Table I. ^{13}C Chemical Shifts[a] (in ppm), Multiplicities,[b] and Deuterium Isotope Effects ($^n\Delta$)[c] (in ppb/deuteron) for Sugar Units and Ethylenediamine Units in Cobalt(III)–*N*-Glycoside Complexes

	C1	C2	C3	C4	C5	C6	en (a)	en (b)	en (c)	
1	97.83	87.40	77.00	91.20	63.96		53.92	46.20	46.70	45.74
	$^2\Delta$ d; 67	–	–	$^4\Delta$ d; 12	–		$^2\Delta$ d; 106	$^2\Delta$ t; 121	$^2\Delta$ t; 119	$^2\Delta$ t; 121
							$^3\Delta$ t; <15	$^3\Delta$ d; 27	$^3\Delta$ t; <21	$^3\Delta$ t, <15
2	97.65	88.68	76.12	87 27	69.24	21.70	53.67	46.38	46.88	45.57
	$^2\Delta$ d; 70	–	–	–	–	–	$^2\Delta$ d; 106	$^2\Delta$ t; 119	$^2\Delta$ t; 119	$^2\Delta$ t; 121
							$^3\Delta$ t; <20	$^3\Delta$ d; <24	$^3\Delta$ t; <18	$^3\Delta$ t; <15

[a] 2-Methyl-2-propanol was used as an internal reference with a chemical shift 31.9 ppm. Each chemical shift value is given for the all-protio form (lowest field component). [b] d = doublet, t = triplet, – = no isotope effect. [c] The magnitudes of all the isotope effects are negative (upfield shifts). Digital resolution is 3 ppb.

(III)–*N*-glycoside complexes in order to obtain the direct evidence of the C–N bond formation.

Partial deuteriation of coordinated NH_2 or NH groups can be easily achieved in a neutral H_2O–D_2O mixture. Generally hydrogen exchange of coordinated amino groups in aqueous solutions is expected to be slow on the NMR time scale. As a result, the isotope effects on the ^{13}C resonances of the α- (two-bond effect: $^2\Delta$) and β- (three-bond effect: $^3\Delta$) carbons to the coordinated nitrogens should give rise to distinct ^{13}C resonances for the individual isotopomers.[6] Some of the ^{13}C resonances of [Co(D-Rib-en[15])(en)]$^+$ (**1**)[4] were observed as multiplets in a neutral ca. 1:1 H_2O–D_2O mixture (Figure 1a). Partial deuteriation of a coordinated NH group produces two species, NH and ND, which cause ^{13}C resonances of α- and β-carbons to appear as doublets. On the other hand, partial deuteriation of a coordinated NH_2 group produces four species, NHH, NHD, NDH, and NDD, which cause ^{13}C resonances of α- and β-carbons to commonly appear as triplets.[6] The magnitude of the two-bond effect (55–128 ppb/deuteron) is usually greater than that of the three-bond effect (0–85 ppb/deuteron)[5,6,16] and that of the four-bond effect ($^4\Delta$) is commonly too small to be observed.[5,6] The resonances of carbon atoms with two possible isotope effects exhibit multiplicities analogous to those due to spin–spin couplings.[5-7,9-13]

The isotopic multiplet patterns in the ^{13}C NMR spectrum of **1** are expected as shown in Figure 1b on the basis of these empirical rules. Unlike the bidentate en ligand, the tetradentate *N*-glycoside ligand contains an NH_2 group and an NH group, which can give fingerprints of the formation of the *N*-glycoside bond. The carbon adjacent to the NH group of the en unit [en (a)] may appear as a doublet of triplets due to the two-bond effect by the NH group and the three-bond effect by the NH_2 group. The carbon adjacent to the NH_2 group of the en unit [en (b)] may appear as a triplet of doublets corresponding to the two-bond effect by the NH_2 group and the three-bond effect by the NH_2 group. C1 of the sugar unit may be observed as a doublet corresponding to the two-bond effect by the NH group and C2 as a doublet due to the three-bond effect by the NH group. The isotopic multiplet patterns for [Co(L-Rha-en[17])(en)]$^+$ (**2**)[4] are expected to be of the same manner.

All the ^{13}C NMR signals from the sugar units of **1** and **2** in D_2O were previously assigned completely by the ^1H–^1H and ^{13}C–^1H shift correlation 2D NMR spectroscopies. Assignments of the observed isotopic multiplets of **1** and **2** are listed in Table I. As expected, the resonances of C1 of the sugar unit and the carbons of the en unit of the *N*-glycoside ligand exhibit multiplet patterns that originate from the presence of the glycosidic NH group. These results reveal evidence for the presence of the C1–NH–C–C–NH_2 moiety. Thus the C–N bond formation on the Co(III) complexes is unambiguously proved by the application of the isotopic multiplets in the ^{13}C NMR spectra.

It has been difficult to identify the C–N bond formation by means of conventional NMR techniques. There is considerable obscurity in estimation by ^{13}C NMR chemical shifts or vicinal ^1H–^1H spin–spin couplings (H–N–C–H), and observation of ^{15}N–^{13}C spin–spin couplings commonly requires preparations of ^{15}N-labeled samples. In conclusion, the approach by use of the isotopic multiplet technique appears to be uniquely suited for such identification because of its clarity and simplicity.

(15) The full name is 1-(2-aminoethyl)amino-1-deoxy-D-ribose.
(16) The magnitudes of the two- and three-bond isotope effects are empirically greater for the less-substituted carbon atoms.[6,9,11] The magnitude of the three-bond isotope effect depends on the dihedral angle C(obsd)–C–X–H(D) (X = C or N).[6,14]
(17) The full name is 1-(2-aminoethyl)amino-1,6-dideoxy-L-mannose.

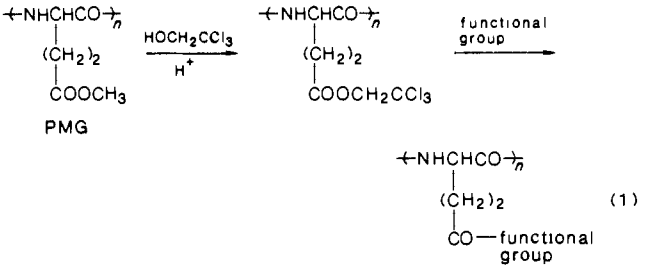
Synthesis and Polymerization of γ-Trichloroethyl-L-glutamate *N*-Carboxyanhydride: A Polypeptide That Can Be Functionalized with a Nucleophilic Agent

Kazunori Ishikawa and Takeshi Endo*

Research Laboratory of Resources Utilization
Tokyo Institute of Technology
Nagatsuta, Midori-ku, Yokohama 227, Japan

Received October 5, 1987

Poly(α-amino acids) are interesting as base materials in the synthesis of functional polymers because they can be induced to assume a conformation such as α-helix, β-sheet, and random coil under certain conditions. The synthesis of functional polymers utilizing these features of poly(α-amino acids) has been reported.[1,2] We have reported methods for the introduction of functional groups into poly(γ-methyl-L-glutamate) (PMG) by transesterification.[3] However, selective incorporation of a nucleophilic functional group into PMG, such as an amino group, is difficult due to low reactivity of the methyl ester and to the fission of the PMG main chain by nucleophilic attack by the amino group. Therefore, we have devised a method for incorporation amino functional groups into PMG (eq 1), where the methyl ester is



(1)

transesterified with trichloroethanol and the amino group is reacted without main chain fission. PMG functionalized with various groups has been prepared by applying these methods, and polymeric electron transport membranes containing redox functionality such as viologen and lipoic acid have been developed.[4-7]

If a glutamate *N*-carboxyanhydride with a highly activated ester such as trichloroethyl ester could be synthesized, it may be possible

(1) Maeda, M.; Kimura, M.; Hareyama, Y.; Inoue, S. *J. Am. Chem. Soc.* **1984**, *106*, 250.
(2) Sisido, M.; Egusa, S.; Imanishi, Y. *J. Am. Chem. Soc.* **1983**, *105*, 1041.
(3) Tanaka, H.; Endo, T.; Okawara, M. *Nippon Kagaku Kaishi* **1973**, 1770, 1775, 1780.

0002-7863/88/1510-2016$01.50/0

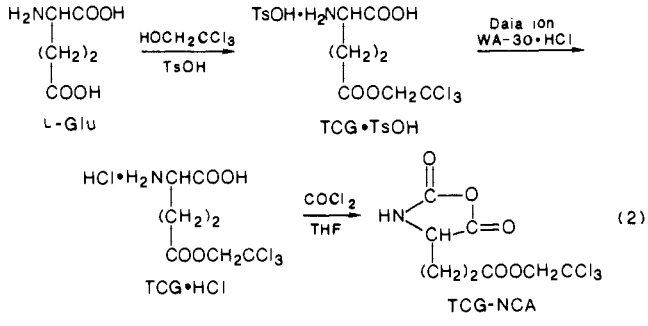
Table I. Polymerization of TCG-NCA[a]

run	initiator	(mol%)	temp[d] (°C)	convn[b] (%)	mol wt[c] $\times 10^{-4}$
1	Et_3N	(2)	rt	75	14
2	n-Bu_3N	(2)	rt	83	13
3	n-Bu_2N	(2)	rt	44	
4	n-$BuNH_2$	(2)	rt	45	
5	pyridine	(2)	rt	45	
6	Et_3N	(2)	10	90	13
7	Et_3N	(2)	40	43	12
8	Et_3N	(0.5)	rt	12	14
9	Et_3N	(5)	rt	100	7

[a] [M] = 0.328 M (EDC). [b] Determined by IR after 24 h based on 1844 and 1744 cm^{-1}. [c] Measured by GPC based on polystyrene (eluent: THF). [d] rt stands for room temperature.

to substitute the active esters in the resulting poly(glutamate) with nucleophilic functional groups under mild conditions. Furthermore, it may be possible to control the active ester contents by copolymerization with other amino acid *N*-carboxyanhydrides (NCA). There have been no reports concerning the synthesis of polypeptides from monomers that have active groups in the side chain. In this article, we describe the synthesis and polymerization of γ-(2,2,2-trichloroethyl)-L-glutamate *N*-carboxyanhydride (TCG-NCA) which is the first amino acid NCA monomer having an active ester ($-COOH_2CCl_3$) in its side chain.

γ-esterification of L-glutamic acid by 2,2,2-trichloroethanol was successfully carried out by using *p*-toluenesulfonic acid (TsOH) (eq 2) as a catalyst and toluene as a solvent.[8] TCG-NCA was



synthesized by passing dry phosgene into a solution of TCG·HCl[9] in dry THF and by heating under reflux for 30 min. After THF was removed under reduced pressure, the resulting residue was recrystallized twice from 1,2-dichloroethane (EDC).[9] TCG-NCA

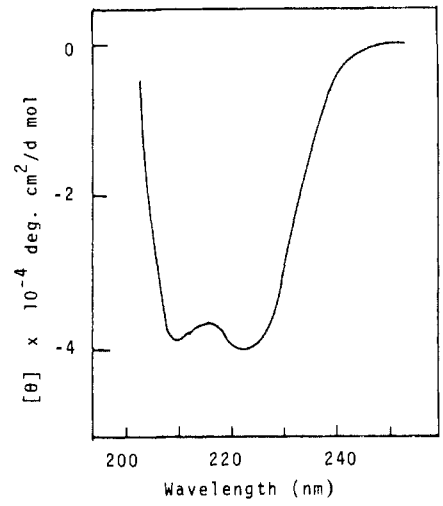
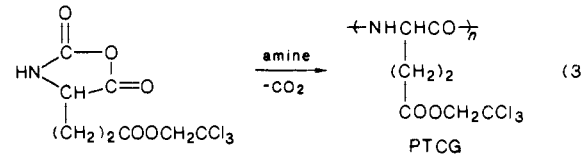


Figure 1. CD spectrum of PTCG in EDC.

was subsequently polymerized with amines under various conditions as shown in Table I to obtain the corresponding high molecular weight polypeptide.[11] The conversion of TCG-NCA



into polymer was higher with use of tertiary amines as initiator than when using secondary or primary amines. Further, the polymerization temperature, solvent, and concentration of the initiator affected the conversion of TCG-NCA remarkably. When THF, dioxane, or acetonitrile was used as a polymerization solvent, the conversion of TCG-NCA was very low (data not shown). As the polymerization temperature was increased, the conversion decreased. High molecular weight poly(γ-trichloroethyl-L-glutamate) was obtained with high conversion of the starting material in spite of having a sensitive group ($-COOCH_2CCl_3$) and by careful optimization of the reaction condition.

The IR spectrum of the PTCG film cast from the EDC solution shows two amide absorptions at 1655 and 1548 cm^{-1} and in the EDC solution at 1653 and 1550 cm^{-1} (independent on concentration), which are characteristic of an α-helical polypeptide even though the trichloroethyl ester group is a bulky side chain. CD curve of PTCG in EDC was shown in Figure 1. This also indicated that PTCG forms the α-helical structure in EDC solution. Additionally, PTCG has good film-forming characteristics. The reactivity of the activated ester of PTCG is now under intensive investigation.

(4) Kudo, A.; Endo, T.; Tosabayashi, Y.; Okawara, M. *J. Polym. Sci., Polym. Lett. Ed.* **1979**, *17*, 789.
(5) Nambu, Y.; Endo, T.; Okawara, M. *J. Polym. Sci., Polym. Chem. Ed.* **1985**, *23*, 223.
(6) Nambu, Y.; Endo, T.; Okawara, M. *J. Polym. Sci., Polym. Lett. Ed.* **1985**, *23*, 49.
(7) Ishikawa, K.; Nambu, Y.; Endo, T. *J. Polym. Sci., Part A* **1988**, in press.

(8) An esterified product at the α-carboxyl group was rarely detected because of low nucleophilicity of trichloroethanol and low reactivity of the α-carboxyl group compared to γ-carboxyl. Although γ-esterification also did not occur at all in benzene under reflux, γ-esterification was successful at a higher temperature by using toluene as a solvent. A mixture of L-glutamic acid (10.0 g, 0.068 mol), 2,2,2-trichloroethanol (12.0 g, 0.080 mol), *p*-toluenesulfonic acid monohydride (19.4 g, 0.102 mol), and toluene (120 mL) was refluxed for 6 h with stirring and removing water by using the Dean–Stark apparatus. After having cooled the reaction mixture, a mixed solvent of diethyl ether and *n*-hexane (1:1 v/v) was added to it. A precipitated crystalline product was recrystallized from a mixed solvent of ethanol and diethyl ether (1:4 v/v) to give TCG·TsOH: yield 85%; mp 148–152 °C; IR (KBr) 1754, 795, 715 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.67 (d, 2 H), 7.16 (d, 2 H), 4.80 (s, CH_2), 4.07 (t, CH), 2.0–3.0 (m, $2CH_2$), 2.5 (s, CH_3). Anal. Calcd for $C_{14}H_{18}NO_7SCl_3$: C, 37.30; H, 4.03; N, 3.11; S, 7.11; Cl, 23.66. Found: C, 37.99; H, 4.00; N, 3.05; S, 6.93; Cl, 23.91.

(9) TCG·TsOH (10.0 g, 0.022 mol) was dissolved in methanol (50 mL), and the solution was passed through a column of a resin Daia ion WA-30 hydrochloride (150 mL, 0.225 mol) by using methanol as an eluent. The eluted methanol solution was evaporated under reduced pressure below 30 °C to give 5.6 g of γ-trichloroethyl-L-glutamate hydrochloride (TCG·HCl) yield 90%; mp 167–169 °C.

(10) TCG-NCA: yield 65%; mp 90–91.5 °C; IR (KBr) 1844, 1774, 1744 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 2.28 (m, CH_2), 2.74 (t, CH_2), 4.46 (t, CH), 4.78 (s, CH_2), 6.30 (s, NH). Anal. Calcd for $C_7H_4NO_4Cl_3$: C, 31.55; H, 2.65; N, 4.60; Cl, 34.93. Found: C, 31.85; H, 2.86; N, 4.65; Cl, 35.20.

(11) PTCG: yield 95% (methanol insoluble part); IR (casted from EDC solution) 3286, 1757, 1655, 795, 715 cm^{-1}; ^1H NMR (CF_3COOH) δ 2.0–3.0 (br, $2CH_2$), 4.83 (br, CH_2, CH), 8.03 (br, NH). Anal. Calcd for $C_7H_8NO_4Cl_3$: C, 32.28; H, 3.09; N, 5.38; Cl, 40.80. Found: C, 33.08; H, 3.28; N, 5.38; Cl, 40.73.