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Facile synthesis of poly-(L-lysine) dendrimers with a pentaaminecobalt(III) complex at the core

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Abstract—The synthesis of second and third generation dendrimers based on poly-(L-lysine) with a pentaamine cobalt(III) metal complex at the core is described. The synthesis and purification of these dendrimers were facilitated by using the metal complex as the C-terminal protecting group.

The synthesis of peptidic dendrimers has lately been the focus of many research groups, due to their utilization as protein and liposomal mimetics as well as biomaterials for biomedical applications.[1,2] The most commonly used peptide-based dendrimers are the ones containing L-lysine in their branching units, due to the important applications of these dendrimers as antimicrobial agents,[3,4] drug-delivery agents,[5] vaccines,[6,7] gene transfectors,[8–10] gold nanoparticles stabilizers,[11] and last but not the least as organogelators.[12,13]

Recently, we have been working on the preparation of stable platinum nanoparticles that are capped by different generations of poly-(L-lysine) dendrimers. Therefore, a facile and time-efficient preparation of such dendrimers is essential to us and others working on the applications of these peptidic dendrimers. However, both the preparation procedures of these dendrimers in the literature based on either solid-phase peptide synthesis[5] or solution phase methods,[14,15] do not afford the higher generation dendrimers on a multi-gram scale, especially the solid-phase preparation method. Moreover, the purification of the Boc-protected dendrimers becomes very difficult especially in non-polar organic solvents due to the formation of gels of the higher generation dendrimers.[14]

Here, we describe the facile synthesis and time-efficient purification of peptide dendrimers based on poly-(L-lysine) following a divergent synthetic approach. The synthesis was carried out (Scheme 1) by first coupling the carboxylic acid group of a $(Boc)_2$-Lys-OH residue to a pentaaminecobalt(III) aquo complex,[16,17] using 1,3-dicyclohexylcarbodiimide (DCC) as the coupling reagent and 1-hydroxybenzotriazole (HOBt) to suppress any racemization of L-lysine residues during the synthesis.[18] This afforded the first generation of the fully protected dendrimer, **Boc–G1Co**. Deprotection of the Boc-groups was accomplished using 50% trifluoroacetic acid (TFA) solution in dry methylene chloride (CH_2Cl_2) for 1 h, which afforded the **G1Co** dendrimer with a ca. 90% total isolated yield. Similarly, the second and third generation dendrimers, **Boc–G2Co** and **Boc–G3Co**,[19] were synthesized by sequential deprotection of the Boc-groups followed by coupling reactions with a slight excess of $(Boc)_2$-Lys-OH. The deprotection of the cobalt(III) group in **Boc–G2Co** and **Boc–G3Co** was easily accomplished using a mild reducing agent such as dithiothreitol (DTT) and *N*-methylmorpholine (NMM), or sodium borohydride ($NaBH_4$) in water/ethanol solution to afford the Boc-protected dendrimers, **Boc–G2OH** and **Boc–G3OH**.[20] Deprotection of the Boc-groups with TFA/CH_2Cl_2 afforded multi-grams (5–10 g) of the pure second and third generation dendrimers **G2OH** and **G3OH**.

The attractive features of using pentaaminecobalt(III) as a C-terminal protecting group of a peptide include the color (reddish-pink), stability (in basic and dry acidic media) and the solubility of the cobalt-protected

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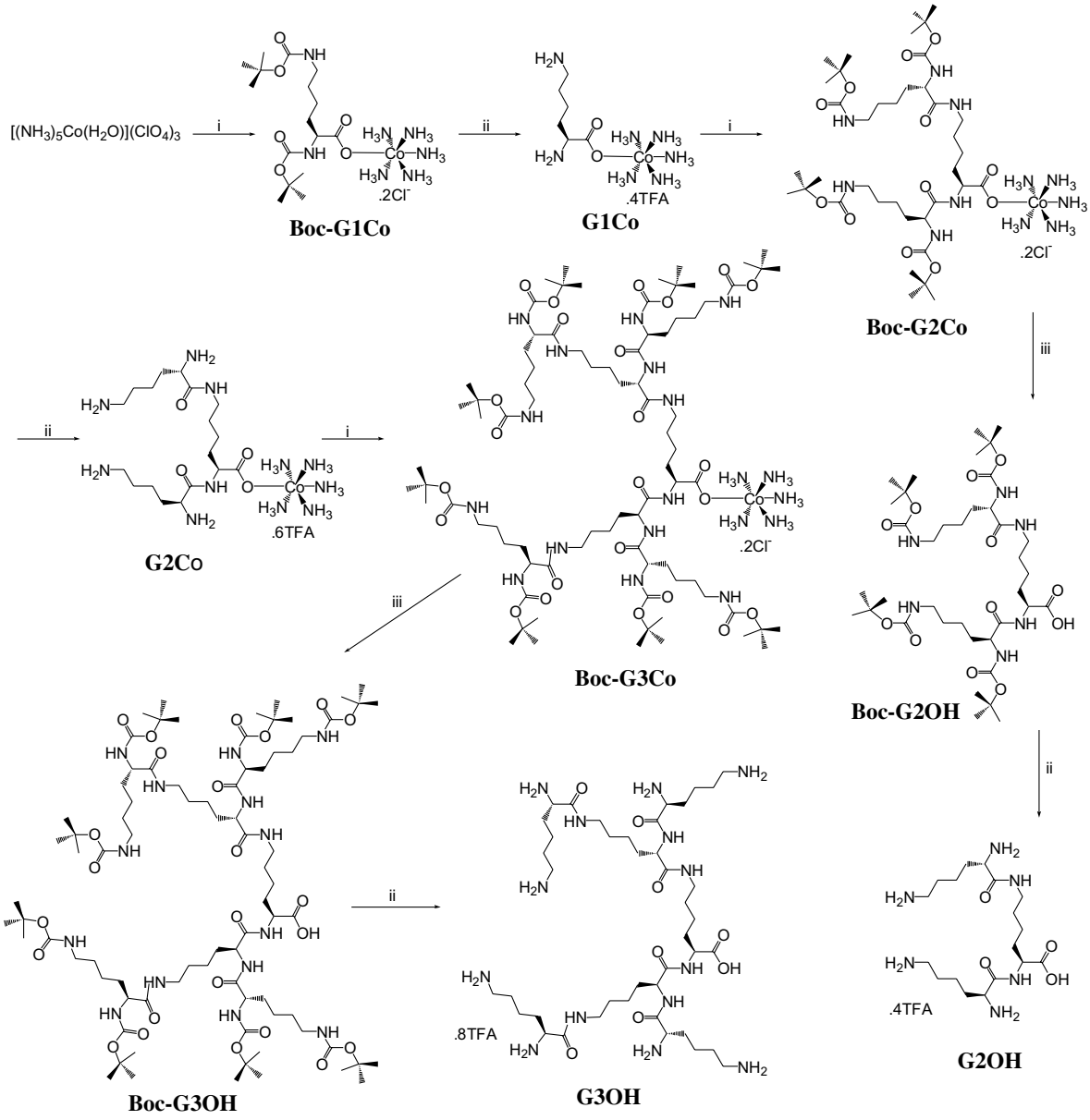

Scheme 1. Reagents and conditions: (i) 1.1 equiv of $(Boc)_2$-Lys-OH, DCC, HOBt, and NMM (per one coupling linkage) in DMF at 0 °C for 20 min then at rt for 12 h, 95% (for **Boc–G1Co**), 93% (for **Boc–G2Co**), 90% (for **Boc–G3Co**), (ii) 1:1 TFA in dry CH_2C1_2, rt, 1 h, 100% (for **G1Co**, **G2Co**, **G2OH**, and **G3Co**), (iii) 4 equiv of $NaBH_4$ in 1:1 water/ethanol at rt for 2 min, 90% (for **Boc–G1OH**), 90% (for **Boc–G2OH**).

peptides in organic and in aqueous solutions,[17] which in turn makes the purification steps easier than when using conventional C-terminal protecting groups, such as methyl esters. The latter feature, however, was the most important to us, since the purification of all dendrimers after the coupling steps was accomplished by first applying the cobalt-protected dendrimer to a preparative cation-exchange column (Sephadex, SP-C25), then to a preparative liquid chromatography C-18 column (YMC, ODS-AQ), where the eluents were aqueous NaCl/ethanol and water/ethanol mixtures, respectively. In such polar protic solvents no gelation of the dendrimers occurred during the chromatographic purification steps, and most importantly, all traces of the coupling reaction side-product dicyclohexylurea (DCU), HOBt, and the excess $(Boc)_2$-Lys-OH were removed during the first purification step on the cation exchange resin. All of the coupling steps after 12 h of reaction time were about 100% yield when monitored by HPLC (at 190 and 490 nm, where the peptide branches and the cobalt complex absorb, respectively), with isolated yields of more than 90% after purification. No side reactions, incomplete coupling steps or loss of the cobalt protecting group was detected at any stage of the synthesis. The purity and identity of the synthesized dendrimers were checked by HPLC (Fig. 1 and Table 1) and high resolution ESI mass spectrometry (Fig. 2), respectively.

NMR spectroscopy was not of a great help for identifying the cobalt-protected peptides, since broad peaks were seen, most probably due to the presence of small amounts of paramagnetic Co(II) species. However, the NMR spectra of the cobalt-free dendrimers (**Boc–G2OH**, **Boc–G3OH**, **G2OH**, and **G3OH**) agreed well

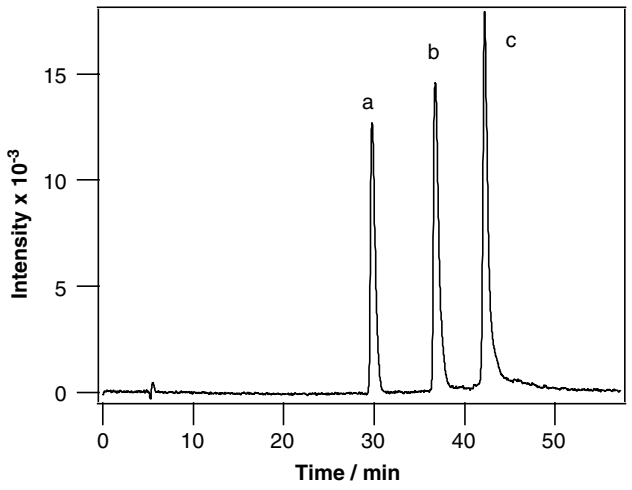

Figure 1. HPLC chromatogram of a mixture of (a) **Boc–G1Co**, (b) **Boc–G2Co**, and (c) **Boc–G3Co** monitored at $\lambda = 490$ nm.

Table 1. Specific rotation measurements and HPLC retention times

Entry	Dendrimer	$[\alpha]_D$ (deg cm^3 g^{-1} dm^{-1})[a]	HPLC (min)
1	**Boc–G1Co**	−78.5	29.7[b]
2	**Boc–G2Co**	−62.2	36.7[b]
3	**Boc–G2OH**	−36.7	40.7[b]
4	**Boc–G3Co**	−49.2	42.2[b]
5	**Boc–G3OH**	−27.5	46.5[b]
6	**G2OH**	+26.2	2.7[c]
7	**G3OH**	+27.1	3.1[c]

[a] The measurements were done at 22 °C in methanol ($c = 1$).
[b] The gradient used is (50% A–50% B) to (0% A–100% B) in 30 min. Solvent A is 10% CH_3OH/H_2O and solvent B is 85% CH_3OH/H_2O with 0.1% NaTFA/TFA, pH = 3.5.
[c] The solvent is 10% CH_3OH/H_2O with 0.1% NaTFA/TFA, pH = 3.5.

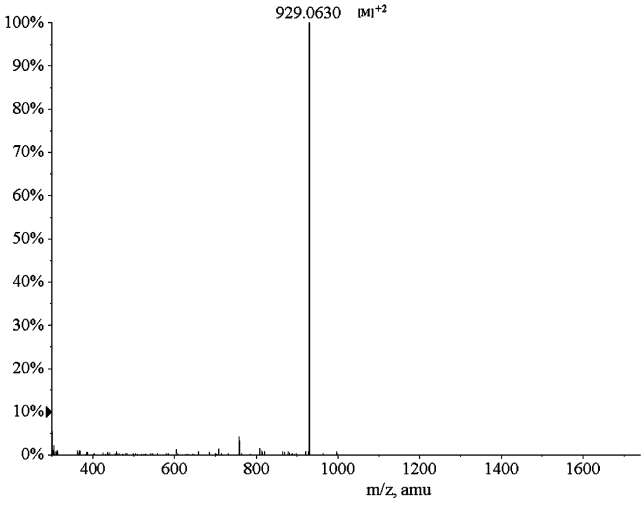

Figure 2. High resolution ESI mass spectrum of **Boc–G3Co**.

with reported spectra in the literature.[14,15] The specific rotation $[\alpha]_D$ was measured for all of the dendritic peptides in methanol, Table 1. The $[\alpha]_D$ values of the dendritic peptides were negative and non-zero for the Boc-protected dendrimers and positive non-zero for the free dendritic peptides (**G2OH** and **G3OH**), which suggested that using the pentaamine cobalt complex at the core does not cause racemization of the L-lysine residues during the synthesis.

In summary, we report the synthesis of poly-(L-lysine) based peptide dendrimers with a pentaamine cobalt complex at the core. The use of the cobalt complex serves as the C-terminal protecting group, which in turn facilitates purification of these dendrimers due to the enhancement of the dendrimers' solubility in aqueous and organic solvents and due to the inhibition of the gelation of the Boc-protected dendrimers in most common solvents.

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19. *Procedure for the synthesis of* **Boc–G3Co**:**Boc–G2Co** (7.1 g, 7.0 mmol) was added to 50 ml of 1:1 TFA/CH_2Cl_2 and left stirring at room temperature for 1 h. The volatiles

were removed by rotary evaporation, and the hygroscopic deprotected cobalt-peptide, **G2Co**, was dried under vacuum for 12 h. $(Boc)_2$Lys·DCHA (16.25 g, 30.83 mmol) in 100 ml DMF was cooled to 0 °C for 15 min. DCC (6.33 g, 30.72 mmol) and HOBt (4.15 g, 30.74 mmol) were added and the solution was stirred at 0 °C for 20 min. The dry **G2Co** was then added to the above mentioned solution, and the coupling was initiated by the addition of *N*-methylmorpholine (3.4 ml, 30.97 mmol). The solution was stirred at room temperature for 12 h. The reaction was quenched by the addition of 200 ml of water, and the reddish-pink solution containing the cobalt(III)-peptide (**Boc–G3Co)** was passed through a cation exchange (Sephadex SP-C25) column (10 × 5 cm), and washed with copious amounts of water. The pink cobalt complex adsorbed on the cation exchange column was eluted with 1 M NaCl in 1:2 ethanol/water solution. The pink eluent obtained was further purified by passing it through a preparative C_{18} (YMC) column (20 × 5 cm). The *N*-protected cobalt-peptide, **Boc–G3Co**, was washed with 40% ethanol/water solution, then eluted with 80% ethanol/water solution. The volatiles were then evaporated under vacuum to afford 12.1 g of **Boc–G2Co** (90% yield).

20. *Selected data for* **Boc–G3OH**: Crystalline solid, mp 100–120 °C; $[\alpha]_D = -27.5$ ($c = 1.0$ in CH_3OH); ^{1}H NMR (300 MHz, d_6-DMSO): δ 4.39 (m, 2H; COCHN), 4.24 (m, 1H; COCHN), 4.07 (m, 2H; COCHN), 3.97 (m, 2H; COCHN), 3.15 (m, 6H; CH_2N), 2.89 (m, 8H; CH_2N), 1.90–1.28 (m, 114H; CH_2 and CH_3); ^{13}C NMR: δ 173.3, 171.8, 171.5, 171.1, 156.1, 155.5, 155.2, 77.9, 77.2, 56.6, 55.9, 54.8, 54.5, 54.2, 52.0, 51.7, 33.2, 32.0, 31.4, 29.8, 29.1, 28.8, 28.7, 28.2, 28.1, 23.4, 22.7, 19.1, 18.5; HRMS (ESI) $[M+H]^+$: calculated for $C_{82}H_{151}N_{14}O_{24}$ $M = 1716.1026$: found *m*/*z* (%): 1716.1065 (20); $[M+2H]^{+2}$: calculated for $C_{82}H_{152}N_{14}O_{24}$ $M = 858.5526$: found *m*/*z* (%): 858.5438 (100).