

Amino acid functionalisation using the 2-phosphaethynolate anion. A facile route to (phosphanyl)carbonyl-amino acids.

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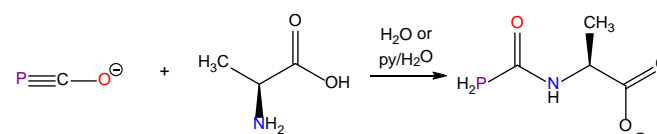
We describe the reactivity of the 2-phosphaethynolate anion (PCO^-) towards enantiomerically pure α -amino acids (AAs) resulting in the formation of novel salts of phosphinecarboxamides bearing chiral functionalities. These transformations occurred quantitatively with all but one of the amino acids trialled (the basic amino acid arginine was found to be unreactive). The resulting ionic species can be readily protonated to afford *N*-(phosphanyl)carbonyl-amino acids, a novel group of amino acids bearing primary phosphine functionalities.

The reaction of amino acids with cyanate salts in protic media is known to afford α -carbamido acids (or *N*-carbamoyl amino acids). This transformation has been well established for over a hundred years, and was extensively employed in pioneering studies focused on the isolation of amino acids from urine and blood serum.^{1–6} *N*-carbamoyl amino acids have also been proposed as possible prebiotic precursors to peptides through the formation of *N*-carboxyanhydrides.^{7–10} Given the relevance of this transformation, we recently sought to explore related reactions employing a heavier phosphorus-containing congener of the cyanate anion, i.e. the 2-phosphaethynolate anion (PCO^-).

We have recently shown that the 2-phosphaethynolate anion reacts with ammonia, primary, and (to a lesser extent) secondary amines, in the presence of a proton source to afford phosphinecarboxamides.^{11–13} The resulting species are novel primary phosphines which have been shown to be remarkably tolerant to air and moisture in contrast to the vast majority of related compounds.¹⁴ Early attempt to prepare such species by reaction of phosphine gas with isocyanates afforded tertiary phosphines even when the reactions were carried out in a 1:1 ratio.¹⁵ Given the stability of the resulting products, and the functional group tolerance they exhibit, we decided to explore the reactivity of this remarkable anion towards biologically relevant substrates. Herein we report one such study exploring the reactivity of PCO^- salts towards several examples of enantiomerically pure α -amino acids including alanine, serine, cysteine, asparagine, glutamine, arginine and proline (all as the *L*-stereoisomers).

We first investigated the reactivity of $[\text{K}(18\text{-crown-6})][\text{PCO}]$ towards *L*-alanine on the grounds that it is one of the simplest

amino acids available and no competing reactivity with the side chain was envisaged.¹⁶ The reaction was carried out in water, or in a mixture of water and pyridine (py), to give quantitative formation of $[\text{K}(18\text{-crown-6})][\text{H}_2\text{PC}(\text{O})\text{NHCHMeCO}_2]$ ($[\text{K}(18\text{-crown-6})][\mathbf{1a}]$) as evidenced by ^{31}P NMR spectroscopy (Scheme 1).



Scheme 1. Reaction of PCO^- with *L*-alanine to afford $[\text{K}(18\text{-crown-6})][\mathbf{1a}]$.

This reaction is not significantly different from previous transformation involving the 2-phosphaethynolate anion with ammonium salts,^{11–13} especially as the amino acid is likely to exist in its zwitterionic form in water. The amino acid starting material is chiral and enantiopure resulting in the formation of a chiral phosphinecarboxamide salt. The product can be isolated as a solid by removal of the solvent *in vacuo*. Crystals suitable for single crystal X-ray diffraction were grown by slow diffusion of hexane into a 1:1 THF/pyridine solution of the product (Figure 1).

The product crystallised with four distinct molecules of $[\text{K}(18\text{-crown-6})][\mathbf{1a}]$ in the asymmetric unit (space group $P2_1$). In all cases the anion is coordinated to a potassium ion through both oxygen centres of the carboxylate group. The bond length data for the four different anions in the asymmetric unit are all the same within statistical error, due to the relatively large standard deviations associated with each measurement (see Electronic Supplementary Information (ESI) for details). The average picture of $\mathbf{1a}$ in the solid state matches that of the previously characterised phosphinecarboxamides. The P1–C1 bond length (1.862(av) Å) is indicative of a single bond (cf. 1.80–1.86 Å), and C1–N1 (1.336(av) Å) has multiple bond character (cf. 1.44–1.46 Å for a C–N single bond).^{17,18} The latter is shorter than the N1–C2 bond length (1.455(av) Å), which is purely a single bond. The

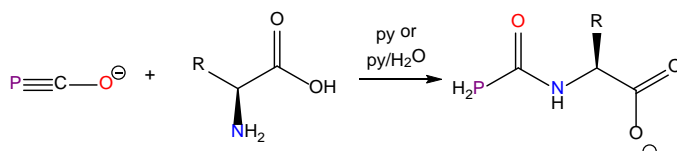
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The stability of the **1a** was probed by exposing a *d*₅-pyridine solution of the compound (with a drop of water to aid solubility) to atmospheric air. The solution was monitored by ¹H and ³¹P{¹H} NMR spectroscopy over several days. After fifteen days the ³¹P{¹H} NMR spectrum revealed no evidence of a phosphine oxide and approximately 2% of PH₃ relative to the

This preliminary result prompted us to explore the reactivity of sodium salts of the 2-phosphaethynolate anion ([Na(dioxane)_{1.78}(PCO)]) towards several examples of amino acids with varying functional groups.¹⁹ Reactions were all carried out in pyridine or in pyridine/water mixtures when the solubility of reagents and/or products was found to be limited. The reactions proceed quantitatively to afford the novel anionic phosphinecarboxamides as pictured in Scheme 2. All of the amino acids trialled were found to react with PCO⁻ in a 1:1 ratio with the exception of arginine, which was found to be entirely unreactive under the conditions employed due to its high *pK_a* (12.48).²⁰ This is in no doubt due to the fact that the guanidine residue in arginine is known to exist in its protonated form even in highly basic media, therefore precluding the protonation of the PCO⁻ anion (which is believed to be the first step prior to formation of the phosphinecarboxamide product).



All of the reactions were monitored by multi-element *in situ* NMR studies which revealed the formation of the corresponding phosphinecarboxamides. These appear as characteristic pseudo-triplet resonances in the ^{31}P NMR spectra, collapsing to singlets on proton decoupling. The resonances all occur in a narrow chemical shift range (–130.6 to –131.4 ppm). Given the chiral centre present in the amino acid backbone, the two phosphine protons are rendered diastereotopic (*vide supra*). This was observed in all cases with the exception of the cysteine product, **1c**, where the broad nature of the resonance in the $^1\text{H}\{^{31}\text{P}\}$ NMR spectrum precludes the observation of two distinct resonances for the phosphine protons. Selected NMR spectroscopic data for all of the species studied are presented in Table 1. These data are consistent with a *trans*- arrangement of the PH_2 and CH(R)COO^- groups about the C–N bond, as observed in the single crystal X-ray structure of **1a**. Previous studies on the parent compound $\text{PH}_2\text{C(O)NH}_2$, revealed that $^3J_{\text{H-P}}$ coupling between the phosphorus centre and the amide protons could only be resolved for the proton in the *trans*- position to the PH_2 functionality ($^3J_{\text{H-P}} = 12 \text{ Hz}$).¹¹ Calculations at the density functional level of theory (DFT) reveal that the *trans*- isomers for compounds **1a–1e** are all lower in energy than the corresponding *cis*- isomers, albeit only by 7–13 kJ mol^{–1}. These

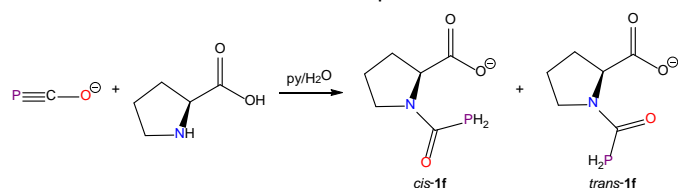
studies suggest that the *trans*- isomers may be favoured for steric reasons on attack of the amine at the protonated (unobserved) HPCO intermediate.²¹

The choice of *d*₅-pyridine as a solvent for the NMR experiments is due to evidence of H/D exchange at the phosphine and amide protons in more acidic deuterated solvents. Dissolution of the sodium salts of **1a–1e** in D₂O affords the *d*₃-isotopologues within minutes, as evidenced by the appearance of characteristic 1:2:3:2:1 quintets in the ³¹P NMR spectra, and the loss of resonances in the ¹H NMR spectra arising from the PH₂ and C(O)NH functionalities. As a representative example, when Na[**1a**] is dissolved in D₂O, a 1:2:3:2:1 quintet is observed at –134.1 ppm in the ³¹P NMR spectrum with a ¹J_{D–P} coupling constant of 33 Hz, as anticipated based on the different gyromagnetic ratios of ²H and ¹H ($\gamma_{\text{H}}/\gamma_{\text{D}} \approx 6.5$).²²

Table 1. Selected NMR spectroscopic data for sodium salts of **1a–1e** (chemical shifts given in ppm, coupling constants in Hz). All NMR spectra were collected in *d*₅-pyridine (or *d*₅-pyridine and a drop of degassed, deionised water if solubility was limited). Only ¹H and ¹³C NMR resonances for the PH₂C(O)– functional groups are listed. Full spectroscopic details are available in the Supporting Information.

AA	Product	³¹ P	¹ H	¹ J _{H–P}	² J _{H–H}	¹³ C	¹ J _{C–P}
L-Ala	1a	–131.0	3.93, 3.90	211	12	175.0	8
L-Ser	1b	–131.4	3.84, 3.83	210	12	174.2	8
L-Cys	1c	–130.6	3.86	210	N.A.	174.6	8
L-Asn	1d	–131.0	3.92, 3.91	211	12	175.8	9
L-Gln	1e	–131.3	3.86, 3.85	210	12	174.5	8

The preference for all of the aforementioned linear α -amino acids to give rise to products with a *trans*- arrangement of the PH₂ and CH(R)COO[–] groups prompted us to explore similar reactivity studies with the cyclic amino acid L-proline. In this case, two different isomers of the anionic phosphinecarboxamide could be observed in the NMR spectra consistent with the two structures presented in Scheme 3.



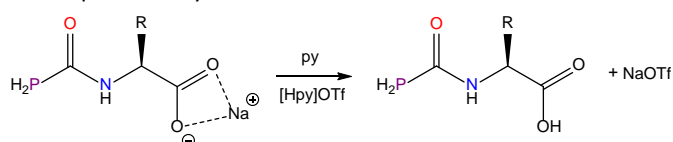
Scheme 3. Reaction of PCO[–] with L-proline to afford compound **1f**.

The ³¹P NMR spectrum of a reaction mixture containing equimolar amounts of [Na(dioxane)_{1.78}(PCO)] and L-proline revealed two pseudo-triplet resonances at –127.6 (¹J_{H–P} = 217 Hz) and –131.2 (¹J_{H–P} = 213 Hz) ppm which collapse to singlets in the proton-decoupled spectrum. These two resonances integrate in a 1:0.35 ratio and arise due to the *cis*- and *trans*- isomers of **1f**, respectively. The resonances arising from each of the two isomers were assigned with the assistance of ¹H–¹³C HMBC and ¹H–³¹P HMBC NMR experiments (see ESI for full assignment). These data are consistent with DFT calculations which show that the *cis*-isomer is slightly higher in energy than the *trans*- but only by 4 kJ mol^{–1}, which is within the error of the calculations.

Negative ion mode electrospray ionization mass spectrometry measurements on solutions of Na[**1a–1f**] (in 10% water, 89.9% methanol and 0.1% formic acid) all reveal the presence of the molecular ions **1a–1f** as the free ion or in ion pairs with Na⁺ cations (i.e. Na_{*n*}[**1a–1f**]_{*n+1*} where *n* = 0 – 7). Interestingly, in all the spectra recorded there is also the presence of the corresponding amino acid anions, which are formed during the ionization process along with, presumably, HPCO or PCO[–] and H⁺ (which were unobserved as the lower detection limit of the experiments was set at 80 Da).

All of anionic species synthesised, **1a–1f**, can be readily protonated to afford the neutral (phosphanyl)carbonyl-amino acids using a strong acid such as pyridinium trifluoromethanesulfonate (see Scheme 3). Protonation significantly increases the solubility of the resulting amino acids in non-aqueous media. Thus while **1a–1f** are only sparingly soluble in pyridine, the parent acids are much more soluble. The most notable changes to the NMR spectra of the resulting products (**2a–2f**) are to the carboxylate ¹³C NMR resonances which are upfield shifted by approximately 3 ppm on protonation. Compounds **2a–2f** all exhibit a broad acidic protic resonance (12.26–16.27 ppm) consistent with the carboxylic proton. Selected NMR data for all of these compounds are provided in Table 2.

The air-stability of **2a** was probed by exposing a *d*₅-pyridine solution of the compound to atmospheric air. After fifteen days there was no evidence of oxidation of the (phosphanyl)carbonyl-amino acid, however significant decomposition was observed. ¹H NMR spectroscopy revealed that approximately 40% of **2a** had decomposed L-alanine, which presumably results from loss of HPCO.



Scheme 3. Synthesis of neutral (phosphanyl)carbonyl-amino acids **2a–2e** by protonation of **1a–1e**, respectively, using pyridinium trifluoromethanesulfonate ([Hpy]OTf).

Table 2. NMR spectroscopic data for carboxyl functionalities of **1a–1f** and **2a–2f** (chemical shifts given in ppm). All NMR spectra were collected in *d*₅-pyridine.

AA	Product	¹³ C (COO [–])	Product	¹³ C (COOH)	¹ H (COOH)
L-Ala	1a	179.7	2a	176.5	12.79
L-Ser	1b	177.2	2b	174.3	12.26
L-Cys	1c	176.8	2c	174.0	16.03
L-Asn	1d	177.7	2d	175.1	16.27
L-Gln	1e	178.6	2e	175.9	12.42
L-Pro	1f	178.7 (<i>cis</i>), 178.2 (<i>trans</i>)	2f	175.6 (<i>trans</i>), 175.3 (<i>cis</i>)	16.15

Conclusions

Compounds **2a–2f** are a novel group of amino acids bearing an *N*-carbamoyl phosphine residue. The transformations used to access such systems can be employed for the *N*-labelling of amino acids with an NMR active functional group, and for

further reactivity studies aimed at incorporating phosphorus nuclei into peptides.

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Acknowledgements

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