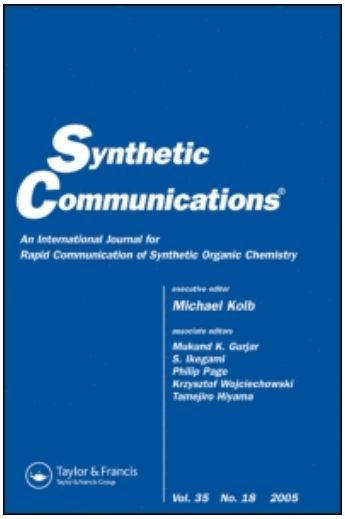

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B. S. Lele[a]; M. A. Gore[a]; M. G. Kulkarni[a]
[a] Polymer Science and Engineering Unit, Chemical Engineering Division, National Chemical Laboratory, Pune, India

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DIRECT ESTERIFICATION OF POLY (ETHYLENE GLYCOL) WITH AMINO ACID HYDROCHLORIDES

Lele, B.S.*, Gore, M.A., Kulkarni, M.G.

Polymer Science and Engineering Unit, Chemical Engineering Division, National Chemical Laboratory, Pune 411008, India

ABSTRACT: Diesters of poly (ethylene glycol) with various α amino acid hydrochlorides were synthesized using dicyclohexyl carbodiimide as coupling agent. The use of hydrochloride as amino protecting group in dicyclohexyl carbodiimide mediated condensation reactions of amino acids was demonstrated for the first time.

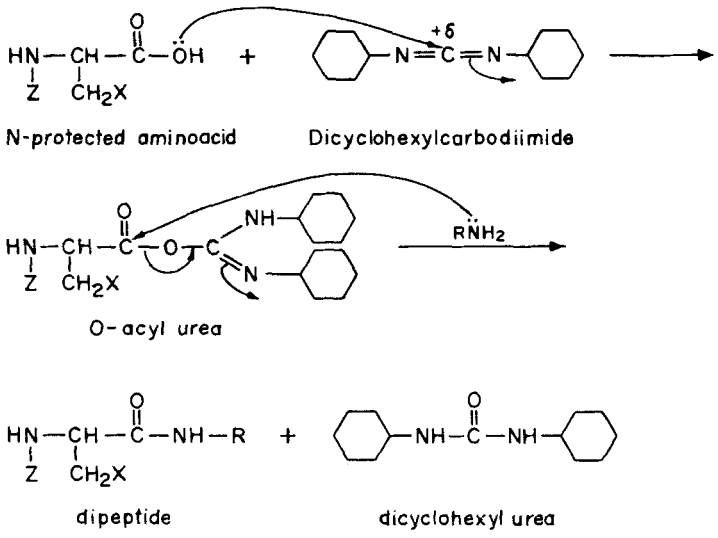
Dicyclohexyl carbodiimide (DCC) is widely used as coupling agent in syntheses of ester, amide and peptide derivatives of α amino acids [1]. This requires protection of α amino group with various protecting groups such as N-carbobenzoxy (N-cbz), N- tertiary butyloxycarbonyl (N-tboc) etc.; followed by the attack of nucleophile in the presence of DCC. In contrast to these speciality protecting groups, the use of hydrochloride as amino protecting group in DCC

* To whom correspondence should be addressed.

mediated condensation reactions of α amino acids has not been reported. In this communication we report one step synthesis of diesters of poly (ethylene glycol) 6000 (PEG 6000) with various amino acid hydrochlorides using DCC as coupling agent. Additionally, esters of amino acid hydrochlorides with simple alcohol i.e. ethylene glycol monomethyl ether were synthesized to confirm the reaction.

Esters and amides of PEG derivatives with amino acids are important monomers used in the synthesis of biodegradable polymers [2, 3]. Esters of amino acids are generally synthesized and stored as hydrochloride salts since free amino group is prone to undergo diketopiperazine formation. While working on our program to develop amino acid based biodegradable polymers we observed that conventional reaction of alcohol, amino acid and thionyl chloride to afford amino acid ester hydrochloride is not feasible for PEG because of its bulky chain length [4]. Thus amino acid ester hydrochloride of PEG is synthesized in two steps. In the first step p-toluene sulfonate salt of the diester is synthesized by conventional Dean- Stark type esterification using stoichiometric amounts of amino acid, PEG and p-toluene sulfonic acid monohydrate. In the second step, p-toluene sulfonate is deblocked by the treatment of triethylamine or sodium bicarbonate. The free ester so synthesized is then converted into hydrochloride salt by passing dry HCl in its etheral solution. Thus it is desirable to have a single step method for synthesis of amino acid ester hydrochlorides of PEG.

DCC is a strong condensing agent which activates carboxyl groups by forming unstable O-acyl urea with N-protected amino acid and facilitates the attack of



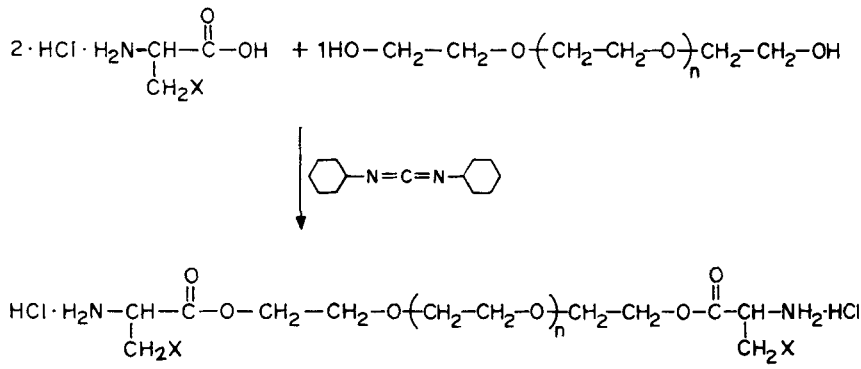
Scheme-1

Mechanism of DCC coupling

nucleophilic molecule [1]. Thus it brings about condensation reaction by quenching water molecule and forming dicyclohexyl urea. (Scheme 1).

In the present case we expected activation of carboxyl groups in amino acid hydrochloride by DCC and the reaction between carboxyl groups of amino acid hydrochloride and hydroxyl groups of PEG. Unlike amino group, hydroxyl groups of PEG cannot abstract proton from hydrochloride. Therefore the hydrochloride salt was expected to act as amino protecting group in the condensation reaction. Also, consistent with the reports of Kohn et al [5] phenolic – OH and sulphhydryl groups in trifunctional amino acids like tyrosine and cysteine respectively need no protection (Scheme 2).

With this assumption, DCC mediated coupling of PEG 6000 with hydrochlorides of two trifunctional amino acids viz. tyrosine and cysteine and



Scheme-2

DCC Mediated coupling of PEG and amino acid hydrochloride

four difunctional amino acids viz. glycine, isoleucine, 6 amino caproic acid and 11 amino undecanoic acid was conducted as described in the experimental section. Diesters so synthesized were characterized by melting point, IR, NMR, acid values and elemental analysis. As an example, IR spectrum of bis (tyrosyl hydrochloride) poly (ethylene glycol) 6000 diester (Bis Tyr. HCl –PEG 6000) is shown in Figure 1. The spectrum shows only ester carbonyl peak at 1720 cm^{-1} and not for amide carbonyl. This shows that condensation between carboxyl groups of Tyr.HCl and hydroxyl groups of PEG 6000 proceeded smoothly with amino groups protected in the form of hydrochloride. Similar IR spectra for other diesters were also observed. To substantiate the protection of $-NH_2$ by hydrochloride, quantitative estimation of hydrochloride in all the diesters was done by finding out their acid values. Data for the acid values are listed in Table 1.

It can be seen from the data that calculated and found acid values are in close agreement, taking into consideration polydispersity in commercial PEG

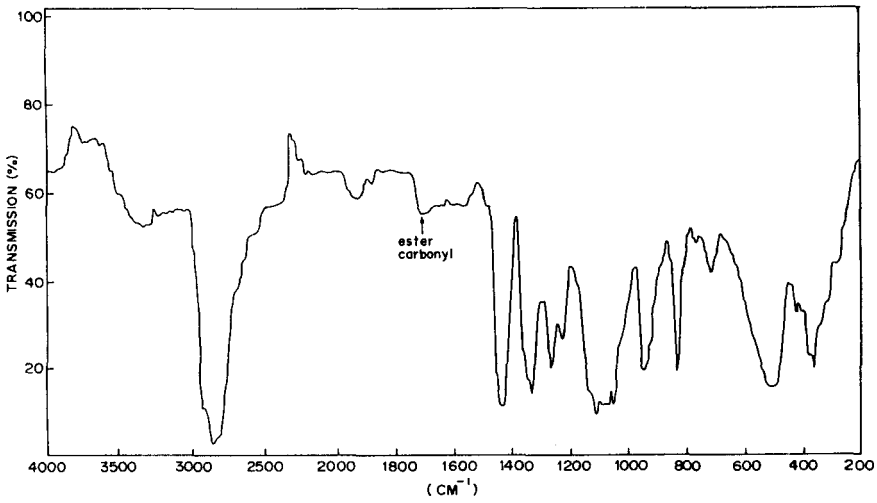


FIG. 1 - FT-IR SPECTRUM OF Bis Tyr .HCl- PEG 6000.

Table 1

Calculated and found values of hydrochloride in diesters

No.	Diester	Acid value, milimoles of HCl /g (calculated)*	Acid value, milimoles of HCl /g (found)
1	Bis Tyr.HCl – PEG 6000	0.2 to 0.3	0.224
2	Bis Cyst.HCl – PEG 6000	0.2 to 0.3	0.29
3	Bis Isoleu.HCl – PEG 6000	0.2 to 0.3	0.213
4	Bis 6ACA.HCl – PEG 6000	0.2 to 0.3	0.224
5	Bis Gly.HCl – PEG 6000	0.2 to .03	0.254
6	Bis 11AU.HCl – PEG 6000	0.2 to 0.3	0.26

*Acid values calculated for PEG 6000 with molecular weight range 6000 to 7500.

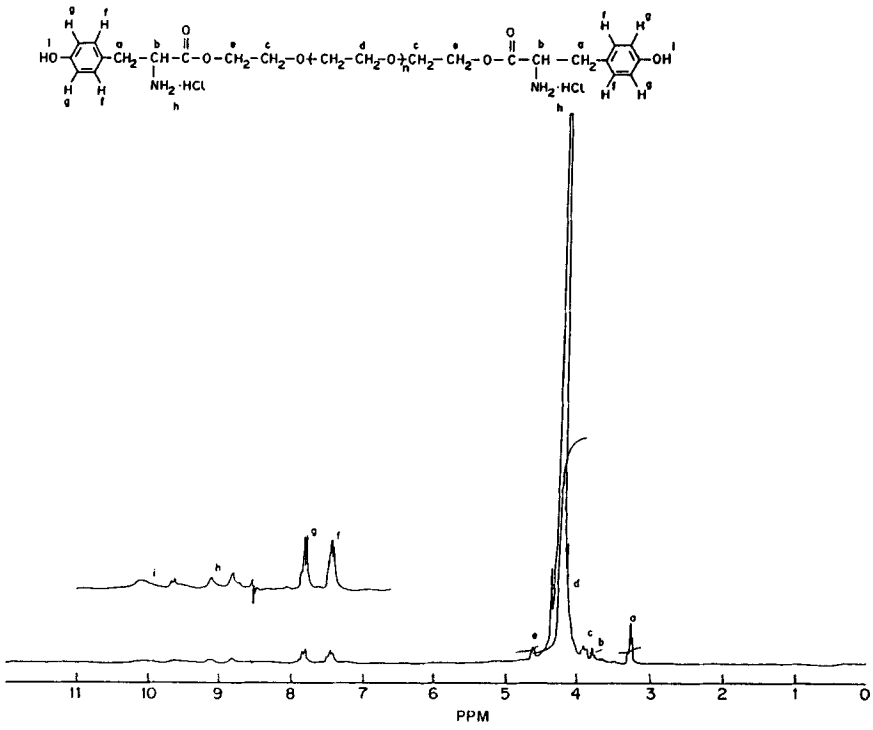


FIG.2- ^{1}H NMR SPECTRUM OF Bis Tyr· HCl PEG-6000

6000 sample. (Molecular weight range 6000 to 7500). ^{1}H NMR spectrum of Bis Tyr.HCl – PEG 6000 is shown in Figure 2.It shows that the signals in the spectrum are in accordance with the proposed structure of the diester. ^{1}H NMR spectra of other diesters were also in agreement with their proposed structures. Diesters were also characterized by elemental analyses. From the data listed in the experimental section, it can be seen that calculated and found values for elemental analyses are in close agreement.

Although the objective of this work was to synthesize amino acid ester hydrochlorides of PEG, the alcohol used i.e. PEG 6000 is polydisperse. Therefore

to validate the present reaction it was desired to synthesize esters using simple alcohols. Ethylene glycol monomethyl ether (2 methoxy ethanol) was selected for this. It was reacted with tyrosine.hydrochloride, leucine.hydrochloride and 11 amino undecanoic acid.hydrochloride in the presence of DCC as per the general procedure of the present reaction. 2 methoxy ethyl ester hydrochlorides of amino acids so synthesized were characterized by IR, ^{1}H NMR and elemental analyses. The spectral data listed in the experimental section is in agreement with the proposed structure of products. This further supports the reaction i.e. formation of diesters of PEG 6000 with amino acids having hydrochloride as $-NH_2$ protecting group, by DCC mediated condensation. Experimental results obtained using various amino acids confirm the general applicability of this reaction in esterification of amino acids.

Experimental

PEG 6000, all amino acids, dimethyl formamide (DMF), diethyl ether, methanol, were obtained from local suppliers. DCC was from Aldrich. Hydrochlorides were prepared by treating amino acids with hydrochloric acid. Solvents were distilled prior to use as per standard procedures. IR spectra were recorded on Perkin Elmer FT-IR 1600 spectrometer. Elemental analyses were done on Perkin Elmer elemental analyzer [6]. ^{1}H NMR spectra were recorded on Varian 200 MHz spectrometer [7]. Acid values were estimated as follows.

Estimation of acid values

0.2 g of material was dissolved in 10 ml distilled water. To this solution, 2 drops of phenolphthalein indicator was added and the solution was titrated against

0.01680 N KOH (normality determined from titration with potassium hydrogen phthalate) till colourless to faint pink end point was obtained. Acid values were calculated using the formulae,

Acid value (mg KOH /g) = 56.1 * (ml of KOH required) * normality of KOH / weight of the material (g).

Acid value so calculated is in terms of mg of KOH required to neutralize one g of the material. Acid value in terms of milimoles of HCl was calculated as

Acid value (milimoles HCl /g) = acid value (mg KOH) /56.1

Synthesis of bis (tyrosyl hydrochloride) poly (ethylene glycol) 6000 diester (Bis Tyr.HCl -PEG6000)

In a 100 ml capacity conical flask, 6 g PEG 6000 (0.001 M), 0.435g (0.002 M) Tyr.HCl, and 10 ml DMF were taken. The contents of the flask were gently heated to dissolve the solids and obtain a clear solution. To this solution, 0.412 g DCC (0.002 M) dissolved in 5 ml DMF was added in a single portion. The reaction mixture was stirred at room temperature (25°C) for 24 hours. It was then filtered to separate out dicyclohexyl urea (DCU) formed and the clear solution was poured in 200 ml diethyl ether to precipitate out white powdery product. The product was isolated and purified by reprecipitation from methanol into diethyl ether.

Yield: 78%. **Melting point:** 58°C. **Acid value:** (Found) 0.224 milimoles HCl /g, (Calculated) 0.2 to 0.3 milimoles HCl /g. **IR (nujol):** 1720 cm^{-1} (ester carbonyl), 3500 cm^{-1} (-OH, NH stretching). **^{1}H NMR (DMSO d_6):** 3.3 δ doublet (-CH_2-Ph of Tyr), 3.8 δ triplet (Tyr-CH-COO-PEG), 3.9 δ triplet (-OCH_2 of PEG next to

terminal $-OCH_2$), 4.2 δ broad singlet $(-CH_2-CH_2-O-)_n$ of PEG chain, 4.6 δ terminal $-CH_2-O-$ of PEG), 7.4 δ doublet (Tyr ring protons), 7.8 δ doublet (Tyr ring protons), 8.8 δ singlet ($-NH_2$), 9.6 δ singlet (-OH). **Elemental analysis:** Calculated for ($C_{290}H_{566}O_{141}N_2Cl_2$) C 54.36%, H 8.84%, N 0.43 %. Found C 53.99%, H 8.50%, N 0.43%.

All compounds were synthesized by following the above- described procedure using the same stoichiometric amounts of alcohol and amino acid hydrochlorides. Characterization data are listed in the following.

Bis (cystyl hydrochloride) poly (ethyelene glycol) 6000 diester (Bis Cyst.HCl –PEG 6000)

Yield: 70%. **Melting point:** 59°C. **Acid value:** (Found) 0.29 milimoles HCl /g, (Calculated) 0.2 to 0.3 milimoles HCl /g. **IR (nujol):** 1700 cm^{-1} (ester carbonyl), 3500 cm^{-1} (-NH stretching). **^{1}H NMR (DMSO d_6):** 2.5 δ doublet ($-CH_2-SH$ of cysteine), 3.3 δ triplet (Cyst –CH-COO-PEG), 3.6 δ broad singlet $(-CH_2-CH_2-O-)_n$ of PEG chain. **Elemental analysis:** Calculated for ($C_{278}H_{558}O_{147}N_2S_2Cl_2$) C 52.05%, H 8.70%, N 0.43%. Found C 53.12%, H 9.0%, N 0.35%.

Bis (isoleucyl hydrochloride) poly (ethylene glycol) 6000 diester (Bis Isoleu. HCl –PEG 6000)

Yield: 68%. **Melting point:** 129°C. **Acid value:** (Found) 0.213 milimoles HCl /g, (Calculated) 0.2 to 0.3 milimoles HCl /g. **IR (nujol):** 1740 cm^{-1} (ester carbonyl), 3520 cm^{-1} (-NH stretching). **^{1}H NMR (DMSO d_6):** 0.9 δ doublet ($-CH_3$ of $-CH_2-CH_3$), 1.1 δ multiplet ($-CH_2$ of $-CH_2-CH_3$), 1.6 δ multiplet (-CH of -CH-CH_3),

2.5 δ doublet (-CH_3 of –CH-CH_3), 3.3 δ doublet (isoleuCH-COO-PEG), 3.6 δ broad singlet (-CH_2-CH_2-O-)$_n$ of PEG chain. **Elemental analysis:** Calculated for ($C_{284}H_{570}O_{141}N_2Cl_2$) C 54.08%, H 9.04%, N 0.44%. Found C 53.41%, H 8.39%, N 0.41%.

Bis (6 amino caproyl hydrochloride) Poly (ethylene glycol) 6000 diester (Bis 6ACA.HCl -PEG 6000)

Yield: 80%. **Melting point:** 59°C. **Acid value:** (Found) 0.224 milimoles HCl /g, (Calculated) 0.2 to 0.3 milimoles HCl /g. **IR (nujol):** 1710 cm^{-1} (ester carbonyl), 3400 cm^{-1} (-NH stretching). **^{1}H NMR (DMSO d_6):** 1.2 δ multiplet (C_5 methylene of 6ACA), 1.6 δ multiplet (C_4 methylene of 6ACA), 2.2 δ multiplet (C_2 methylene of 6ACA), 3.6 δ broad singlet (-CH_2-CH_2-O-)$_n$ of PEG chain. **Elemental analysis:** Calculated for ($C_{294}H_{570}O_{139}N_2Cl_2$) C 54.94%, H 8.87%, N 0.43%. Found C 53.38%, H 8.72%, N 0.30%.

Bis (glycyl hydrochloride) Poly (ethylene glycol 6000) diester (Bis Gly.HCl – PEG 6000)

Yield: 82 %. **Melting point:** 58°C. **Acid value:** (Found) 0.254 milimoles HCl /g, (Calculated) 0.2 to 0.3 milimoles HCl /g. **IR (nujol):** 1690 cm^{-1} (ester carbonyl), 3450 cm^{-1} (-NH stretching). **^{1}H NMR (DMSO d_6):** 2.4 δ singlet (-CH_2 of gly), 3.6 δbroad singlet (-CH_2-CH_2-O-)$_n$ of PEG chain, 8.2 δ doublet (-NH_2). **Elemental analysis:** Calculated for ($C_{276}H_{554}O_{139}N_2Cl_2$) C 53.51%, H 8.95%, N 0.45%. Found C 53.15%, H 8.78%, N 0.35%.

***Bis* (11 amino undecanoyl hydrochloride) poly (ethylene glycol 6000) diester (Bis 11 AU –PEG 6000)**

Yield: 80 %. **Melting point:** 137°C. **Acid value:** (Found) 0.26 milimoles HCl /g, (Calculated) 0.2 to 0.3 milimoles /g. **IR (nujol):** 1710 cm^{-1} (ester carbonyl), 3500 cm^{-1} (-NH stretching). **^{1}H NMR (DMSO d_6):** 1.2 δ broad singlet (C_4 to C_9 methylenesof 11AU), 1.5 δ triplet (C_2 methylene of 11 AU), 2.2 δ multiplet (C_{10} methylene of 11AU), 3.5 δ broad singlet $(-CH_2-CH_2-O-)_n$ of PEG chain, 7.8 δ doublet ($-NH_2$). **Elemental analysis:** Calculated for ($C_{294}H_{590}O_{139}N_2Cl_2$) C 52.79%, H 9.56%, N 0.45%. Found C 53.0%, H 9.52%, N 0.40%.

2 Methoxy ethyl tyrosyl ester hydrochloride

Yield: 55%. **Melting point:** 268 ^{0}C (dec.). **Acid value:** (Found) 203 milimoles HCl /g, (Calculated) 203.62 milimoles HCl/g. **IR (nujol):** 1755 cm^{-1} (ester carbonyl), 3500 cm^{-1} (-OH, -NH stretching). **^{1}H NMR (DMSO d_6):** 2.0 δ 2H triplet ($-CH_2-CH_2O-$), 2.3 δ 3H singlet ($-OCH_3$), 2.5 δ 2H triplet ($-CH_2O-COOR$), 3.3 δ 2H doublet ($-CH_2-Ph$ of Tyr), 3.8 δ 1H triplet (-CH-COOR of Tyr), 7.4 δ 2H doublet (Tyr ring protons), 7.8 δ2H doublet (Tyr ring protons), 8.6 δ 2H singlet ($-NH_2$). **Elemental analysis:** Calculated for ($C_{12}H_{18}NO_4Cl$) C 52.26%, H 6.53%, N 5.08%. Found C 52.30%, H 6.50%, N 5.07%.

2 Methoxy ethyl leucyl ester hydrochloride

Yield: 50%. **Melting point:** 232^{0}C (dec.) **Acid value:** (Found) 224.8 milimoles HCl/g, (Calculated) 225.5 milimoles HCl/g. **IR (nujol):** 1740 cm^{-1} (ester carbonyl) 3300 cm^{-1} (-NH stretching). **^{1}H NMR (DMSO d_6):** 0.9 δ 6H doublet ($(CH_3)_2$-CH- of Leu), 1.2 δ 1H multiplet (-CH-$(Me)_2$ of Leu), 1.6 δ 2H triplet ($-CH_2$-CH- of Leu), 1.8 δ 1H triplet (-CH-COOR of Leu), 1.9 δ 2H triplet ($-CH_2-CH_2O-$), 2.1 δ 3H singlet ($-OCH_3$), 2.5 δ 2H triplet ($-CH_2O-COOR$), 7.7 δ 2H

singlet (-NH_2). **Elemental analysis:** Calculated for ($C_9H_{20}NO_3Cl$) C 47.89%, H 8.86%, N 6.20%. Found C 47.90%, H 8.88%, N 6.20%.

2 Methoxy ethyl 11 aminoundecanoyl ester hydrochloride

Yield: 80 %. **Melting point:** 127^{0}C. **Acid value:** (Found) 190 milimoles HCl/g, (Calculated) 189.84 milimoles HCl/g. **IR (nujol):** 1750 cm^{-1} (ester carbonyl), 3350 cm^{-1} (-NH stretching). **^{1}H NMR (DMSO d_6):** 1.2 δ18H broad singlet (C_3 to C_{11} methylene protons of 11AU), 1.6 δ 2H triplet (-CH_2-COOR of 11AU), 2.1 δ 2H triplet (-CH_2-CH_2O-), 2.5 δ 3H singlet (-OCH_3), 2.7 δ 2H triplet (-CH_2O-COOR), 7.8 δ 2H singlet (-NH_2). **Elemental analysis:** Calculated for ($C_{14}H_{30}NO_3Cl$) C 56.85%, H 10.15%, N 4.73%. Found C 56.90%, H 10.10%, N 4.71%.

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6) Theoretical percentage of C, H and N were calculated assuming PEG 6000 is monodisperse and its molecular weight is exactly 6000.

7) In PEG based samples signals for amino acid moieties were very weak as compared to PEG chain due to its high molecular weight. Therefore signals are assigned to individual moiety without proton integration.

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