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Abstract:

Use of C_{60} as a protein scaffold is an interesting prospect for investigation of crystal structures of peptides. The synthetic route investigated aims to provide a hexakis template that allows for attachment of up to 12 peptides. Future potential exists to investigate Alzheimer's disease in which folding properties of its Aβ amyloid are elusive. Attachment of key peptides of Aβ amyloid to the hexakis adduct with obtaining its crystal structure could provide insight to the mis-folding and formation of Aβ amyloid in Alzheimer's disease.

Introduction:

Tertiary structures of proteins are determined by arranging to optimize favorable thermodynamic interactions with their environment. Due to the complexity of these folding interactions, it is difficult to predict or control the tertiary structure of a synthetic protein. Use of Template-assembled synthetic proteins (TASPs) provides a molecular template for peptide attachment as to control protein folding toward a specific synthetic goal [1]. Use of spherical C_{60} as a template provides an ability for attachment of up to 12 peptides for possible application in denovo protein synthesis, drug design, and protein function and structure studies [2]. Specifically Alzheimer Disease is of interest for the study of the toxicity of Aβ42 peptide which is believed a central neurotoxic agent in AD, though Aβ42 alone is unstable for isolation [3]. Use of a C_{60} protein scaffold to attach Aβ42 peptides theoretically exposes the amyloid's hydrophobic residues so that studies in its neurotoxicity can be investigated [2].

This project is focused to improve the yield and separation of a hexakis template that can support attachment of up to 12 peptides through coupling a Boc protecting group to amino acid residues. The reaction scheme goes by synthesis of a Boc protected malonate and then bingel reaction with C60.

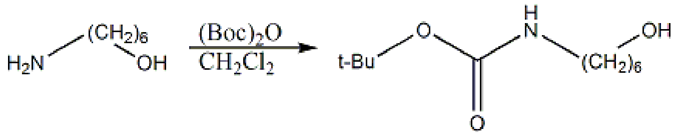
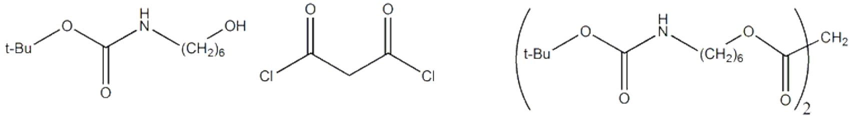
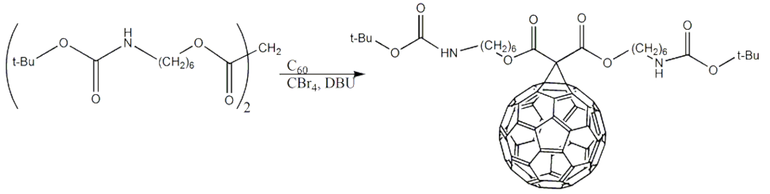
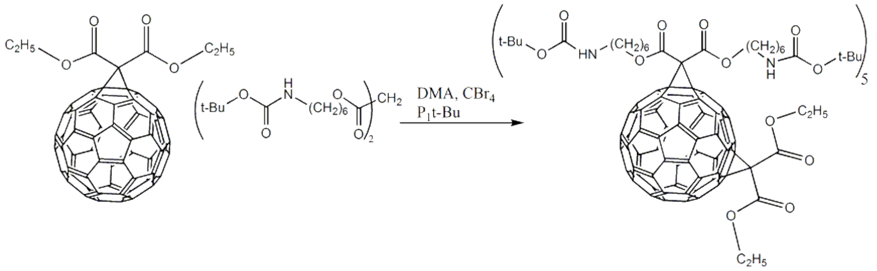
Step 1: Synthesis N-Boc-6-Amino-1-Hexanol (reaction 1 in experimental)	
Step 2: Synthesis Di(boc-aminohexyl) malonate (reactions 2,3, & 4 in experimental)	
Step 3: Synthesis C_{60} monoadduct di(boc-aminohexyl)malonate (reactions 5 &6 in experimental)	
Step 4: Synthesis 5:1 C_{60} hexakis adduct of di(boc-aminohexyl)malonate (5) and diethyl malonate (1). (reaction 7 in experimental)	

Table 1: Reaction Schemes

Experimental

General: Most reagents obtained from Sigma-Aldrich. Dry CH_2Cl_2 obtained by distilling under argon and CaH_2, pyridine distilled and dried by CaH_2, and dry toluene was distilled with Na and benzophenone under argon.

Reaction 1. Boc-6-amino-1-hexanol: Solution of di-tert-butyldicarbonate (10.0119g, 45mmol) in 20mL CH_2Cl_2 added dropwise over 30 minutes to a solution of 6-aminio-1-hexanol (5.048g , 43mmol) in 25mL CH_2Cl_2 under argon gas. Reaction mixture stirred at room temperature overnight. Workup differed significantly from reference[1] as reaction mixture was diluted with 50ml diethyl ether, washed with NH_4Cl (2x25ml), $NaHCO_3$ (25ml), saturated NaCl (25ml), and dried with Mg_2SO_4. Solvent was removed and yellow oil was recrystallized in pentane, vacuum filtered, and placed under hi-vac for 3 hours. 6. 3926g (29mmol) white powder at 70% yield. MP 37-38^{0}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.4 (m, 18H), 3.2 (m, 2H), 3.6 (t, 2H), 4.8 (s, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 26.3, 28.3, 29.9, 32.6, 40.3, 62.4, 76.7, 78.96, 156.04. FTIR (KBr) v(cm^{-1}) 1523 (s), 1687 (s) 2860 (m), 2935 (s), 2979 (m), 3369.03 (s)

Reaction 2. Di(Boc-aminohexyl) malonate: To a solution of 0.6668g N-Boc-6-amino-1-hexanol, 170µL of pyridine, and 30ml dry CH_2Cl_2 added 140µL (1.4mmol) malonyl dichloride in 10mL dry CH_2Cl_2 dropwise over one hour at 0˚C. Stirred at 0˚C for two hours and overnight at room temperature. Extracted with NH_4Cl (2x30mL), dH_2O (30mL), dried Mg_2SO_4, filtered, and removed solvent by rotavap. Flash chromatography with SiO_2 and 6:4 hexane/EtOAc eluent. Fractions 5-12 from column combined and evaporated to yellow oil. Impure by TLC and stored in CH_2Cl_2.

Reaction 3. Di(Boc-aminohexyl) malonate: Similar conditions as described in reaction 2 above with 0.6983g (3.2mmol) N-Boc-6-amino-1-hexanol and 0.14ml (1.439mmol) malonyl dichloride although pyridine was increased to 290ul (3.6mmol). Product was 0.6374g (1.27mmol) yellow oil at 88% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.4 (m, 32H), 3.1 (m, 4H), 3.4 (m, 2H), 4.1 (m, 4H), 4.6 (m, 2H). ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 25.4, 28.4, 29.9, 40.39, 41.6, 65.4, 79.0, 155.9, 166.6. FTIR (KBr) v(cm^{-1}) 3361 (m), 2975 (m), 2935 (s), 2861 (m), 1699 (s), 1521 (s), 1456 (w), 1392 (m), 1365 (m).

Reaction 4. Di(Boc-aminohexyl) malonate: Scale up of reaction 3 conditions with 500ml round bottom flask equipped with stirbar and addition funnel under argon gas with 4.4977g (21mmol) N-Boc-

6-amino-1-hexanol dissolved in 200ml dry CH_2Cl_2. 1.88mL (23mmol) pyridine added through additional funnel and rinsed with CH_2Cl_2. Solution of 0.91ml (9.3mmol) malonyl dichloride and 64ml dry CH_2Cl_2 added dropwise over 1.5 hours at 0^0C. Stirred at 0^0C for two hours and then stirred at room temperature overnight. Extracted with NH_4Cl (2x160ml) and dH_2O (1x160ml), dried with Mg_2SO_4, filtered, and evaporated solvent by rotavap. Flash chromatography with SiO_2 and 60/40: hexane/EtOAc eluent. Yellow oil 2.7072g (5.4mmol) 58.0% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.4 (m, 32H), 3.1 (m, 4H), 3.4 (m, 2H), 4.1 (m, 4H), 4.6 (m, 2H). ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 25.5, 28.4, 29.9, 40.4, 41.6, 65.4, 166.6.

Reaction 5. C_{60} monoadduct di(Boc-aminohexyl)malonate: In 100ml round bottom flask, combined 0.1086g (0.15mmol, 1 equiv) C_{60}, 0.1337g (0.27mmol, 1.5 equiv) di(boc-aminohexyl) malonate, 0.7695g (2.3mmol, 15 equiv) CBr_4, 60ml dry toluene, and stir bar. Applied argon gas and added 67.5ul (0.42mmol, 3 equiv) DBU by syringe and stirred overnight at room temperature. Quenched reaction by eluting reaction mixture through SiO_2 and glass filter with 5:1 CH_2Cl_2/EtOAc eluent. Removed solvent and re-dissolved in toluene. Flash chromatography used SiO_2 with toluene as C_{60} eluent, 20:1 CH_2Cl_2/EtOAc as monoadduct eluent, and 5:1 CH_2Cl_2/EtOAc as bisadduct eluent. Monoadduct fractions combined and evaporated by rotavap. Used minimal chloroform (<2ml) to transfer adduct to three centrifuge tubes, filled with pentane (5-7ml), centrifuged 3 minutes, and poured out pentane. Redissolved monoadduct precipitate in minimal chloroform by sonification, filled tube with pentane, centrifuged 3 minutes, poured out pentane, and repeated. Combined monoadduct centrifuge precipitates using chloroform and evaporated solvent by rotavap and hi-vac.

Monoadduct was 0.0798g (0.065mmol) at 43.3% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.4 (m), 3.1 (m), 4.5 (m), 7.3 (s). ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 25.7, 26.4, 28.5, 30.1, 40.5, 67.3, 71.6, 76.7, 77.0, 77.3, 129.1, 139.0, 140.9, 141.9, 142.2, 143.0, 143.1, 143.9, 144.6, 144.7, 144.7, 144.9, 145.2, 145.2, 145.3, 163.7. Same centrifugation steps were followed for bisadduct flash chromatography fractions to get 0.0706g (0.041mmol) at 27.2% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.5 (m), 1.6 (s), 3.1 (m), 4.4 (m), 7.3 (m). ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 25.7, 26.4, 28.5, 30.0, 67.2, 76.7, 77.0, 77.2, 77.3, 156.0.

Reaction 6. C_{60} monoadduct di(Boc-aminohexyl)malonate. Similar reaction conditions as described in reaction 5 above with a decrease in CBr_4 to use 0.1075g C_{60} (0.15mmol, 1 equiv), 0.1123g malonate (0.22mmol, 1.5 equivalent), 0.0812g CBr_4 (0.25mmol, 1.6 equiv), 60ml toluene, and 67ul (0.42mmol, 3 equiv) DBU. The reaction time was monitored with TLC and quenched at 5 hours and the centrifugation step followed in reaction 5 was not performed.

0.0285g (0.040mmol) of unreacted C_{60} was recovered from the column and was taken into account in yield calculation in which C_{60} limiting reagent is 0.109mmol. Monoadduct isolated was 0.0808g (0.066mmol) at 61% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.2 (m), 1.4 (m), 1.5, 1.8, 2.0, 3.1, 4.5, 7.2. ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 25.7, 26.4, 28.5, 30.0, 67.3, 71.6, 75.7, 77.0, 77.4, 139.0, 141.0, 141.9, 142.2, 143.0, 143.1, 144.6, 144.7, 144.9, 145.2, 145.3, 163.6. MS (MALDI-TOF), m/z: 730.1, 947.1, 1065.2, 1244.4 [M^+]; MW=1221.21g/mol.

Bisadduct isolated was 0.0679g (0.039mmol) at 36.2% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.3, 1.4, 1.6, 3.1, 3.4, 4.1, 7.3. ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 25.5, 26.4, 28.4, 30.0, 40.5, 41.6, 65.5, 76.4, 77.0, 77.4, 79.0, 156.0, 166.7. MS (MALDI-TOF), m/z: 721.0, 730.1, 947.1, 1322.4, 1744.6 [M^+]; MW=1721.9g/mol.

Reaction 7. 5:1 C_{60} hexakis adduct of di(boc-aminohexyl)malonate (5) and diethyl malonate (1).

A solution of 0.0501g (0.057mmol) diethyl malonate C_{60} monoadduct and 20ml dry CH_2Cl_2 was prepared in 100ml round bottom flask with addition funnel and argon gas. 0.1169g (0.57 mmol, 10 equiv) in 15ml CH_2Cl_2 added through addition funnel and stirred for two hours in the dark. A solution of 0.2905g (0.58 mmol, 10 equiv) di(boc-aminohexyl)malonate, 0.1904g (0.57mmol, 10 equiv) CBr_4, and 6ml CH_2Cl_2 were added through addition funnel and stirred for 20 minutes. 217 ul (0.85mmol, 15 equiv) of phosphazene base in 20ml CH_2Cl_2 was added dropwise over one hour and then stirred for two days in the dark. Extracted NH_4Cl (2x40ml) and dH_2O (1x40ml), dried Mg_2SO_4, and filtered. Flash chromatography with CH_2Cl_2 load and 4:1 CH_2Cl_2 eluent. Dissolved in minimal chloroform and precipitated with hexane and centrifugation. 0.1312g (0.039mmol) 68% yield. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.282, 1.296, 1.306, 1.394, 1.651, 1.662, 3.051, 3.060, 4.203, 4.26, 4.228, 4.274, 4.89, 4.303, 4.782. ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 13.9, 24.4, 25.5, 26.3, 27.7, 28.2, 28.3, 29.8, 32.3, 40.4, 41.6, 45.3, 45.4, 62.8, 66.7, 66.9, 68.3, 68.5, 69.0, 69.9, 70.0, 70.7, 76.7, 77.0, 77.2, 78.8, 141.0, 141.9, 142.1, 142.8, 145.6, 145.6, 145.9, 147.7, 155.9, 162.9, 163.3, 163.5, 163.6, 163.7, 163.9, 164.1. MS (MALDI-TOF), m/z: 721.2, 1039.9, 1280.8, 2380.5, 2385.9, 2901.2, 346.2, 3402.2, 3418.1, [M^+]; MW=3881.99g/mol.

Reaction 8 Monoadduct diethyl malonate: To a solution of 8.5122g (0.71 mmol, 1 equiv) C_{60}, 0.1751g diethyl malonate (1.1 mmol, 1.5 equiv), 0.3576g CBr_4 (1.1mmol, 1.5 equiv), and 50mL distilled OCDB, added 319μL of DBU (2.1mmol, 3 equiv) and stirred overnight. Quenched reaction mixture with 156μL TFA (2.1 mmol). Flash chromatography and precipitate by centrifugation in hexane. Impure and incorrect fraction collected.

Reaction 9 monoadduct di(Boc-aminohexyl)malonate: Prepared a solution of 0.1096g C_{60} (0.15mol), 0.0800g CBr_4 (0.24 mmol), and 60ml dry toluene under argon gas at 0˚C. Added 34.6μL diethylmalonate (0.23mmol), stirred at room temperature in the dark for two hours, and added 68.2μL

DBU (0.46mmol). Stirred for six hours in the dark and quenched reaction with 1mL TFA. Flash chromatography and precipitation in hexane and centrifugation. 0.0667g (0.077mmol) 51% yield.

Reaction 10. 5:1 C_{60} hexakis adduct of di(boc-aminohexyl)malonate (5) and diethyl malonate (1): A solution of 30mL dry DCM, 0.0350g (0.040mmol) diethyl malonate monoadduct, and 0.0831g (0.40mmol) DMA was stirred in the dark for 3 hours. Added 0.2086g di(boc-aminohexyl)malonate (0.42mmol), and 0.1406g CBr_4 (0.42 mmol), and 8ml dry DCM and stirred for ten minutes. A solution of 90μL DBU (0.60 mmol) in 20mL dry DCM was added dropwise for 30 minutes and stirred at room temperature in the dark for two days. Flash chromatography (fraction #1 45mg, #2 45mg, #3 20mg, #4 17mg, #5 23mg).

Literature Cited:

1. Mutter, M., Template-assembled synthetic proteins (TASPS) containing two folding domains, *Pept. Synth*. **1989**, *193, 193.*
2. Suzuki, M.; Rubin, Y., First Year Graduate Research Report, Toward Template-Assembled Synthetic Proteins Based on C_{60} Scaffolds
3. Gal B., Marina K., Aleksey L., Sabrina V., George B., and David T., Amyloid β-protein (Aβ) assembly: Aβ40 and Aβ42 oligomerize through distinct pathways, **2003** *100:330-335*
4. Bergbreiter, D. E.; Osburn, P. L.; Li, C. Soluble Polymer-Supported Catalysts Containing Azo Dyes. *Org. Letters.* ***2002***, *4*, 737
5. Chronakis, N.; Hartnagel, U.; Braun, M.; Hirsch, A. A chiral dumbbell shaped bis(fullerene) oligoelectrolyte. *Chem. Comm.* **2007**, *607*
6. Camps, X.; Hirsch, A. Efficient cyclopropanation of C_{60} starting from malonates. *J. Chem. Soc.*, **1997**, *1595*