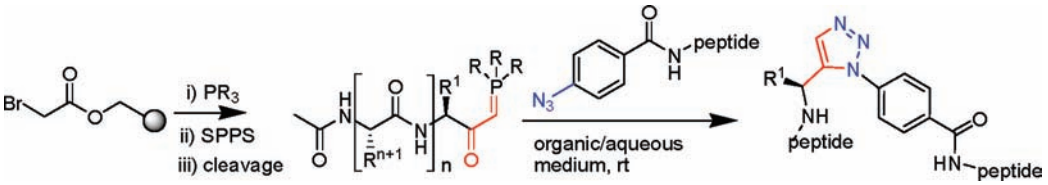
ABSTRACT



Protocols for solid-phase syntheses of soluble peptidyl phosphoranes are presented. Various supported phosphoranylidene acetates were prepared on Rink amide or via alkylation of trialkyl- and triarylphosphines with bromoacetyl Wang ester. C-Acylation was conducted racemization-free with activated Fmoc-amino acids, followed by SPPS (solid-phase peptide synthesis). Acidic conditions released decarboxylated peptidyl phosphoranes into solution. The protocol allowed for the electronic variation of peptidyl phosphoranes which were investigated in ligation reactions with azides in organic and aqueous solvents.

Chemoselective organic reactions have had a tremendous impact on chemical biology during recent years.[1] Ideally, such reactions tolerate the presence of water and other biologically relevant nucleophiles and occur at ambient temperature or up to 37 °C and over a broad range of concentrations. The involved functionalities should be stable under physiological redox conditions and toward reactive groups occurring frequently in biological systems. In the past decade, the copper(I)-catalyzed dipolar cycloaddition of an azide with an alkyne moiety has become an especially prominent chemoselective reaction, mostly denominated simply as a "click reaction".[2] Despite the advantages associated with this reaction in terms of kinetics and regioselectivity, it is not ideal for use in cellular systems or even living organisms due to the toxicity of copper. The scope of click materials is limited by residual traces of copper.[3] In addition, the reactions can be severely impeded in copper-coordinating environments. Therefore, metal-free, biocompatible reactions are highly desirable.

One early example for the development of a metal-free chemoselective ligation reaction has been made by introduction of the Staudinger ligation though this approach is impeded by comparably slow reaction rates and background phosphine oxidation.[4] In addition, strained alkynes have been used in the click reaction though this variant of a metal-free reaction

[†] University of Leipzig.
[‡] Leibniz Institute of Molecular Pharmacology.
[§] German-Jordanian University.

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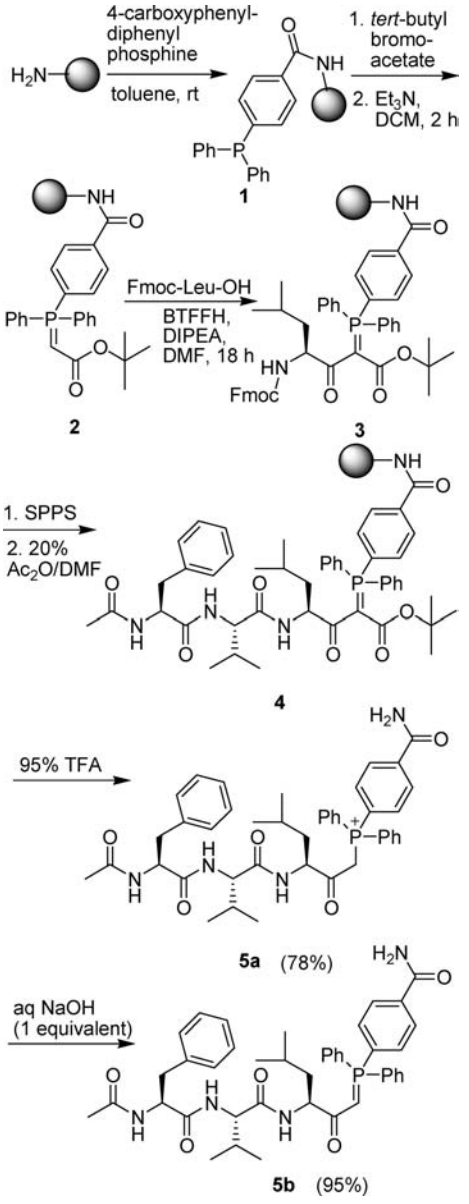
Scheme 1. Synthesis of Soluble Peptidyl Phosphoranes Based on Rink Amide Phosphine Resin **1**



requires considerable synthetic effort for the preparation of cyclooctyne derivatives and is not stereoselective.[5] Recently, the dipolar cycloaddition reactions between azides and polymer-attached peptidyl phosphoranes have been reported.[6] The reaction was found to proceed smoothly, stereoselectively, and without metal catalysis. Both involved functionalities that possess remarkable stability in biological systems. While azides are broadly established in ligation reactions, the biocompatibility of phosphoranes needs more consideration.[7] Differing from phosphines, their direct precursors, peptidyl phosphoranes, are stable toward most oxidants. For cleavage, strong oxidants such as ozone and dimethyldioxirane are required. In principle, acylphosphoranes can react with carbonyl compounds; however, most aldehydes including aromatic and peptidyl aldehydes as well as ketones require heating and prolonged reaction times. Only aliphatic, nonhydrated aldehydes may react at room temperature in a water-free environment. Hydrolysis of peptidyl phosphoranes was found only under strongly basic conditions and under acidic conditions with heating.[7a] Therefore, it appeared to be attractive to investigate the potential of this reaction for chemoselective ligations. As the first step toward this goal, a robust synthesis of soluble peptidyl phosphoranes had to be established. Initially, Rink amide resin was employed for this purpose (Scheme 1). The polymer was acylated with 4-carboxyphenyldiphenylphosphine toward **1** and subsequently alkylated with *tert*-butyl 2-bromoacetate yielding **2**. Acylation with an Fmoc-amino acid using BTFFH for activation delivered **3**, and peptide elongation furnished the peptidyl phosphorane **4**, which released by decarboxylating cleavage under acidic conditions the phosphonium salt **5a** which was isolated in 78% overall yield. Treatment of **5a** with sodium hydroxide precipitated **5b** with 95% yield. In the first ligation reaction, **5b** was reacted with 4-azidobenzoic acid. At room temperature, the reaction proceeded smoothly and delivered the triazole product (Table 1). The ligation proceeded, however, significantly slower when phosphorane **5b** was reacted with azido peptide **14**. Only 50% conversion was obtained in 18 h and did not increase over the extended reaction time of 3 days (see the Supporting Information (Figure S1)).

Variation of the phosphorus substituents was expected to alter the electronic nature and thus the reactivity of the obtained phosphoranes. For this reason, a protocol allowing for the flexible variation of peptide sequence and phosphorus substituents was devised (Scheme 2). The central idea was to incorporate phosphorus by alkylation of trisubstituted phosphines with polymer-bound electrophiles. Thus, 4-hydroxymethyl phenoxide resin ("Wang resin") was O-acylated with bromoacetyl bromide yielding polymer **6**. Triphenylphosphine **7a** was alkylated with the bromoacetate residue, and treatment with Et_3N provided the attached phosphorus ylide **8a**. The latter was C-acylated employing a BTFFH-activated Fmoc-amino acid and yielded the Fmoc-amino acylphosphorane **9a**. Following Fmoc-deprotection, the sequence of a simple peptide was constructed using diisopropylcarbodiimide and 1-hydroxybenzotriazole for activation. The N-terminal amino group was deblocked and acetylated furnishing *N*-acetyl-peptidyltriphenylphosphoranylidene acetate ester **10a**. Linker cleavage with trifluoroacetic acid (TFA) led to the soluble peptidyl phosphonium salt **11a**, and the phosphorane ylide **13a** was precipitated upon addition of an aqueous solution of NaOH in pure form.

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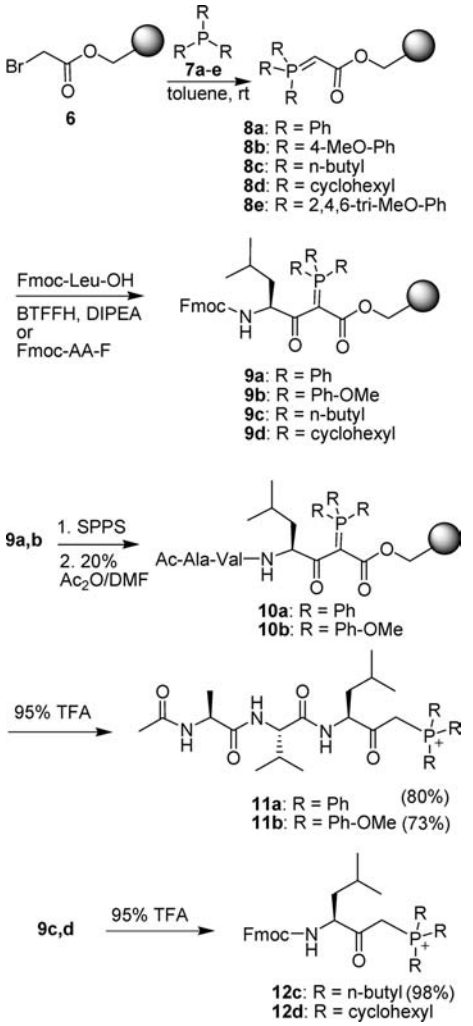
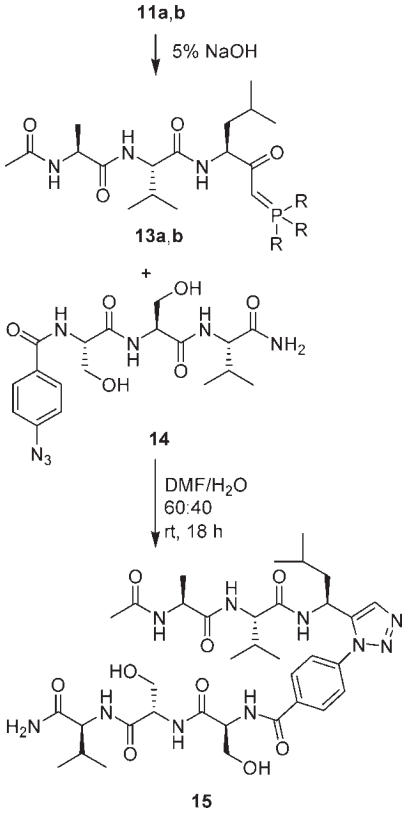
Scheme 2. Synthesis of Soluble Peptidyl Phosphoranes Based on 2-Bromoacetyl Wang Resin **6**



In the first ligation reaction, **13a** was reacted with only 1.2 equiv of 4-azidobenzoic acid. At room temperature, the reaction proceeded smoothly and delivered the triazole product. When, however, the same peptidyl phosphorane was ligated at rt with azido peptide **14** carrying the phenyl azide attached to the amino terminus, the reaction to the ligation product **15** proceeded significantly slower with only 48% conversion. Considering its mechanism, the rate of this reaction should be increased either by lowering the energy level of the lowest unoccupied molecular orbital (LUMO) of the azide dipole or by raising the energy level of the highest occupied molecular orbital (HOMO) of the phosphorane. While the electronic effects on the side of the azide have already been investigated, here we focused on variations on the side of the phosphorane. In order to accelerate the reaction, aromatic phosphines with increased electron density (**7b** with R = 4-methoxyphenyl) and **7e** with R = 2,4,6-trimethoxy) were employed first. In addition, the aliphatic trialkylated phospines **7c** (R = *n*-butyl) and **7d** (R = cyclohexyl) were used. All five alkylation products **8a**−**e** were obtained and investigated for C-acylation. Unexpectedly, acylation of the tri-*n*-butylphosphoranylidene resin **8c**,**d** failed in the first set of experiments despite using a list of activating agents including BTFFH, TFFH, PyBOP, HATU, and MSNT. Fmoc-amino acyl fluorides, however, which were prepared in advance,[8] succeeded in the C-acylation of the ylides **8c**,**d** affording the *N*-Fmoc-aminoacyl-tributylphosphoranylidene acetate **9c** and *N*-Fmoc-aminoacyl-tricyclohexylphosphoranylidene acetate **9d**. Cleavage of the linker with TFA afforded pure *N*-Fmoc-aminoacyl-tributylphosphorane **12c** indicating the complete acylation of the precursor judging from peak integration in the NMR spectrum. While the aminoacyl-tributylphosphorane **12c** was stable toward strong acidic conditions, the supported *N*-Fmoc-aminoacyl-tributylphosphoranylidene acetate **9c** was found to degrade on treatment with 20% piperidine with breakage of the carbon−phosphorus bond. The same was observed for the tricyclohexyl derivative **9d** obtained from tricyclohexylphosphine as the precursor.

Scheme 3. Ligation Reaction of Peptidyl Phosphoranes **13a**,**b** and Azido Peptide **14** in Aqueous Medium



In contrast, the acylation of the tris-(2,4,6-trimethoxyphenyl)-phosphoranylidene acetate **8e** failed either by using the condensation agents or by employing Fmoc-amino acyl fluorides. C-Acylations with the triaryl phosphoranes **8a** and **8b** proceeded with ease using amino acyl fluoride

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Table 1. Effect of Differentially Substituted Phosphoranes and Azide on the Conversion in the Ligation Reactions[a]

no.	phosphorane	azide	conv (%)
1	**5b (PPh_2Ph-$CONH_2$)**	4-azidobenzoic acid	98
2	**5b (PPh_2Ph-$CONH_2$)**	N_3Bz-Ser-Ser-Val-NH_2 (**14**)	45
3	**13a (PPh_3)**	4-azidobenzoic acid	98
4	**5b (PPh_2Ph-$CONH_2$)**	N_3Bz-Asp-Ser-Gly-NH_2	50
5	**13a (PPh_3)**	N_3Bz-Ser-Ser-Val-NH_2 (**14**)	48
6	**13b ($P(4\text{-}MeOPh)_3$)**	N_3Bz-Ser-Ser-Val-NH_2 (**14**)	85

[a] Conversions determined by LCMS. Reaction conditions: DMF/H_2O (60:40), 0.06 M, 18 h, rt, 1.2 equiv of excess of azide.

or BTFFH for activation. Fmoc-removal succeeded for **9a**,**b**, and the liberated amine could be used for peptide synthesis. Following the described protocol (coupling of amino acids, Fmoc-deprotection, N-acetylation of the N-terminus, and acidic cleavage) the peptidyl triphenylphosphorane **11a** (R = Ph) and peptidyl tris-(4-methoxyphenyl)-phosphorane **11b** (R = 4-MeO-Ph) were isolated and fully characterized. The corresponding ylide **13a**,**b** was obtained by treatment with base, and the ligations were investigated with the azido peptide **14** in organic−aqueous solvent mixtures (DMF/H_2O) (Scheme 3). The reactivity was considerably enhanced in the case of tris(4-methoxyphenyl) **13b** compared to the analogous triphenylphosphorane **13a**. More than 85% conversion of the phosphorane was observed with a 1.2 equiv excess of the azide at a 0.06 M concentration over 18 h based on LCMS. Ligation product **15** was purified by preparative HPLC in 58% yield and analyzed by NMR spectroscopy.

In summary, we have presented the first synthesis of soluble peptidyl phosphoranes via solid phase peptide synthesis. The synthetic route is flexible allowing for reactivity control of the peptidyl phosphoranes with altering the trivalent phosphine precursor. The peptidyl phosphoranes have been demonstrated to ligate the peptide component to aryl azide functionalities in the presence of water. Next, we will investigate the potential of soluble peptidyl phosphoranes in target guided synthesis[9] and in template-assisted ligation screening[10] through either azide−phosphorane or Wittig reactions.

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Supporting Information Available. Experimental procedure and characterization data for all new compounds. This material is available free of charge via the Internet http://pubs.acs.org.

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