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Efficient Preparation of Proline *N*-Carboxyanhydride Using Polymer-Supported Bases

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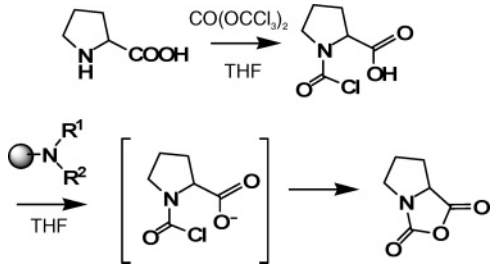
ABSTRACT



A procedure for the preparation of proline *N*-carboxyanhydride in high yield and purity is described using polymer-supported tertiary amines. The polymer-supported amine can be recycled with a basic wash and filtration of the resin. The procedure facilitates the access to the efficient preparation of the polyproline polymer with potential therapeutic interest.

Polymers made out of amino acids (polyamino acids, peptide copolymers) are emerging as promising therapeutic compounds. These polymers are finding widespread application in the field of drug delivery.[1] Drugs are physically entrapped within the polymer matrix or chemically conjugated to the polypeptide for a slow release in the biological millieu. A milestone example of therapeutic polyamino acid carriers is paclitaxel polyglumex,[2] a biologically enhanced version of taxol conjugated to a polyglutamate polymer that has recently been *fast track* designated by the FDA for the treatment of advanced nonsmall cell lung cancer in women. In this context, polyproline polymers are also attracting much attention to their therapeutic potential. Polyproline is soluble in water, and thus, it has been used to solubilize poorly water-soluble proteins[3] obtained by recombinant techniques. Most of these proteins, such as interferons and interleukins, show high therapeutic interest. Polyproline polymers have also found use in affinity chromatography for the purification of platelet profilin.[4] Recently, dendrimers composed of polyproline branches[5] have been shown to be actively internalized by rat kidney cells and to entrap the antibiotic ciprofloxacin.

Polyamino acids are most conveniently synthesized by polymerization of the corresponding amino acid *N*-carboxyanhydride.[6] However, the case of proline is unique among coded amino acids as it has the α-amino group bound to the

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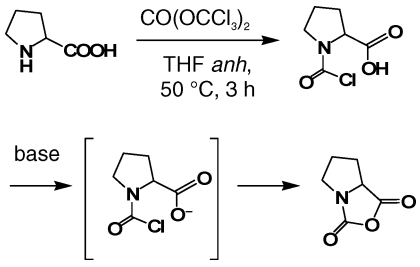
Table 1. Composition of Reaction Crudes in the Cyclization Step at 3 h[a]

entry	amino acid	base	equiv	temp (°C)	Pro-NCA (%)	Pro *N*-carbamoyl chloride (%)	Pro-Pro DKP (%)	Et_3N[c] (%)
1	H-Glu(OBzl)-OH[b]	–	–	50	100	0	0	–
2	H-Pro-OH[b]	–	–	50	3	97	0	–
3	H-Pro-OH	Et_3N	1	−25	59	<1	40, 0	<1
4	H-Pro-OH	Et_3N	1	0	27	38	35	2, 7
5	H-Pro-OH	Et_3N	1	25	41	54	5	6, 6
6	H-Pro-OH	TBD-PS	1	25	13	86	<1	–
7	H-Pro-OH	DMAP-PS	1	25	13	86	<1	–
8	H-Pro-OH	PIP-PS	1	25	70	27	3	–
9	H-Pro-OH	DIEA-PS	1	25	72	25	3	–
10	H-Pro-OH	MPH-PS	1	25	68	30	2	–
11	H-Pro-OH	DEAM-PS	1	25	70	29	<1	–

[a]Data have been calculated (average of triplicates) by integration of proton signals in ^{1}H NMR spectra (see Figure 2). [b] Typical synthesis only with triphosgene. [c] Calculated with respect to the initial amount of Et_3N.

side chain yielding a cyclic secondary amine (pirrolidine) and showing some conformational restrictions. These features probably underlie the poor synthetic yields obtained using currently available methods for α-amino acid *N*-carboxyanhydride formation.[7] Generally speaking, *N*-carboxyanhydrides (NCAs) are obtained by treatment of the corresponding amino acid with phosgene, the so-called Fuchs method.[8] In the case of proline, the *N*-carbamoyl intermediate does not cyclize spontaneously as it takes place with other amino acids, and the use of a nonnucleophilic base, typically a tertiary amine, is required for the cyclization to the *N*-carboxyanhydride (Scheme 1).

Scheme 1. Synthesis of Proline NCA



Procedures described in the literature require slow addition of solutions of phosgene at low temperature and the use of tertiary amines, such as triethylamine,[7,9] that are difficult to eliminate and appear in variable amounts in the final crystallized proline NCA product. In the present article, we describe the preparation of Pro-NCA in high yields and purities[10] using solid triphosgene[11] and polymer-bound bases[12] (Figure 1).

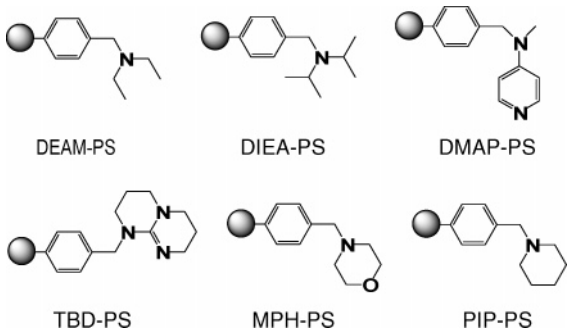


Figure 1. Polymer-bound tertiary amines.

Six polymer-supported bases were chosen to catalyze NCA cyclization (Figure 1). The percentages of different species present in their action crude after 3 h, namely, Pro-NCA, *N*-carbamoyl chloride, and the Pro-Pro diketopiperazine byproduct (Pro-Pro DKP), are summarized in Table 1. For comparison purposes, the yield of formation of the *N*-carboxyanhydride of the benzyl-protected glutamic acid, Glu(OBzl)-NCA, and the yield of formation of Pro-NCA, both in the absence of a base, are shown in entries 1 and 2 of Table 1. As expected, the yield of Glu(OBzl)-NCA is quantitative whereas the yield of Pro-NCA is practically nil

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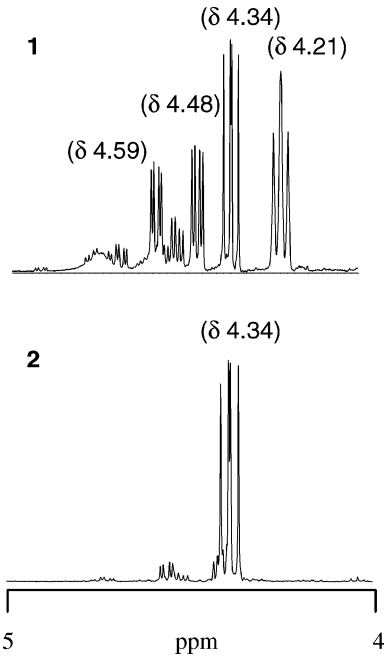

Figure 2. ^{1}H NMR spectra (400 MHz, $CDCl_3$, 298 K) of entries 4 (**1**) and 11 (**2**) of Table 1. Characteristic α-proton signals are identified for Pro-Pro DKP at 4.21 ppm, Pro-NCA at 4.34 ppm, and Pro and *N*-carbamoyl chloride at 4.48 ppm cis and 4.59 ppm trans.

(3.5%). The *N*-carbamoyl chloride intermediate is the major product.

The use of 1 equiv of triethylamine as the base (entries 3−5) rendered a low conversion of the *N*-carbamoyl chloride to the expected Pro-NCA, together with the presence of the Pro-Pro diketopiperazine byproduct. However, the use of resin-bound tertiary amines (1 equiv) provided much higher yields of Pro-NCA (around 70%, entries 8−11), together with very low percentages of the diketopiperazine byproduct and, obviously, the total absence of tertiary amine contamination. The resin DEAM-PS (entry 11) was the one that provided virtually no diketopiperazine byproduct. Resins DMAP-PS and TBD-PS furnished very low reaction yields (entries 6 and 7).

Table 2. Yield of Crystallized Pro-NCA (Average of Triplicates) Depending on the Base Used[a]

entry	base	equiv	Pro-NCA (%)
1	Et_3N	1	21
2	PS-TBD	1	<1
3	PS-DMAP	1	<1
4	PS-PIP	1	43
5	PS-DIEA	1	53
6	PS-MPH	1	54
7	PS-DEAM	1	59
8	PS-DEAM	2	75
9	PS-DEAM	3	93

[a]The percentage represents the yield showing a purity higher than 99%.

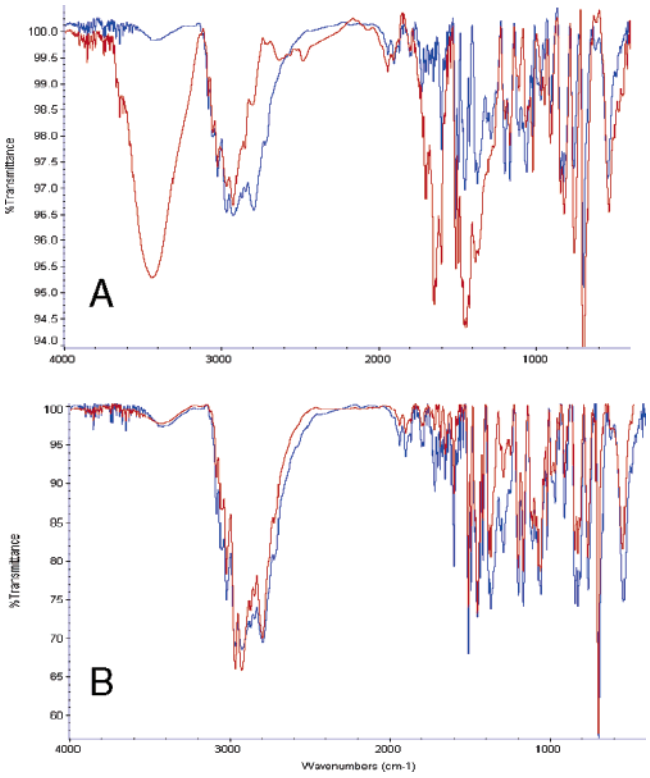

Figure 3. Overlaid IR spectra of: (A) fresh DEAM-PS resin (blue) and the same resin after a cyclization reaction (red) and (B) the resin regenerated with piperidine/DMF 1:4 after a cyclization reaction (red) and compared again with original fresh DEAM-PS resin (blue).

In Figure 2, the ^{1}H NMR spectra of two representative reaction crudes (corresponding to entries 4 and 11) are shown as an example. The characteristic α-proton signals of Pro carbamoyl chloride (two conformers), Pro-Pro DKP, and Pro-NCA are assigned. The corresponding integrations of those proton signals in the different experiments were used to estimate the yield of each product.

Another advantage of using polymer-supported bases is the ease of crystallization of the desired Pro-NCA. Because the base hydrochloride is removed by filtration, purities above 99% were regularly obtained for the different batches of Pro-NCA. Finally, an optimization process was carried out to obtain Pro-NCA not only in high purity but also in high yield. The use of a moderate excess (3×) of the supported amine DEAM-PS afforded a high recovery yield (up to 93%) maintaining a high purity level (>99%, see Table 2). The use of excesses of triethylamine in the solution preparation of Pro-NCA does not increase the yield above 35%, and the crystallized product appears contaminated with the base. The polymer-supported base can be also regenerated for further use. A washing step of the resin with piperidine in DMF (1:4) neutralizes the ammonium salt and furnishes the free base again.

The resin is finally reswollen in dry THF. To test the efficacy of the regenerating process, IR spectra were carried

out before and after the reaction and after the neutralization wash.

In Figure 3, an overlay of the IR spectra before the reaction and after the regeneration shows that there are essentially no differences between them. Finally, the recycled DEAM-PS resin was submitted to several consecutive cycles of *N*-carboxyanhydride formation reaction/regeneration. In Figure 4, the kinetics of Pro-NCA formation is plotted for fresh and recycled DEAM-PS. As it can be seen, the reaction yields and the kinetics of the reaction remain approximately the same.[13]

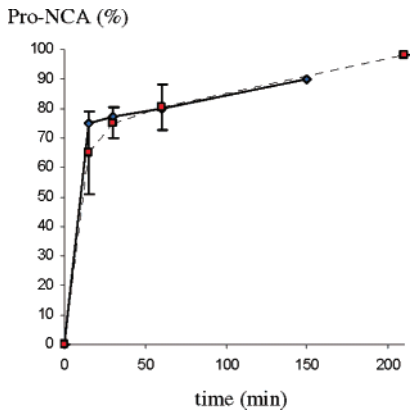

Figure 4. Kinetics of Pro-NCA synthesis with 3 equiv of fresh DEAM-PS (blue) and the average of three consecutive cyclization reactions with the same batch of resin (red).[13]

In conclusion, an efficient procedure for the obtention of proline *N*-carboxyanhydride in high yield and purity using polymer-supported tertiary amines is reported. The resin can be recycled by regenerating the free base after a neutralization step with piperidine in DMF and a filtration of the resin. This efficient method would facilitate the preparation of polyproline-based polymers.

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Supporting Information Available: Experimental procedure for the preparation of Pro-NCA and its characterization. This material is available free of charge via the Internet at http://pubs.acs.org..

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(13) Fresh DEAM-PS resin (3 equiv) was used to synthesize Pro-NCA. The same batch of resin was recycled with piperidine/DMF 1:4 and used again for synthesis of Pro-NCA. Regeneration of the same batch of this resin was carried out twice more to synthesise Pro-NCA three consecutive times with the same batch of resin. Finally, yields of Pro-NCA, of these three consecutive reactions, are plotted in red in Figure 4 and compared with yields obtained in the first synthesis using the original batch of fresh DEAM-PS resin. Data were calculated by integration of α-proton signals in ^{1}H NMR spectra obtained from reaction crudes, as it has been explained before.