The Synthesis of 1,4,7-Triazacyclononane Conjugated Amyloid-phillic Compound and Its Binding Affinity to the β-Amyloid Fibril

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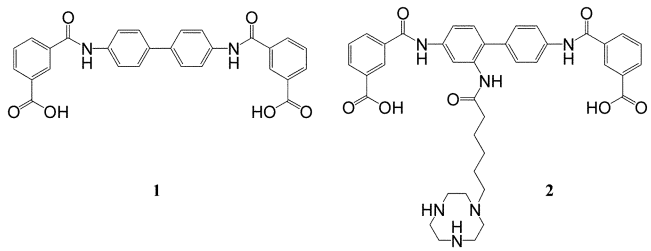
Received July 7, 2003

Key Words : Alzheimers disease, β-Amyloid fibril, Amyloid-philic molecule

The pathological hallmark of AD is a deposition of amyloid plaques in the brains of patients. β-amyloid protein is a major protein component of Alzheimer's plaques. When aggregated into amyloid fibrils, the peptide is toxic to neuronal cells.[1,2] The neurotoxicity of Aβ fibril is closely related to the β-sheet conformation and aggregation of Aβ peptide.[3] Various compounds which have affinity for β-amyloid fibril have shown ability of preventing neurotoxicity of β-amyloid fibril and inhibiting aggregation of β-amyloid fibril.[4] Therefore, the development of new compounds which have affinity for the β-amyloid fibril would lead to the new compounds that could have therapeutic effects on AD. Previously, we generated new amyloid-phillic amide derivative of Chrysamine G **1** and found that this compound protect human astrocyte cells against Aβ-induced toxicity.[5,6] As conjugation of amyloid-philic molecules with suitable metal chelating ligands could lead to new diagnostic molecules for *in vivo* quantification of amyloid deposition[7,8] and new probes for amyloid structure,[9,10] we designed the compound **2**, which was conjugate of 1,4,7-triazacyclononane and the amyloid-philic compound **1**. Here, we would like to report the synthesis of compound **2** and its binding property of β-amyloid fibril. The synthesis of compound **2** was achieved by combining three fragments the biphenyl amine **9**, isophthalic acid **5** and 1,4,7-triazacyclononane **20**.

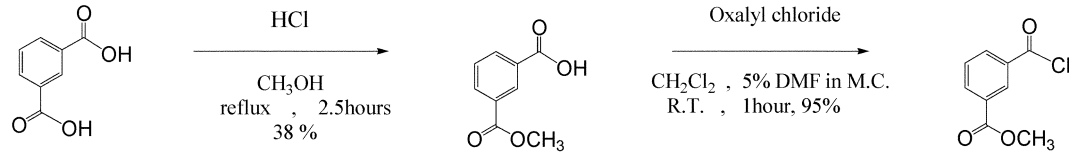


1 **2**

The synthesis of isophthalic acid part **5** commenced with esterfication of isophthalic acid in methanol with HCl to produce the monoester **4** in 38% yield. Treatment of the monoester **4** with oxalyl chloride afforded the acyl chloride **5** in 95% yield (Scheme 1).

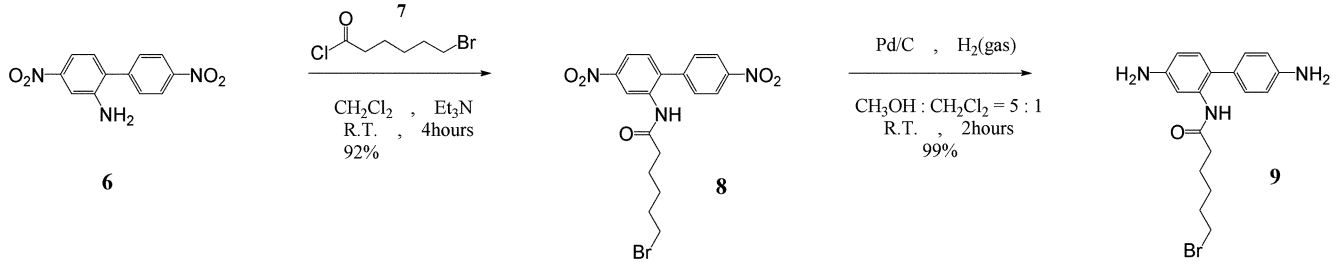
The synthesis of biphenyl amine part **9** began with the reaction between 4,4-dinitro-2-biphenylamine **6** and 6-bromohexanoyl chloride **7** in dried methylene chloride to afford compound **8** in 92% yield. Hydrogenation of the compound **8** at elevated pressure with Pd/C gave the biphenyl amine **9** in 99% yield (Scheme 2).

The synthesis of 1,4-bis(tert-butylcarbonate)-1,4,7-triazacyclononane **20** started from tosylation of diethylenetriamine **12**. Therefore, the reaction between diethylenetriamine and tosylchloride gave the tosylated diethylenetriamine **13** in 93 % yield. Then tosylated diethylenetriamine **13** was refluxed



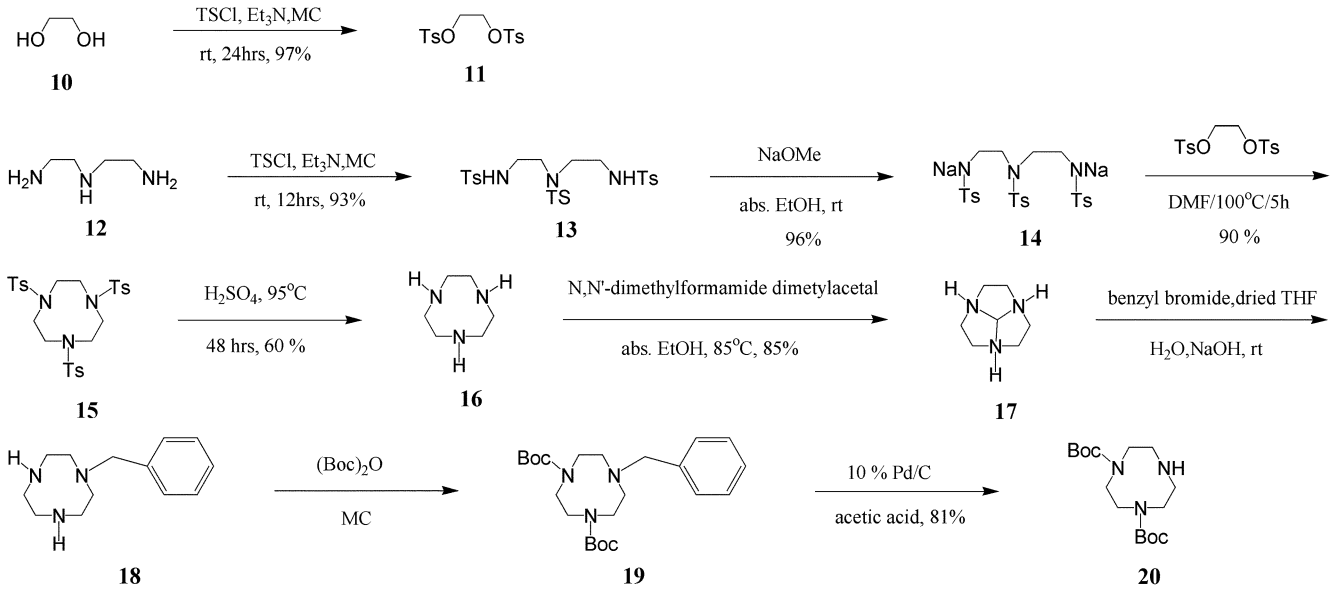
3 **4** **5**

Scheme 1



6 **8** **9**

Scheme 2

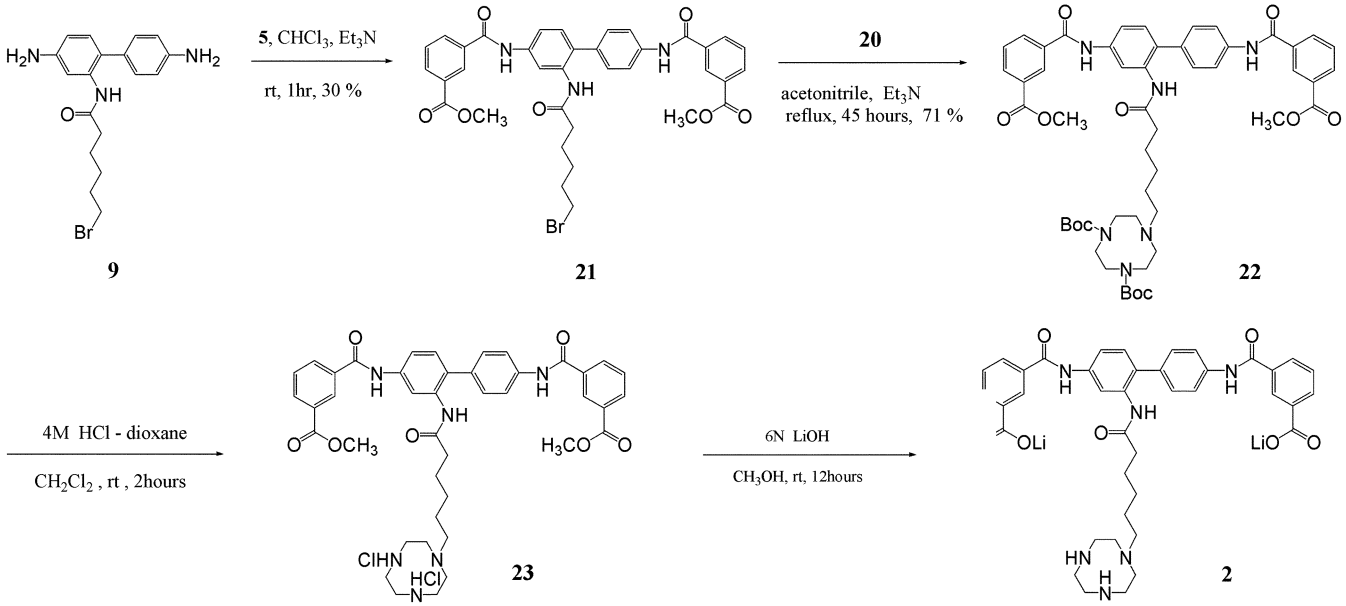


Scheme 3

in absolute ethanol with sodium methoxide to give sodium metallated compound **14** in 96% yield. Metallated compound **14** was reacted with tosylated ethylene glycol **11** in hot and dried DMF to give tritosylated-1,4,7-triazacyclononane **15** in 90% yield. Detosylation of the compound **15** was performed in hot sulfuric acid for 48 hours to give the 1,4,7-triazacyclononane **16** in 60% yield. The 1,4,7-triazacyclononane **16** was reacted with N,N'-dimethylformamide dimethylacetal to give the protected 1,4,7-triazacyclononane **17** in 85% yield. The protected 1,4,7-triazacyclononane **17** was reacted with benzyl bromide in dried THF to give the 1-benzyl-1,4,7-triazacyclononane **18** in quantitative yield. Then the compound **18** was reacted with dibutyldicarbonate in methylene chloride to give the compound **19** in quantitative yield. Debenzylation of the compound **19** with 10% Pd/C in acetic acid gave the desired 1,4-bis(tert-butylcarbonate)-1,4,7-triazacyclononane **20** in 81% yield (Scheme 3).

The coupling of three fragments was accomplished by the reaction of the biphenyl compound **9** and the acyl chloride **5** to afford the molecule **21** in 30% yield. Refluxing the compound **20** and **21** in acetonitrile gave the compound **22** in 71% yield. Deprotection reaction of **22** in 4 M HCl-dioxane and neutralization of the deprotected compound with 6 N LiOH gave the final compound **2** as a lithiated form (Scheme 4).

As lithiated salt form, the compound **2** was readily soluble in water. For the binding study, 80 μL of various concen-



Scheme 4

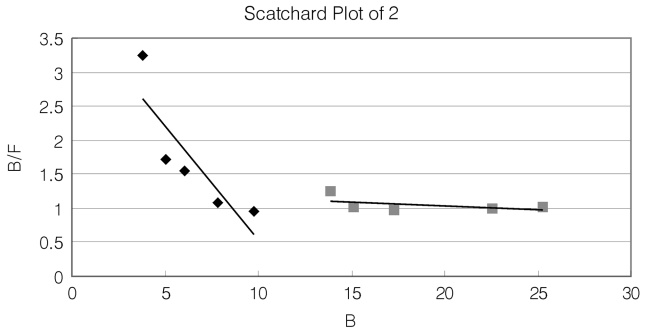


Figure 1. The Scatchard analysis of the compound **2** for the β-amyloid fibril 1-40.

trations of the compound **2** in water (5-100 μM) were added to the 20 μL of 100 μM β-amyloid 1-40 fibril. After 2 hours at 37 °C, the incubating eppendorf tubes were centrifuged (16,000 rpm, 20 min) to spin down the β-amyloid fibril and the compound bound to the β-amyloid fibril. Then the concentrations of unbound compound in the solution were measured by UV spectrometer at 290 nm. Figure 1. shows Scatchard analysis of the binding of **2** to the β-amyloid 1-40 fibril. Like compound **1**, **2** has two binding sites. The higher affinity binding site appears to have a K_d of 2.98 μM and a B_{max} of 0.58 moles per mole of amyloid 1-40 peptide. The lower affinity binding site appears to have a K_d of 88.50 μM and a B_{max} of 5.60 moles per mole of β-amyloid 1-40 peptide. As the compound **1** showed K_d = 0.13, B_{max} = 0.36 at higher affinity binding site and K_d = 10.11 B_{max} = 0.60 at lower affinity binding site, the compound **2** has comparable affinity with the compound **1** or Chrysamine G.[5,6] The coupling of 1,4,7-triazacyclononane to the compound **1** did not affect the binding affinity of the molecule **2**. The molecule **2** could be used to as a new diagnostic molecule for quantification of amyloid deposition or new probes for amyloid structure after metal chelation. The possibilities are currently under investigation.

Experimental Section

Monomethyl isophthalate 4. To a solution of 2 g isophthalic acid in 60 mL of methyl alcohol was added 3 drops of concentrated HCl and refluxed for 2.5 hours. After evaporation of methyl alcohol the reaction mixture was chromatographed on silicagel with 7% MeOH-MC to afford 839 mg product **4** in 38% yield. ^{1}H NMR (200 MHz, $CDCl_3$) 8.8 (t, 1H, *J* = 1.6) 8.3 (dd, 2H, *J* = 8.0, *J* = 1.6) 7.6 (t, 1H, *J* = 8.0) 4.0 (s, 3H) MS calculated for $C_9H_8O_4$ 180.1 found for 179.0 (M-H).

Monomethylester isophthaloyl chloride 5. To a solution of 730 mg **4** in 15 mL of dichloromethane was added 3.5 mL of oxalyl chloride and 150 μL of 5% DMF-MC. After stirring an hour, evaporation of all solvent and oxalyl chloride at reduced pressure produced the compound **5** in 95 % yield. ^{1}H NMR (200 MHz, $CDCl_3$) 8.6 (t, 1H, *J* = 1.6) 8.2 (m, 2H) 7.5 (t, 1H, *J* = 7.8) 3.9 (s, 3H).

Compound 8. To a solution of 2 g 4,4-dinitro-2-biphenylaniline and 1.3 mL triethylamine in 80 mL of dried dichloromethane was added 1.4 mL 6-bromohexanoyl chloride and stirred for 4 hours. Then the reaction mixture was extracted with 100 mL of water three times. The organic layer was dried with $MgSO_4$ and filtered. Evaporation of filtrate and silicagel chromatography (hexane : dichloromethane = 4 : 1) gave 550 mg of product **8** in 92% yield. ^{1}H NMR (200 MHz, $CDCl_3$) 9.1 (d, 1H, *J* = 2.2) 8.4 (d, 2H, *J* = 6.8) 8.1 (dd, 1H, *J* = 8.4, *J* = 2.2) 7.6 (d, 2H, *J* = 6.8) 7.4 (d, 1H, *J* = 8.4) 7.0 (s, 1H) 3.4 (t, 2H, *J* = 6.5) 2.3 (t, 2H, *J* = 7.1) 1.8 (quin, 2H, *J* = 7.0) 1.7 (quin, 2H, *J* = 8.0) 1.5 (quin, 2H, *J* = 6.6) MS calculated for $C_{18}H_{18}BrN_3O_5$ 435.0 found for 434.8.

Compound 9. A solution of 1g compound **8** and 250 mg 10% Pd/C in 20% methanol in dichloromethane was stirred with H_2 gas at elevated pressure for 2 hours. The solution was filtered through celite and the filtrate was evaporated to afford 862 mg product **9** in 99% yield. ^{1}H NMR (200 MHz, 5% CD_3OD-$CDCl_3$) 7.5 (d, 1H, *J* = 2.3) 7.0 (d, 2H, *J* = 8.3) 6.9 (d, 1H, *J* = 8.2) 6.7 (d, 2H, *J* = 8.3) 6.5 (dd, 1H, *J* = 8.2, *J* = 2.3) 3.3 (t, 2H, *J* = 5.7) 2.1 (t, 2H, *J* = 7.0) 1.7 (quin, 2H, *J* = 6.6) 1.5 (quin, 2H, *J* = 7.3) 1.3 (quin, 2H, *J* = 6.4) MS calculated for $C_{18}H_{22}BrN_3O$ 375.1 found for 296.1 (M-HBr).

Ditosyl ethylene glycol 11. To a solution of 29 g triethylamine and 55 g tosyl chloride in 200 mL of dichloromethane at 0 °C was added 8 g ethylene glycol for an hour. Then the reaction mixture was stirred for 24 hours. The reaction mixture was poured into 2 L water and extracted with 100 mL of dichloromethane 5 times. The organic layer was dried with $MgSO_4$ and filtered. Evaporation of dichloromethane produced 47 g of compound **11** in 97% yield. ^{1}H NMR (200 MHz, $CDCl_3$) 7.7 (d, 4H, *J* = 8.2) 7.3 (d, 4H, *J* = 8.2) 4.2 (s, 4H) 2.5 (s, 6H).

Tritosyl diethylenetriamine 13. To a solution of 25.2 g triethylamine and 8 g diethylenetriamine in 350 mL dichloromethane was added 45.8 g tosylchloride at 0 °C. Then the reaction mixture was stirred for 12 hours. Evaporation of the solvent and recrystallization in methanol gave 41.4 g of product **13** in 93% yield. ^{1}H NMR (200 MHz, $CDCl_3$) 7.6 (d, 4H, *J* = 8.2) 7.3 (d, 4H, *J* = 8.2) 5.1 (t, 2H) 3.1 (t, 8H *J* = 6.8) 2.4 (s, 9H).

Compound 14. To a solution of 32 g of the compound **13** in 310 mL ethanol was added 23 g of 28% MaOMe and refluxed for 2 hours. Filtration of the solid afforded the compound **14** in 96% yield. ^{1}H NMR (200 MHz, $CDCl_3$) 7.46 (d, 6H, *J* = 8.0) 7.28 (d, 2H, *J* = 8.0) 7.11 (d, 4H, *J* = 8.0) 2.83 (m, 4H) 2.62 (m, 4H) 2.83 (m, 4H) 2.62 (m, 4H) 2.34 (s, 3H).

Tritosyl 1,4,7-triazacyclononane 15. To a solution of 28 g of the compound **14** in 180 mL dried DMF at 100 °C was added 17 g of the compound **11** in 150 mL dried DMF for 2 hours. Then the reaction mixture was stirred for 2 hours more at 100 °C. Evaporation of the DMF at reduced pressure and recrystallization in water gave the 24.5 g of the compound **15** in 90% yield. ^{1}H NMR (200 MHz, $CDCl_3$) 7.65 (d, 6H, *J* = 8.0) 7.28 (d, 6H, *J* = 8.0) 3.40 (s, 12H) 2.41 (s, 9H).

1,4,7-Triazacyclononane 16. The solution of 25.6 g of the compound **15** in 55 mL concentrated H_2SO_4 was heated at 95 °C for 48 hours. Then the reaction mixture was slowly poured into 300 mL of cold diethyl ether and ethanol (1 : 1) The solid was filtered and dissolved in water and pH was adjusted until 8 with 6 N KOH. Then all of the water was evaporated and the residue was dissolved in small amount of methanol. The undissolved KOH was removed by filtration. This method gave the product **16** in 60% yield. ^{1}H NMR (200 MHz, $CDCl_3$) 2.7 (s, 12H) 1.9 (s, 1H).

Compound 17. To a solution of 0.2 g compound **16** in 18 mL dried acetonitrile was added 11 mL N,N'-dimethylformamide dimethyl actal. The reaction mixture was stirred for 3 hours at 85 °C. Evaporation of the solid gave 0.17 g of the product **17** in 85% yield. ^{1}H NMR (200 MHz, $CDCl_3$) 5.0 (s, 1H) 2.6-3.0 (m, 12H).

1-Benzyl-1,4,7-triazacyclononane 18. To a solution of 6.6 g of the compound **17** in 65 mL dried THF was added 6.7 mL benzyl bromide. Then the reaction mixture was stirred for an overnight. After the solvent was evaporated, 55 mL of water and 2 g NaOH was added to the reaction mixture and refluxed for 2 days. The reaction mixture was extracted with chloroform and the organic layer was dried with $MgSO_4$. Filtration and evaporation of the filtrate gave the product **18** in quantitative yield. The product obtained in this stage was crude. Without further purification this material was used for the next reaction.

1,4-Bis(tert-butylcarbonate)-7-benzyl-1,4,7-triazacyclononane 19. To a solution of 8.9 g of the compound **18** in 250 mL dichloromethane was added 35 g dibutyldicarbonate. Evaporation of the solvent and chromatography (hexane : ethyl acetate = 8 : 1) on silicagel gave product **19** in quantitative yield. ^{1}H-NMR (200 MHz, $CDCl_3$) 7.2 (m, 5H) 3.63 (s, 2H) 3.43 (t, 4H) 3.14 (m, 4H) 2.61 (m, 4H) 1.38 (s, 9H).

1,4-Bis(tert-butylcarbonate)-1,4,7-triazacyclononane 20. To a solution of 1 g of the compound **19** in 25 mL acetic acid was added 0.1 g 10% Pd/C and stirred under H_2 gas atmosphere for 3 hours. The reaction mixture was filtered through celite and the filtrate was evaporated. The residue was dissolved in small amount of water and pH was adjusted until 12 with 1 N NaOH. The water layer was extracted with 50 mL chloroform 3 times. The organic layer was dried with $MgSO_4$. Filtration and evaporation of the filtrate gave 0.6 g of the compound **20** in 81% yield. ^{1}H-NMR (200 MHz, $CDCl_3$) 3.47 (t, 4H) 3.32 (b, 4H) 2.97 (m, 4H) 1.47 (s, 9H).

Compound 21. To a solution of 1.0 g of the compound **9** and 1.1 mL triethylamine in 10 mL chloroform was added compound **5**. The reaction mixture was stirred for an hour. Evaporation of the solvent and chromatography on the silicagel (methanol : dichloromethane = 7 : 493) gave 578 mg of the product **21** in 30% yield. ^{1}H NMR (10% CD_3OD-$CDCl_3$) 8.5 (s, 2H) 8.0 (td, 4H, J = 7.8 J = 1.3) 7.9 (d, 1H, J = 2.0) 7.8 (dd, 1H, J = 8.4, J = 2.0) 7.7 (d, 2H, J = 8.5) 7.5 (td, 2H, J = 7.8, J = 1.3) 7.3 (d, 2H, J = 8.5) 7.2 (d, 1H, J = 8.4) 3.8 (s, 6H) 3.3 (t, 2H, J = 6.7) 2.2 (t, 2H, J = 7.1) 1.8 (quin, 2H, J = 7.3) 1.5 (quin, 2H, J = 7.3) 1.3 (quin, 2H, J = 5.9) MS calculated for $C_{36}H_{34}BrN_3O_7$ 699.1 found for 619.9 (M-Br).

Compound 22. To a solution of 550 mg of the compound **21** and 0.3 mL triethylamine in 20 mL of actonitrile was added 259 mg of the compound **20**. The reaction mixture was refluxed for 45 hours. Evaporation of the solvent and chromatography on the silicagel (4% methanol in dichloromethane) afforded 113 mg of the compound **22** in 71% yield. ^{1}H NMR ($CDCl_3$) 8.5 (d, 2H, J = 1.6) 8.2 (s, 1H) 8.1 (m, 4H) 7.9 (d, 2H, J = 8.2) 7.5 (td, 2H, J = 7.9, J = 1.6) 7.4 (d, 1H, J = 7.8) 7.3 (d, 2H, J = 8.2) 7.2 (d, 1H, J = 8.4) 3.9 (s, 6H) 3.4 (s, br, 4H) 3.1 (s, 4H) 2.5 (s, br, 4H) 2.4 (m, 2H) 2.2 (m, 2H) 1.5 (m, 6H) 1.4 (m, 18H) MS calculated for $C_{52}H_{64}N_6O_{11}$ 949.1 found for 950.1 ($M+H^+$).

Compound 23. 30 mg of the compound **22** was dissolved in 4 M HCl in dioxane (0.4 mL) and stirred for an hour. Evaporation of the solvent gave 26 mg of the product **23** in 99% yield. ^{1}H NMR (CD_3OD) 8.6 (t, 2H, J = 1.5) 8.2 (t, 4H, J = 7.7) 8.0 (d, 1H, J = 2.0) 7.8 (d, 2H, J = 8.6) 7.7 (td, 2H, J = 7.7, J = 1.5) 7.6 (d, 1H, J = 2.1) 7.5 (d, 2H, J = 8.6) 7.4 (d, 1H, J = 4.4) 4.0 (d, 6H, J = 0.8) 3.6 (s, 4H) 3.3 (4H) 3.0 (m, 4H) 2.8 (t, 2H, J = 7.9) 2.4 (t, 2H, J = 6.9) 1.7 (m, 4H) 1.3 (m, 2H).

Compound 2. To a solution of 20 mg of the compound **23** was added 60 mL of 6 N LiOH and stirred for an overnight. Filtration of the precipitated solid afforded 17 mg of lithiated product **2** in 96% yield. ^{1}H NMR (CD_3OD) 8.5 (m, 2H) 8.2 (d, 2H, J = 7.6) 8.1 (d, 2H, J = 7.6) 8.0 (s, 1H) 7.8 (d, 2H, J = 8.5) 7.7 (d, 1H, J = 8.8) 7.5 (t, 2H, J = 7.7) 7.45 (d, 2H, J = 6.6) 7.4 (d, 1H, J = 6.4) 2.6 (m, 12H) 2.3 (t, 2H, J = 7.3) 1.5 (m, 4H) 1.3 (m, 2H) MS calculated for $C_{40}H_{42}Li_2N_6O_7$ 732.3 found for 733.3 ($M+H^+$).

Acknowledgement. This study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea. (01-PJ1-PG3-21500-0017)

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