

A Simple Quick Route to Fullerene Amino Acid Derivatives[†]

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Supplementary Information

Experimental

Protected amino acids were purchased from Chem-Impex International, Inc., unless otherwise specified, and used without further purification. Fullerene (99.5 % purity) was purchased from MER Corp. and was purified chromatographically with toluene prior to use to remove trace C₆₀ oxides. All other chemicals and reagents were purchased from Sigma-Aldrich. All solvents were dried and distilled prior to use using standard techniques unless otherwise specified. When necessary, products were purified by flash column chromatography on silica gel (230-400 mesh). ¹H spectra were recorded on Bruker Avance 400 and 500 MHz spectrometers. MALDI measurements were performed on Bruker Reflex with TOF detection. DCTB (2-[(2E)-3-(4-tert-butylphenyl)-2-methylprop-2-enylidene]malononitrile) and elemental S were used as the matrices to characterize all fullerene derivatives by MALDI/TOF (negative mode). UV-Vis spectra were recorded on a Varian Cary 5000 spectrometer. Cyclic voltammetry data was recorded on CV-50W Voltammetric Analyzer.

The pure product is obtained by filtration through a large silica plug, eluting with toluene in hexanes (4:1) to remove un-reacted C₆₀ followed by a stepped gradient to elute the product. Detailed purification notes to follow. The purification of the aza-fulleroids is a significant improvement over **Baa**¹, in both time and solvent consumption. Also, 70% of the original C₆₀ is recovered. The purity of the product off the column is sufficient for SPPS protocols.

General diazo-transfer reaction

Imidazole-sulfonyl-azide·HCl (ISA·HCl)¹⁶ was used as the diazotransfer reagent. The ISA·HCl was the superior diazotransfer reagent as it is crystalline, easily prepared on the gram scale, and has a long shelf life as opposed to the customary triflyl azide. The subsequent diazo-transfer takes place with a divalent catalyst and a base at room temperature. The choice of catalyst and base were substrate dependant.

Detailed synthesis notes:

Boc-Phe(4-N₃)-OH

1.2 equiv. of ISA·HCl (1.25 g, 6 mmol), 1 equiv. of Boc-Phe(4-NH₂)-OH (1.40 g, 5 mmol), and 4 mole % of ZnCl₂ (27.3 mg, 0.2 mmol) were dissolved in MeOH:MeCN (3:2, 300 mL) in a 500 mL Schlenk flask. Then 2 equiv. of triethylamine (1.39 mL, 10 mmol) were added. The reaction

was stirred in the dark at room temperature for 12 hrs and monitored by thin film IR and TLC. Upon completion, the reaction was concentrated, diluted with diethylether and washed with AcOH (10%, 3x) and brine (1x), dried with MgSO₄, filtered and concentrated. At the gram scale, flash chromatography was used to yield the pure product. Yield: 1.3 g, 85 %; ¹H NMR (400 MHz, CDCl₃): δ 7.07 [2H, d, J(H-H) = 8.97 Hz, Ar-H], 6.97 [2H, d, J(H-H) = 8.63 Hz, Ar-H], 4.93 [1H, d, J(H-H) = 8.63 Hz, NH], 4.58 [1H, dd, J(H-H) = 6.64, J(H-H) = 14.92 Hz, αCH], 3.18 [1H, dd, J(H-H) = 5.12, J(H-H) = 13.92 Hz, CH₂], 3.05 [1H, dd, J(H-H) = 6.41, J(H-H) = 13.64 Hz, CH₂], 1.42 [9H, s, t-Bu]; IR ν_{N3} = 2115 cm⁻¹.

Fmoc-Phe(4-N₃)-OH

1.2 equiv. of ISA·HCl (1.25 g, 6 mmol), 1 equiv. of Fmoc-Phe(4-NH₂)-OH (2.01 g, 5 mmol), and 4 mole % of ZnCl₂ (27.3 mg, 0.2 mmol) were dissolved in MeOH:MeCN (3:2, 300 mL) in a 500 mL Schlenk flask. Next 2 equiv. of triethylamine (1.39 mL, 10 mmol) were added. The reaction was stirred in the dark at room temperature for 12 hrs and monitored by thin film IR and TLC. Upon completion, the reaction was concentrated, diluted with diethylether and washed with AcOH (10%, 3x) and brine (1x), dried with MgSO₄, filtered and concentrated. At the gram scale, flash chromatography was used to yield the pure product. [10 -25 % ethyl acetate (with 1% AcOH) in hexanes]. Yield: 1.71 g, 80 %; ¹H NMR (400 MHz, CDCl₃): δ 7.78-7.32 [8H, m, Ar-H (Fmoc)], 7.09 [2H, d, J(H-H) = 8.1 Hz, Ar-H (Phe)], 6.93 [2H, d, J(H-H) = 8.55 Hz, Ar-H (Phe)], 5.24 [1H, d, J(H-H) = 8.64, NH], 4.64 [1H, dd, J(H-H) = 4.68, J(H-H) = 12 Hz, αCH], 4.47 [1H, dd, J(H-H) = 7.03, J(H-H) = 11.0 Hz, CH₂ (Fmoc)], 4.37 [1H, dd, J(H-H) = 6.85, J(H-H) = 10.89 Hz, CH₂ (Fmoc)], 4.19 [1H, t, J(H-H) = 6.81, CH (Fmoc)], 3.18 [1H, dd, J(H-H) = 5.39, J(H-H) = 14.24, αCH-CH₂-Ar], 3.06 [1H, dd, J(H-H) = 6.07, J(H-H) = 14.29, αCH-CH₂-Ar]; IR ν_{N3} = 2113 cm⁻¹.

Boc-Lys(N₃)-OH

1.2 equiv. of ISA·HCl (1.43 g, 8.4 mmol), 1 equiv. of Boc-Lys(4-NH₂)-OH (1.72 g, 7 mmol), and 4 mole % of ZnCl₂ (27.3 mg, 0.2 mmol) were dissolved in MeOH:MeCN (3:2, 300 mL) in a 500 mL Schlenk flask. Next 2 equiv. of triethylamine (1.39 mL, 10 mmol) were added. The reaction was stirred in the dark at room temperature for 12 hrs and monitored by thin film IR and TLC. Upon completion, the reaction was concentrated, diluted with diethylether and washed with AcOH (10%, 3x) and brine (1x), dried with MgSO₄, filtered and concentrated. At the gram scale, flash chromatography was used to yield the pure product (0-3% MeOH in dichloromethane). Yield: 1.5 g, 80 %; ¹H NMR (400 MHz, CDCl₃): δ 5.15 [1H, d, J(H-H) = 8.59, NH], 4.33 [1H, dd, J(H-H) = 6.72, J(H-H) = 12.8 Hz, αCH], 3.29 [2H, t, J(H-H) = 7.43 Hz, CH₂-N₃], 1.90 [1H, m, αCH-CH₂], 1.72 [1H, m, αCH-CH₂], 1.64 [2H, m, CH₂], 1.51 [2H, m, CH₂], 1.45 [9H, s, t-Bu]; IR ν_{N3} = 2099 cm⁻¹.

Aza-C₆₀-Boc-Phe (1a)

In a 500 mL Schlenk flask, 1 equiv. of C₆₀ (1 g, 1.38 mmol) and 1 equiv. of the Boc-protected azido Phe (425.39 mg, 1.38 mmol) were vacuum dried for 45 min., then 350 mL of dry ODCB was added. The reaction temperature was maintained around 160 °C and monitored by RP HPLC for the formation of the multiple addition products (usually within 4 hours). Due to loss of product from precipitation, no work-up was performed. The product was purified by filtering through a large SiO₂ plug followed by stepped-gradient elution with Tol, followed by 5% EA in Tol, followed by 2.5% EA/1% AcOH in Tol to elute the product. The pure isomers may be obtained by increasing the length of the column. Yield: 371.8 mg, 27 %; ¹H NMR (400 MHz, CDCl₃): δ 7.53 [2H, d, J(H-H) = 8.35 Hz, Ar-H], 7.24 [2H, d, J(H-H) = 8.97 Hz, Ar-H], 5.02 [1H, d, J(H-

H) = 7.47 Hz, *NH*], 4.62 [1H, m, α CH], 3.22 [1H, dd, $J(\text{H-H}) = 5.71$, $J(\text{H-H}) = 12.74$ Hz, CH_2], 3.15 [1H, dd, $J(\text{H-H}) = 7.91$, $J(\text{H-H}) = 13.67$ Hz, CH_2], 1.42 [9H, s, t-Bu] MALDI/TOF, DCTB matrix, (M-) requires m/z 998.13, found m/z , 998.16

Note: The ratio of open to closed isomers can be shifted to approximately 1:1 if the reaction is performed at 160-180°C but the overall yield is still around 30%. Under these conditions the reaction is complete within 4 hours with less formation of multiple addition products.

Aza-C₆₀-Fmoc-Phe (2a)

In a 500 mL Schlenk flask, 1 equiv. of C₆₀ (1.68 g, 2.3 mmol) and 1 equiv. of the Fmoc-protected azido Phe (1.0 g, 2.3 mmol) was vacuum dried for 45 min., then 350 mL of dry ODCB was added. The reaction temperature was maintained around 80 °C and monitored by RP HPLC for the formation of the multiple addition products (usually in 24 hours). Due to loss of product from precipitation, no work-up was performed. The product was purified by filtering through a large SiO₂ plug followed by stepped-gradient elution with Tol (150 mL), followed by 5% EA/0.5 % AcOH in Tol (~500 mL) to elute the aza product and 5% EA/1.5% AcOH in Tol (~500 mL) to elute the closed product. Yield: 825 mg, 32 %; ¹H NMR (400 MHz, CDCl₃): δ 7.78-7.32 [8H, m, Ar-*H* (Fmoc)], 7.10 [2H, d, $J(\text{H-H}) = 7.99$ Hz, Ar-*H* (Phe)], 6.93 [2H, d, $J(\text{H-H}) = 7.81$ Hz, Ar-*H* (Phe)], 5.19 [1H, d, $J(\text{H-H}) = 7.06$, *NH*], 4.66 [1H, m, α CH], 4.47 [1H, m, CH₂ (Fmoc)], 4.39, [1H, m, CH₂ (Fmoc)], 4.20 [1H, t, $J(\text{H-H}) = 6.75$, CH (Fmoc)], 3.19 [1H, dd, $J(\text{H-H}) = 4.93$, $J(\text{H-H}) = 13.88$, α CH-CH₂-Ar], 3.06 [1H, dd, $J(\text{H-H}) = 4.79$, $J(\text{H-H}) = 13.97$, α CH-CH₂-Ar]; MALDI/TOF, DCTB matrix, (M-) requires m/z 1120.14, found m/z 1120.23.

Aza-C₆₀-BOC-Lys (3a)

In a 500 mL Schlenk flask, 1 equiv. C₆₀ (1.0 g, 1.39 mmol) and 1 equiv. of the Boc-protected azido Lys (272.15 mg, 1.39 mmol) were vacuum dried for 45 min. then 200 mL of dry ODCB was added by cannula. The reaction temperature was kept around 60 °C throughout and monitored by RP HPLC for the formation of the multiple addition products (usually within 72 hours). Due to loss of product from precipitation, no work-up was performed. The product was purified by filtering through a large SiO₂ plug, followed by stepped gradient elution with Tol to remove unreacted C₆₀, then dichloromethane (DCM). Next DCM:Tol (3:2) +0.5% AcOH for the aza-derivative, followed by DCM:Tol (4:1) + 1% AcOH for the aziridino product. Yield: 187.5 mg, 14 %; MALDI/TOF, DCTB matrix, (M) requires m/z 964.14, found m/z 964.06. ¹H NMR(**3a**) (500 MHz, CDCl₃): δ 5.09 [1H, d, $J(\text{H-H}) = 7.18$, *NH*], 4.43 [1H, m, α CH], 3.81 [2H, t, $J(\text{H-H}) = 7.20$ Hz, CH₂-N], 2.06 [1H, m, CH₂], 1.89 [1H, m, CH₂], 1.82 [2H, m, CH₂], 1.57 [2H, m, CH₂], 1.48 [9H, s, t-Bu of Boc]; ¹H NMR(**3b**) (500 MHz, CDCl₃): δ 5.09 [1H, d, $J(\text{H-H}) = 7.76$, *NH*], 4.42 [1H, m, α CH], 3.69 [2H, t, $J(\text{H-H}) = 6.90$ Hz, CH₂-N], 2.05 [2H, m, CH₂], 1.91 [2H, m, CH₂], 1.82 [2H, m, CH₂], 1.62 [2H, m, CH₂], 1.48 [9H, s, t-Bu of Boc].

This compound has a tendency to stick on a SiO₂ column. The yield can be improved by precipitating the product with hexanes, then washing the precipitate (3x, 4:1 Hex:Tol) to remove C₆₀. The pellet was then dissolved in CHCl₃ and filtered through a SiO₂ plug. Elute with DCM, then with toluene. Use 4:1 DCM:Tol + 1 % AcOH to elute both isomers.

Deprotection of Aza-C₆₀-Boc-Phe (4)

The Boc protecting group on compound **1a** (25 mg, 0.02 mmols) was removed by 25 % TFA in dry dichloromethane. The residue was stirred with an excess of the reagent for 6-8 hours. Cold diethylether (Et₂O) was added to the vial and the product was allowed to precipitate in the freezer

overnight. The precipitate was washed with Et₂O (3x) and centrifuged. Yield: 16.2 mg, 90 %; ¹H NMR (500 MHz, DMSO-d₆): δ 8.28 2H, bs, NH₂, 7.67 [2H, d, J(H-H) = 8.54 Hz, Ar-H], 7.67 [2H, d, J(H-H) = 8.66 Hz, Ar-H], 4.21 1H, m, αCH, 3.15 [1H, dd, J(H-H) = 5.82 Hz, J(H-H) = 13.98 Hz, CH₂], 3.09 [1H, dd, J(H-H) = 7.05 Hz, J(H-H) = 13.74 Hz CH₂]. MALDI/TOF, (M) requires m/z 898.07, found m/z 898.02.

Aza-C₆₀-Phe-Gly-OtBu (5)

The Fmoc-aza-C₆₀-Phe (2a) derivative was coupled to t-butyl ester protected glycine to determine its reactivity. The coupling was performed in anhydrous DMF with 1 equiv. of HBTU and 2 equiv. of triethylamine under inert conditions. After 2 hours, MALDI/TOF (CHCA matrix) showed only starting materials and product formation at m/z 1234.11. Calculated (M + 1): m/z 1234.23.

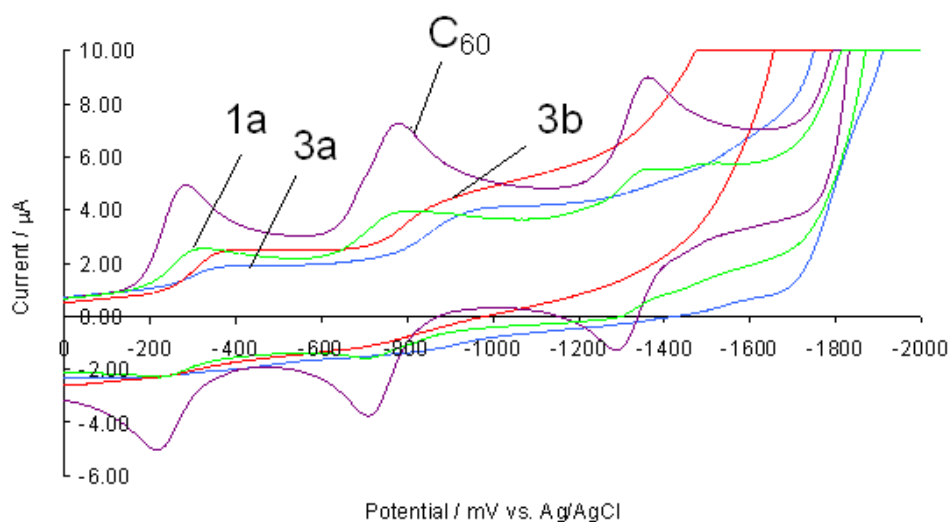


Figure S1. Cyclic voltammograms of C₆₀ (purple), aza-C₆₀-Boc-Lys (blue, **3a**), and aziridino-C₆₀-Boc-Lys (red, **3b**), and aza-C₆₀-Boc-Phe (green, **1a**) in 0.1 M [TBA][BF₄] in DMF/toluene (3:2) at room temperature. Scan rate, 100 mV s⁻¹.