Initiators for End-Group Functionalized Polypeptides via Tandem Addition Reactions

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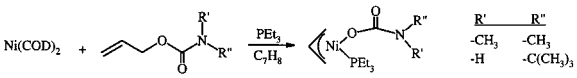
Received March 22, 1999

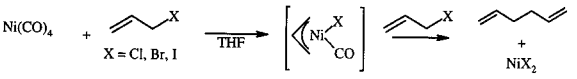
Structural materials constructed of polypeptides are being considered for a variety of biomedical problems such as tissue engineering and drug delivery.[1] For these applications, incorporation of end-group functionality onto the chains is essential for targeting of the drug-delivery complexes as well as substrate-specific anchoring of these materials.[1] We have discovered that amido-amidate nickelacycles are the active intermediates in the controlled polymerization of α-amino acid-*N*-carboxyanhydrides (NCAs).[2] Generation of these species from zerovalent nickel precursors allows formation of block copolypeptides with defined molecular weights, narrow molecular weight distributions, and controlled sequences and compositions of the different amino acid components.[3] All of these features are useful for controlling both the structure and properties of polypeptide materials. A limitation of this methodology is that the active propagating species are generated *in situ* and thus do not allow for controlled functionalization of the polypeptide chain ends.[2] For this reason, we pursued alternative methods for the direct synthesis of these types of initiators. Here we describe new tandem addition reactions that allow the general synthesis of amido-amidate nickelacycles useful for preparation of polypeptides containing a variety of defined end groups.

As a method for preparation of amido nickel species, the reaction of allyloxycarbonyl (Alloc)[4] protected amines with zerovalent nickel was utilized to prepare nickel carbamates, which can be viewed as precursors to nickel amides upon elimination of CO_2 (eq 1).[5]



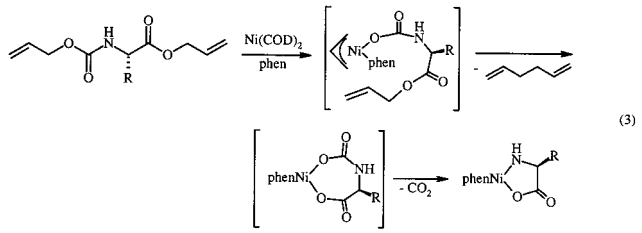
(1)

This reaction was found to be a facile method for preparation of nickel carbamates. However, these species, because of the absence of stabilizing chelates, were found to be ineffective in controlling NCA polymerizations. To eliminate the π-allyl group and in its place incorporate a chelating amidate, we pursued a tandem cyclization strategy. This method relied on chemistry found in the well-known coupling of allylic halides into dienes using zerovalent nickel (eq 2).[6]



(2)

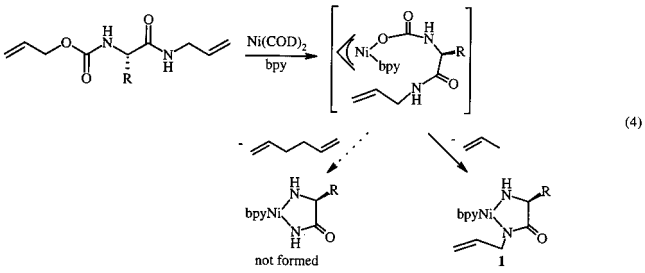
The byproduct of this reaction is a divalent nickel halide. We were intrigued by the possibility of replacing the halides with carbamate and carboxylate groups, tethered in a single molecule, to now form an amido-carboxylate metallacycle as the desired product, with the diene as the byproduct (eq 3).



(3)

Addition of N_α-alloc-amino acid allyl esters to nickel should occur initially at the more reactive allyl carbamate group, to yield π-allyl nickel(II) carbamate species, as we have demonstrated. This reaction would then be followed by addition of the allyl carboxylate, a common substrate for oxidative addition with nickel,[7] to give the desired metallacycle after elimination of 1,5-hexadiene and CO_2. The reaction might proceed through monovalent and trivalent nickel intermediates,[8] yet the final product should be the divalent nickel amido-carboxylate complex. When N_α-Alloc-L-leucine allyl ester was heated with phenNi(COD)[9] in DMF at 80 °C for 20 h, the desired phenNiNHC($CH_2CH(CH_3)_2$)HC(O)O metallacycle was formed in overall 68% isolated yield. The formation of byproduct 1,5-hexadiene was also verified by ^{1}H NMR. This reaction demonstrated that our tandem cyclization strategy was feasible; however, the amido-carboxylate species was not useful since it did not efficiently initiate NCA polymerizations.

To form the desired amido-amidate nickelacycles, we attempted the related reaction where N_α-Alloc-amino acid allyl amides were used as substrates (eq 4).



(4)

This reaction was not expected to be highly successful since the oxidative addition of allylic amides to nickel is without precedent.[7] A surprising result was therefore obtained in that the reaction of bpyNi(COD)[9] with N_α-Alloc-L-leucine allyl amide was found to produce an amido-amidate species in good yield (60% isolated). The product, however, was not the expected one, as evidenced by the lack of byproduct 1,5-hexadiene. The product nickelacycle was found to result from initial addition across the Alloc C−O bond, followed by a second addition across the N−H bond of the amide, not the allylic N−C bond (eq 4). As a result, the product metallacycle, **1**, retained the allyl substituent on nitrogen, as determined by FAB/MS, ^{1}H NMR of the hydrolysis product from reaction with HCl, and ^{13}C labeling studies.[5] The N−H addition was also verified by use of a N_α-2-hexenyloxycarbonyl-amino acid allyl amide in the reaction which resulted in the formation of byproduct hexenes that were identified by ^{13}C {^{1}H} NMR. The reaction of readily synthesized N_α-Alloc-amino acid allyl amides with zerovalent nickel was found to be general for different substituents (R′ and R″) and donor ligands, allowing the use of many combinations of amino acids and primary amines

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(2) Deming, T. J. *J. Am. Chem. Soc.* **1998**, *120*, 4240−4241.

(3) Deming, T. J. *Nature* **1997**, *390*, 386−389.

(4) Stevens, C.; Watanabe, R. *J. Am. Chem. Soc.* **1950**, *72*, 725−727.

(5) See Supporting Information.

(6) (a) Webb, I. D.; Borcherdt, G. T. *J. Am. Chem. Soc.* **1951**, *73*, 2654−2662. (b) Corey, E. J.; Semmelhack, M. F.; Hegedus, L. S. *J. Am. Chem. Soc.* **1968**, *90*, 2416−2417.

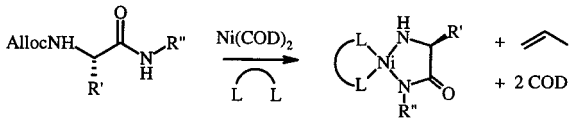
(7) Collman, J. P.; Hegedus, L. S.; Norton, J. R.; Finke, R. G. *Principles and Applications of Organotransition Metal Chemistry*, 2nd ed.; University Science: Mill Valley, CA, 1987.

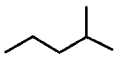
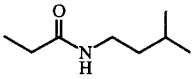
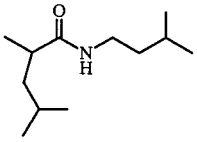
(8) Tsou, T. T.; Kochi, J. K. *J. Am. Chem. Soc.* **1979**, *101*, 6319−6324.

(9) COD = 1,5-cyclooctadiene; phen = 1,10-phenanthroline, bpy = 2,2′-bipyridyl; dmpe = 1,2-bis(dimethylphosphino)ethane; depe = 1,2-bis(diethylphosphino)ethane.

10.1021/ja990905g CCC: $18.00
Published on Web 07/23/1999

Table 1. Example Initiators Synthesized from Different *N*-Alloc-α-amino Amides, Bidentate Ligands, and $Ni(COD)_2$



Initiator	L L	R'	R"	Yield(%)[a]
1	bpy			60(95)
2	phen			72(94)
3	depe	—CH_3		42(98)
4	depe			39(97)
5	phen	—H		59(94)
6	phen			62(96)

[a] Isolated yield of initiator. Yield of crude product, as determined by FTIR spectroscopy, is given in parentheses.

Table 2. Synthesis of Polypeptides and Block Copolypeptides Using **4** in DMF at 20 °C. Lys NCA = ϵ-CBZ-L-lysine-N-carboxyanhydride

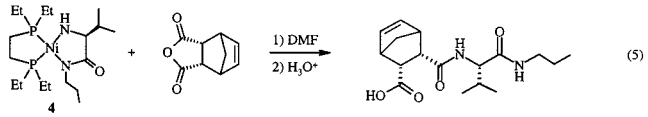
first monomer[a]	second monomer[a]	first segment[b] M_n	first segment[b] M_w/M_n	diblock copolymer[c] M_n	diblock copolymer[c] M_w/M_n	yield[d] (%)
25 Glu NCA	none	5190	1.37			88
50 Glu NCA	none	13 450	1.31			83
200 Glu NCA	none	49 340	1.24			90
25 Glu NCA	71 Lys NCA	5190	1.37	28 880	1.18	75
25 Lys NCA	87 Glu NCA	8760	1.06	25 600	1.15	77

[a] First and second monomers added stepwise to the initiator; number indicates equivalents of monomer per **4**. [b] Molecular weight and polydispersity index after polymerization of the first monomer. [c] Molecular weight and polydispersity index after polymerization of the second monomer. [d] Total isolated yield of polypeptide or block copolypeptide.

in the construction of initiator complexes (Table 1). This method is therefore amenable to incorporation of a wide variety of end-group functionalities onto polypeptides through amide linkages.

At this point, it was necessary to determine if these functional groups, once attached to the initiating complex, were then quantitatively incorporated as end groups on polypeptide chains. Polymerizations of γ-benzyl-L-glutamate NCA (Glu NCA) using nickel complexes containing different bidentate donor ligands revealed that alkyl phosphine ligands (dmpe and depe) promoted the most efficient initiation. These initiators were able to prepare block copolypeptides of defined sequence and composition (Table 2). It was also found that the initiators could be used for polymerization without isolation from the crude reaction mixture. This feature greatly simplifies the use of these complexes, which are formed in near-quantitative yield but can be tedious to isolate from the reaction solvent. Hence, polymerizations were conducted using either isolated or in situ initiators with no noticeable differences in results.

Concerning the degree of functionalization of the polymers, reaction of initiator **4** with one equivalent of *cis*-5-norbornene-*endo*-2,3-dicarboxylic anhydride, which should add to the initiator like an NCA monomer but not form polymer, followed by hydrolysis of the product with HCl, resulted in complete consumption of **4** to yield the addition product (eq 5).[10]



(5)

No unreacted monopeptide from hydrolysis of unreacted **4** was detected, showing that all metal centers were active. Furthermore, polymerization studies using initiator **3** gave polypeptides with a 1-naphthyl end group. These end groups were then quantitated using fluorescence spectroscopy, which showed that the number of end groups increased commensurately with the number of polymer chains.[5] These fluorescent tags are useful for monitoring polypeptide location and mobility, desirable for applications such as the monitoring of drug-delivery complexes in vitro.[11] Finally, MALDI-MS analysis of phenylglycine oligomers prepared using a nickel complex containing a leucine isoamylamide initiating group revealed that nearly all chains were end-functionalized with the leucine residue of the initiator.[5] Only very small peaks were observed for nonfunctionalized oligo(phenylglycines), indicating that the degree of chain functionalization was greater than 98%.

We have demonstrated an unprecedented general method for synthesis of amido-amidate nickelacycles. These complexes are valuable for the synthesis of polypeptides and block copolypeptides with well-defined and functional end groups. Potential applications range from labeling of polypeptide chains to the incorporation of ligands on chains for substrate-specific binding or recognition.

Acknowledgment. This work was supported by the U.S. Army Research Office Multidisciplinary University Research Initiative under award no. DAAH04-96-1-0443 and the National Science Foundation CAREER award no. CHE-9701969 and partially supported by the MRSEC program of the National Science Foundation under award No. DMR-9632716. T. J. D. is grateful for Alfred P. Sloan and Beckman Young Investigator Fellowships.

Supporting Information Available: Details of all reactions and polymerizations. This material is available free of charge via the Internet at http://pubs.acs.org.

JA990905G

(10) The anhydride IR stretch of the starting material (1780 cm^{-1}) was observed to completely disappear over the course of the reaction. The only amino acid containing compound present after hydrolysis was the coupled product (FAB MS) MH^+: 323.8 calc'd, 323 found).

(11) Singhal, A.; Huang, L. In *Gene Therapeutics: Methods and Applications of Direct Gene Transfer*; Wolff, J. A., Ed.; Birkhauser: Boston, 1994.