Electronic Supplementary Information

Oxidative damage of aromatic dipeptides by the environmental oxidants NO_2• and O_3

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Table of Contents

1. Experimental Section

1.1 General procedures

^{1}H and ^{13}C spectra were recorded on a Varian Unity Inova 500 spectrometer [499.688 MHz (^{1}H), 125.646 (^{13}C)] in deuterated dimethylsulfoxide (DMSO-d_6) or deuterated chloroform ($CDCl_3$). If possible, the assignment of the chemical shifts was confirmed through 2D NMR techniques, including HSQC and COSY.

HR-MS was conducted by ionising the samples via ESI into a Thermo-Finnigan LTQ FT-ICR hybrid mass spectrometer or an Agilent 6520 LC/Q-TOF mass spectrometer with an electrospray ionizing source coupled to an Agilent 1100 LC system.

The crude products were purified by reverse-phase HPLC (Phenomenex C18, 150 × 21.2 mm, 5 micron, preparative column, 8 ml/min) using an Agilent 1100 LC system by running a gradient from 0.1 % TFA in water to 0.1 % TFA in acetonitrile within 2-3 hours. Purity was assessed by analytical RP HPLC on a Phenomenex Aeris Peptide XB-C18 3.6um 100Å 250 × 4.6 mm column (Gradient: 100% water buffered with 0.1% TFA to 100% acetonitrile buffered with 0.1% TFA over 25 minutes, 4%/min, 1mL/min).

1.1.1 General procedure for the methylation of amino acids

Thionyl chloride (8 mL, 110 mmol) was added dropwise to a stirred suspension of the amino acid (63 mmol) in methanol (200 mL) at 0^{o}C. The reaction mixture was stirred overnight at room temperature, followed by removal of the solvent by rotary evaporation to give the amino acid as the methyl ester hydrochloric acid salt.

1.1.2 General procedure for the peptide coupling

The *N*-acetyl protected amino acid (10 mmol), amino acid methyl ester hydrochloride salt (10 mmol) and *N,N,N′,N′*-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) (4.17 g, 11 mmol) were suspended in anhydrous DMF (15 mL) and cooled to 0ºC. Triethylamine (4.2 mL, 30 mmol) was added slowly and the reaction mixture warmed to room temperature. After stirring overnight, the mixture was partitioned between 1M HCl (100 mL) and ethyl acetate (100 mL). The aqueous phase was extracted with ethyl acetate (2 x 100 mL), and the combined extracts were washed with aq. $NaHCO_3$ (5% w/v, 100 mL) followed by brine (100 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to give a sticky foam. The residue was purified by silica chromatography or recrystallisation in ethyl acetate.

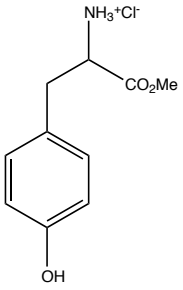
1.1.3 General procedure for the reaction with nitrogen dioxide and ozone

The reactions were performed at +10^{o}C under exclusion of moisture in acetonitrile (100 mL) by adding a measured excess amount of liquid NO_2 (0.5 mL) to the amino acid or peptide (1 mmol), with a stream of O_3 in O_2 passing through the solution. Consumption of the amino acids was usually complete after 20 mins of reaction time. After quenching with aqueous saturated

sodium bicarbonate solution (50mL), the reaction products were isolated and purified by preparative HPLC, followed by NMR and ESI-MS analysis. In addition, control reactions were performed under identical conditions, where the amino acids were separately treated with either $NO_{2(l)}/N_2O_4$ or an O_2/O_3 mixture, respectively.

1.2 Synthesis of starting materials

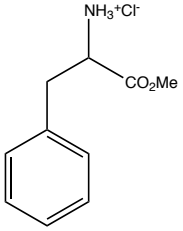
L-Tyrosine methyl ester hydrochloride



The compound was prepared according to the general procedure for the methylation of amino acids. The residue was dried under high vacuum to give the hydrochloride salt of L-tyrosine methyl ester (14.3 g, 98 %) as an off-white solid.

^{1}H NMR (600 MHz, DMSO-d_6) δ 9.51 (s, 1H, O*H*), 8.65 (s, 3H, NH_3), 7.00 (d, *J* = 8.4 Hz, 2H, 2,6-*H*), 6.72 (d, *J* = 8.4 Hz, 2H, 3,5-*H*), 4.12 (dd, *J* = 7.4, 5.5 Hz, 1H, C*H*), 3.65 (s, 3H, COOCH_3), 3.07 (dd, *J* = 14.1, 5.7 Hz, 1H, CH_2), 2.99 (dd, *J* = 14.1, 7.2 Hz, 1H, CH_2).

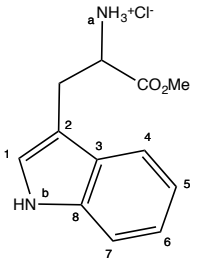
L-Phenylalanine methyl ester hydrochloride



The compound was prepared according to the general procedure for the methylation of amino acids. The residue was dried under high vacuum to give the hydrochloride salt of L-phenylalanine methyl ester (13.4 g, 99%) as an off-white solid.

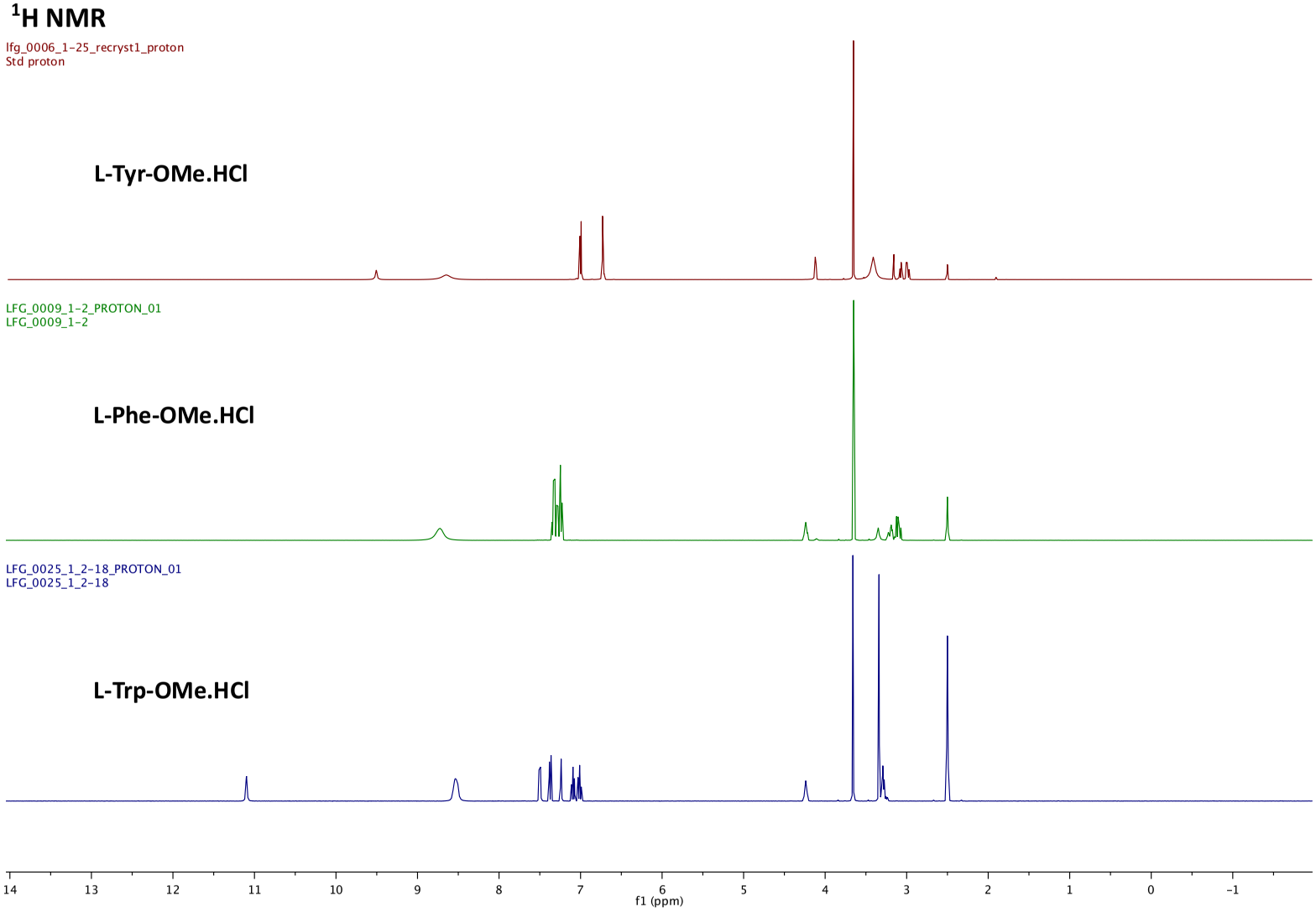
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.73 (s, 3H, NH_3), 7.38 – 7.21 (m, 5H, Ar-*H*), 4.24 (ddd, *J* = 7.3, 5.7, 3.0 Hz, 1H, C*H*), 3.65 (s, 3H, COOCH_3), 3.20 (ddd, *J* = 14.0, 5.7, 2.8 Hz, 1H, CH_2), 3.10 (dd, *J* = 14.0, 7.5 Hz, 1H, CH_2).

L-Tryptophan methyl ester hydrochloride

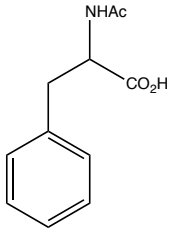


The compound was prepared according to the general procedure for the methylation of amino acids. The residue was dried under high vacuum to give the hydrochloride salt of L-tryptophan methyl ester (11.7 g, 99%) as an off-white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.10 (s, 1H, N_b*H*), 8.53 (d, *J* = 11.7 Hz, 3H, $N_a$$H_3$), 7.50 (d, *J* = 7.9 Hz, 1H, 4-*H*), 7.37 (d, *J* = 8.5 Hz, 1H, 7-*H*), 7.24 (d, *J* = 2.7 Hz, 1H, 1-*H*), 7.09 (t, *J* = 7.3 Hz, 1H, 5-*H*), 7.01 (t, *J* = 7.4 Hz, 1H, 6-*H*), 4.23 (td, *J* = 6.2, 3.2 Hz, 1H, C*H*), 3.66 (s, 3H, COOCH_3), 3.32 – 3.22 (m, 2H, CH_2).


^{1}H NMR
lfg_0006_1-25_recryst1_proton
Std proton
L-Tyr-OMe.HCl
LFG_0009_1-2_PROTON_01
LFG_0009_1-2
L-Phe-OMe.HCl
LFG_0025_1_2-18_PROTON_01
LFG_0025_1_2-18
L-Trp-OMe.HCl
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1
f1 (ppm)

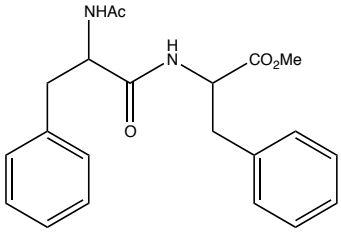
_N_-Acetyl-L-phenylalanine



L-Phenylalanine (9.92 g, 60.1 mmol) was suspended in aq. $NaHCO_3$ (5% w/v, 150 mL) at cooled to 0ºC. To this solution acetic anhydride (6.8 mL, 72 mmol) was added dropwise over a period of 1 hr. The mixture was stirred at room temperature for two hours followed by acidification to pH 2-3 with 6M HCl and was cooled over ice/water. The resulting white precipitate was filtered, washed with water and dried to give *N*-acetyl-L-phenylalanine (9.37 g, 75 %) as a white powder.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.19 (d, *J* = 8.1 Hz, 1H, N*H*), 7.40 – 7.12 (m, 5H, 2,3,4,5,6-*H*), 4.40 (ddd, *J* = 9.6, 8.0, 4.9 Hz, 1H, C*H*), 3.04 (dd, *J* = 13.8, 4.9 Hz, 1H, CH_2), 2.83 (dd, *J* = 13.8, 9.6 Hz, 1H, CH_2), 1.78 (s, 3H, NHCOCH_3).

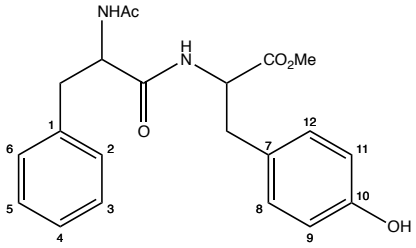
_N_-Acetyl-L-phenylalanyl-L-phenylalanine methyl ester (7)



The compound was prepared according to the general procedure for peptide coupling. The crude residue was purified by recrystallisation in ethyl acetate to give the product as a white solid (1.37 g, 37 %).

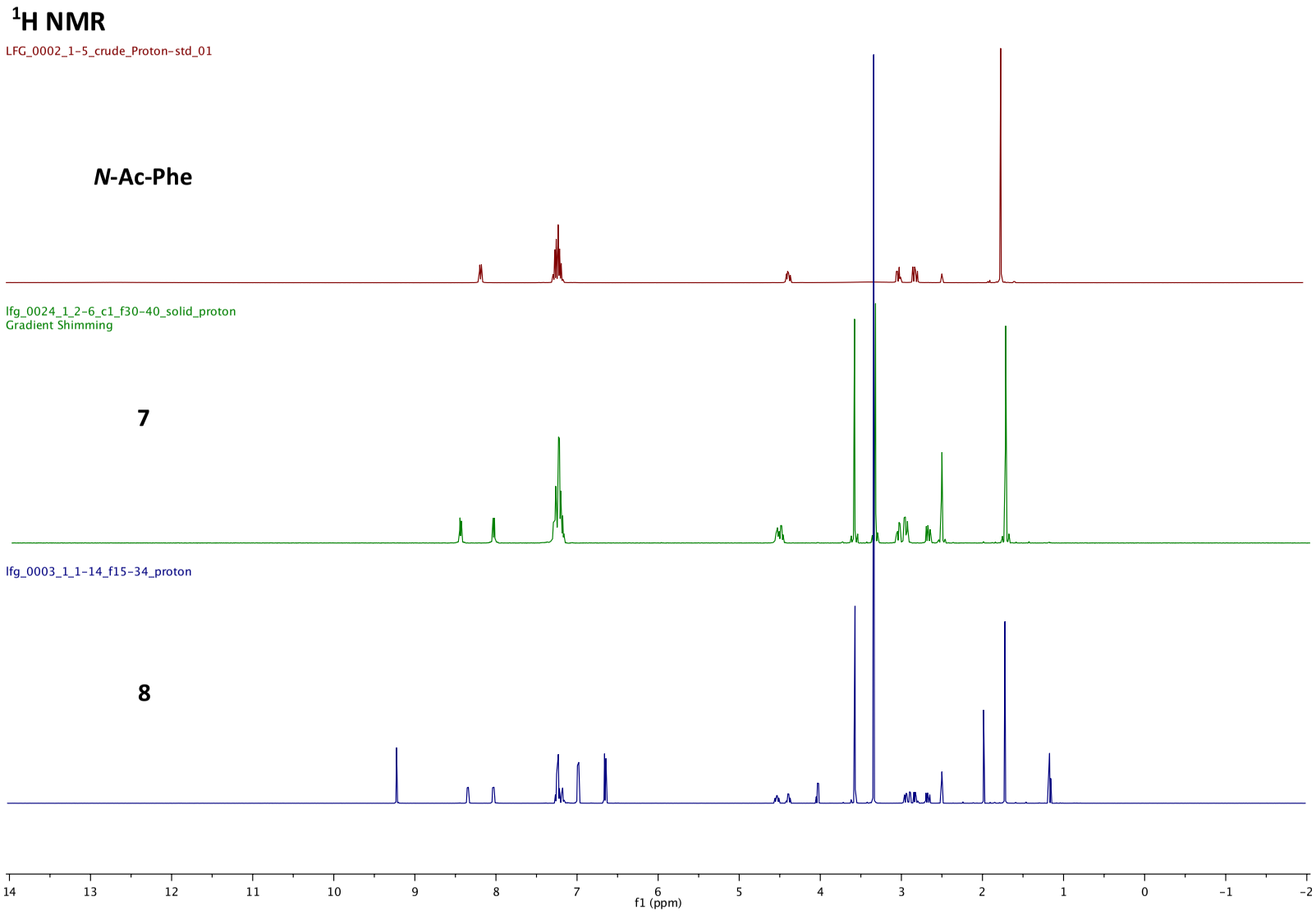
^{1}H NMR (500 MHz, DMSO-d_6) δ 8.44 (d, *J* = 7.5 Hz, 1H, N*H*), 8.03 (d, *J* = 8.6 Hz, 1H, N*H*), 7.23 (dtd, *J* = 25.7, 13.7, 12.3, 7.2 Hz, 10H, Ar-*H*), 4.51 (dtd, *J* = 23.9, 8.7, 8.1, 5.2 Hz, 2H, C*H*), 3.58 (s, 3H, COOCH_3), 3.04 (dd, *J* = 13.8, 5.9 Hz, 1H, CH_2), 2.99 – 2.89 (m, 2H, CH_2), 2.67 (dd, *J* = 13.9, 10.0 Hz, 1H, CH_2), 1.71 (s, 3H, NHCOCH_3).

_N_-Acetyl-L-phenylalanyl-L-tyrosine methyl ester (8)

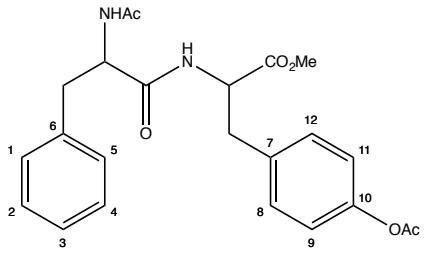


The compound was prepared according to the general procedure for peptide coupling. The crude residue was purified by silica column chromatography (100:4 CH_2Cl_2:MeOH, R_f = 0.17) to give the product as a white foam (2.33 g, 61 %).

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.23 (s, 1H, O*H*), 8.35 (d, *J* = 7.5 Hz, 1H, N*H*), 8.03 (d, *J* = 8.5 Hz, 1H, N*H*), 7.28 – 7.21 (m, 4H, 2,3,5,6-*H*), 7.20 – 7.16 (m, 1H, 4-*H*), 6.99 (d, *J* = 8.4 Hz, 2H, 8,12-*H*), 6.65 (d, *J* = 8.4 Hz, 2H, 9,11-*H*), 4.53 (ddd, *J* = 9.9, 8.5, 4.5 Hz, 1H, C*H*), 4.39 (td, *J* = 8.1, 6.1 Hz, 1H, C*H*), 3.57 (s, 3H, COOCH_3), 2.95 (dd, *J* = 13.9, 4.5 Hz, 1H, CH_2), 2.91 (dd, *J* = 14.0, 6.0 Hz, 1H, CH_2), 2.82 (dd, *J* = 13.9, 8.4 Hz, 1H, CH_2), 2.67 (dd, *J* = 13.9, 10.0 Hz, 1H, CH_2), 1.72 (s, 3H, NHCOCH_3).


^{1}H NMR
LFG_0002_1-5_crude_Proton-std_01
N-Ac-Phe
lfg_0024_1_2-6_c1_f30-40_solid_proton
Gradient Shimming
7
lfg_0003_1_1-14_f15-34_proton
8
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2
f1 (ppm)

_N_-Acetyl-L-phenylalanyl-_O_-acetyl-L-tyrosine methyl ester (9)



The previously prepared *N*-Acetyl-L-phenylalanyl-L-tyrosine methyl ester (1.44 g, 3.75 mmol) and DMAP (5 mg, 0.04 mmol) were dissolved in pyridine (20 mL). Acetic anhydride (8 mL, 85 mmol) was added dropwise and the mixture was stirred for two hours at room temperature. The reaction was quenched with ethanol (10 mL) and concentrated in vacuo to give a white solid. The crude solid was recrystallised from ethyl acetate to give the product as a white powder (0.78 g, 49 %).

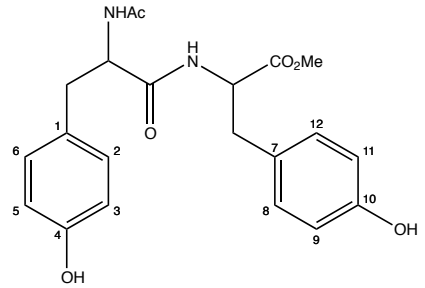
^{1}H NMR (500 MHz, DMSO-d_6) δ 8.46 (d, *J* = 7.4 Hz, 1H, N*H*), 8.03 (d, *J* = 8.5 Hz, 1H, N*H*), 7.35 – 7.20 (m, 6H, 1,2,4,5,8,12-*H*), 7.21 – 7.14 (m, 1H, 3-*H*), 7.02 (d, *J* = 8.5 Hz, 2H, 9,11-*H*), 4.53 (ddd, *J* = 10.0, 8.5, 4.6 Hz, 1H, C*H*), 4.48 (td, *J* = 8.2, 6.0 Hz, 1H, C*H*), 3.58 (s, 3H, COOCH_3), 3.04 (dd, *J* = 13.9, 5.8 Hz, 1H, CH_2), 3.01 – 2.88 (m, 2H, CH_2), 2.67 (dd, *J* = 13.9, 10.0 Hz, 1H, CH_2), 2.24 (s, 3H, OCOCH_3), 1.72 (s, 3H, NHCOCH_3).

^{13}C NMR (101 MHz, DMSO-d_6) δ 171.63, 171.58, 169.13, 168.96, 149.17 (*C*-10), 137.90 (*C*-1), 134.51 (*C*-7), 130.07 (*C*-3,5), 129.10 (*C*-12,18), 127.97 (*C*-2,6), 126.18 (*C*-4), 121.51 (*C*-9,11), 53.49 (2x*C*H), 51.85 (*C*OOCH$_3$), 37.46 (*C*H$_2$), 35.81 (*C*H$_2$), 22.37 (NHCO*C*H$_3$), 20.85 (OCO*C*H$_3$).

HRMS (ESI) $C_{12}H_{15}NO_3$+H^+ (M+H^+) Calculated for 427.1864, found 427.1872.

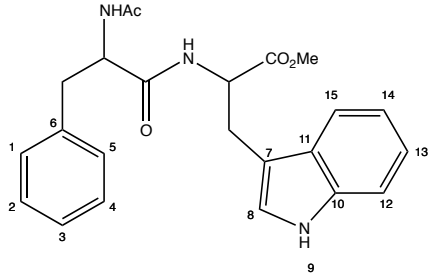
$C_{12}H_{15}NO_3$+Na^+ (M+Na^+) Calculated for 449.1683, found 449.1687.

_N_-Acetyl-L-tyrosyl-L-tyrosine methyl ester (10)



The compound was prepared according to the general procedure for peptide coupling. The crude residue was purified by silica column chromatography (10:1 EtOAc:Pet. Spirit, R_f = 0.29) to give the product as a white foam (2.12 g, 53 %).

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.22 (s, 1H, O*H*), 9.14 (s, 1H, O*H*), 8.29 (d, *J* = 7.5 Hz, 1H, N*H*), 7.95 (d, *J* = 8.5 Hz, 1H, N*H*), 6.99 (t, *J* = 8.6 Hz, 4H, 2,6,8,12-*H*), 6.63 (dd, *J* = 9.1, 8.4 Hz, 4H, 3,5,9,11-*H*), 4.44 (ddd, *J* = 10.1, 9.0, 4.7 Hz, 1H, C*H*), 4.38 (td, *J* = 8.1, 6.2 Hz, 1H, C*H*), 3.57 (s, 3H, COOCH_3), 2.89 (dd, *J* = 13.9, 6.0 Hz, 1H, CH_2), 2.86 – 2.77 (m, 2H, CH_2), 2.55 (dd, *J* = 13.9, 9.9 Hz, 1H, CH_2), 1.73 (s, 3H, NHCOCH_3).

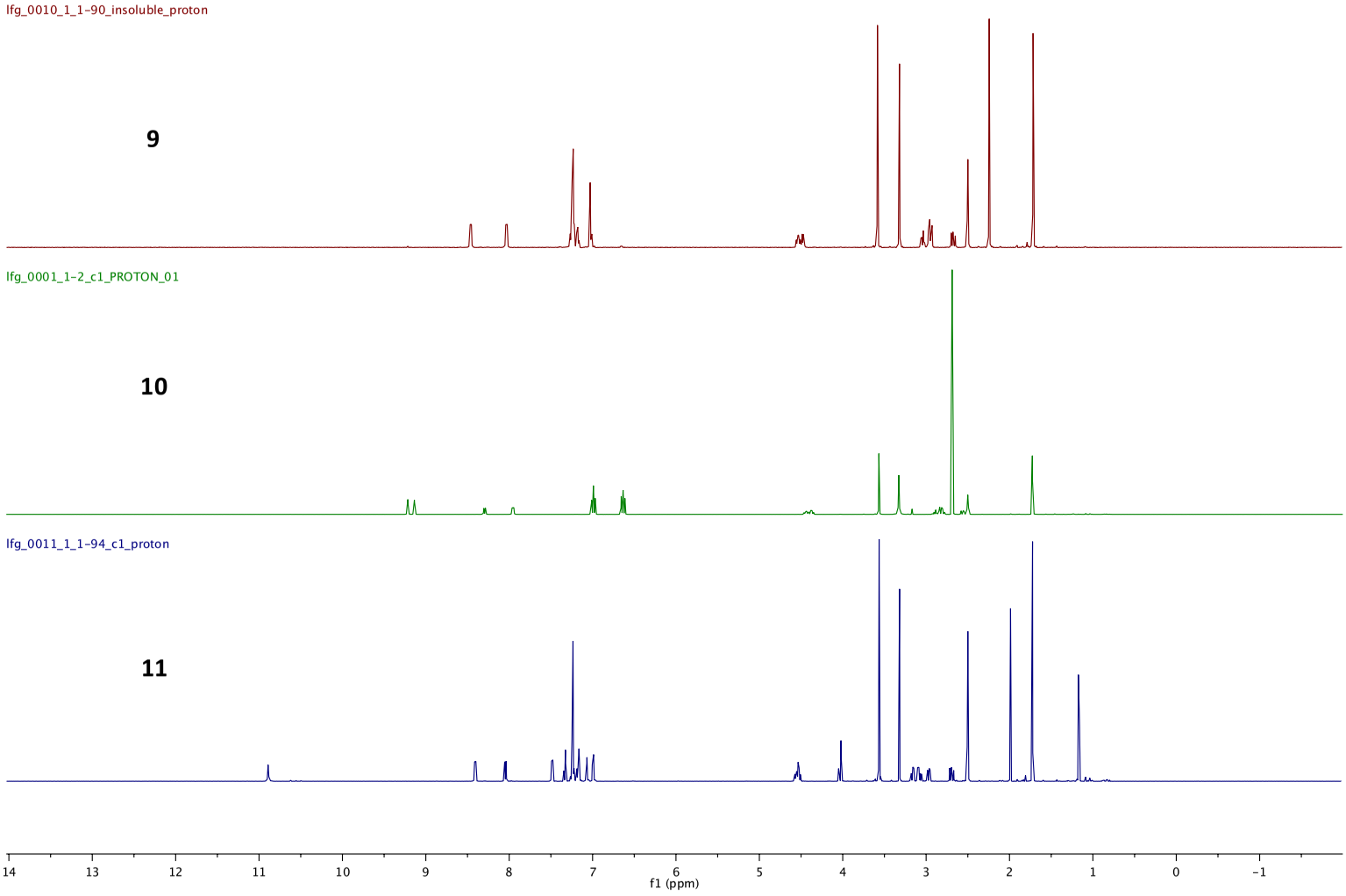


_N_-Acetyl-L-phenylalanyl-L-tryptophan methyl ester (11)

The compound was prepared according to the general procedure for peptide coupling. The crude residue was purified by silica column chromatography (4:1 EtOAc:Pet. Spirit, R_f = 0.23) to give the product as an off-white foam (1.05 g, 40 %).

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.89 (s, 1H, 9-N*H*), 8.41 (d, *J* = 7.4 Hz, 1H, N*H*), 8.05 (d, *J* = 8.5 Hz, 1H, N*H*), 7.48 (d, *J* = 7.1 Hz, 1H, 15-*H*), 7.33 (dt, *J* = 8.1, 0.9 Hz, 1H, 12-*H*), 7.30 – 7.14 (m, 6H, 1,2,3,4,5,8-*H*), 7.07 (ddd, *J* = 8.2, 7.0, 1.2 Hz, 1H, 13-*H*), 6.99 (ddd, *J* = 8.0, 7.0, 1.1 Hz, 1H, 14-*H*), 4.66 – 4.42 (m, 2H, C*H*), 3.56 (s, 3H, COOCH_3), 3.17 (dd, *J* = 14.7, 5.8 Hz, 1H, CH_2), 3.08 (dd, *J* = 14.6, 7.9 Hz, 1H, CH_2), 2.97 (dd, *J* = 13.8, 4.4 Hz, 1H, CH_2), 2.69 (dd, *J* = 13.8, 10.0 Hz, 1H, CH_2), 1.72 (s, 3H, NHCOCH_3).

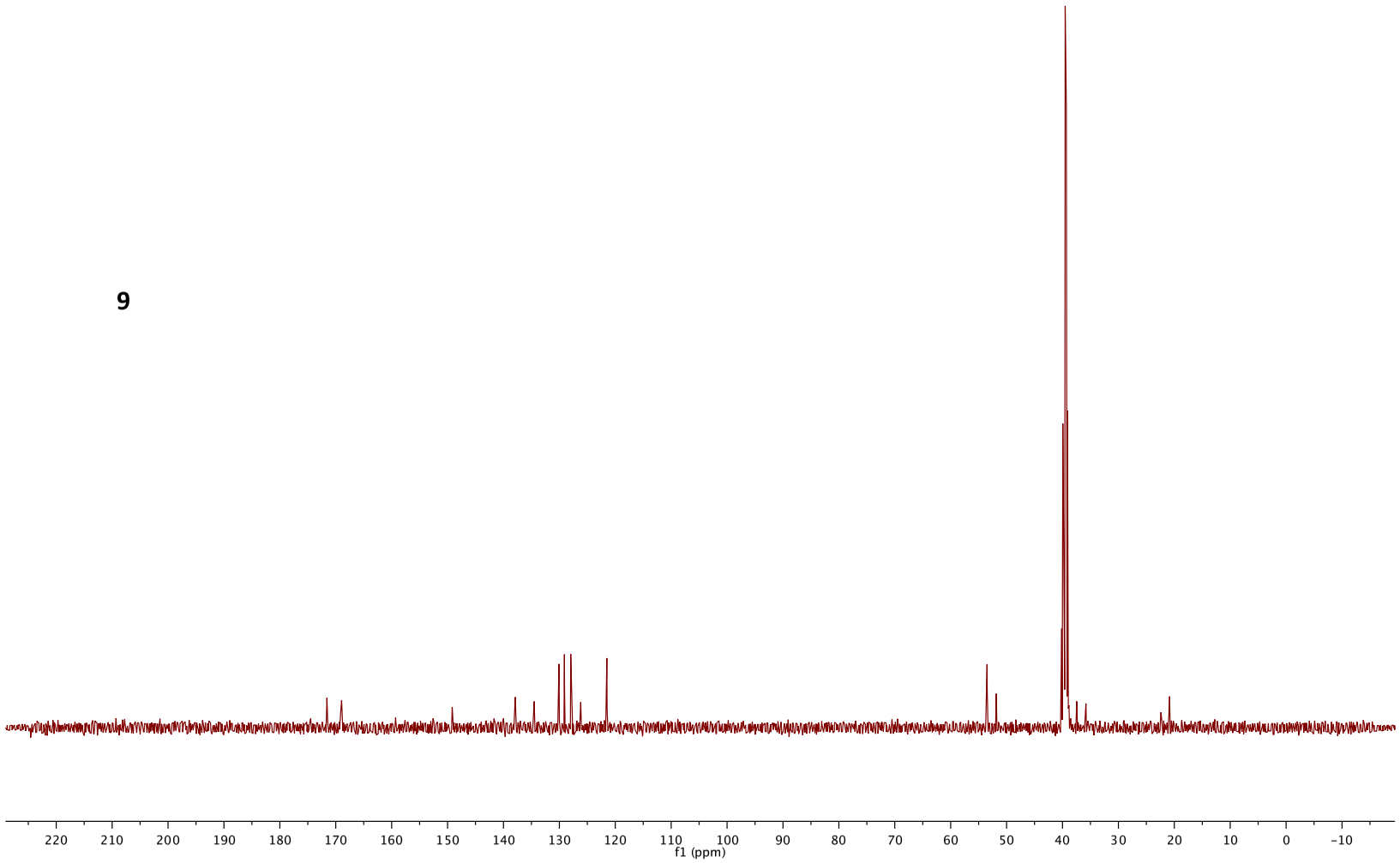
^{1}H NMR


lfg_0010_1_1-90_insoluble_proton
9
lfg_0001_1-2_c1_PROTON_01
10
lfg_0011_1_1-94_c1_proton
11
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
f1 (ppm)

^{13}C NMR

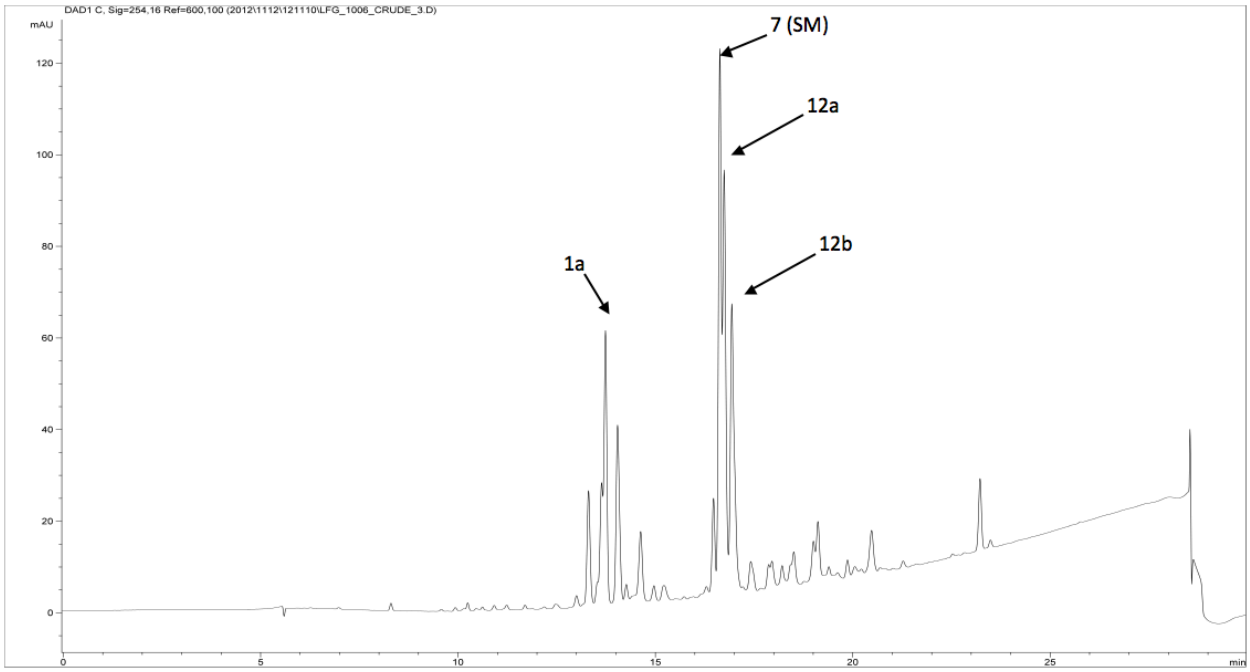
LFG_1002_2_P1A1-A3_CARBON_01

9



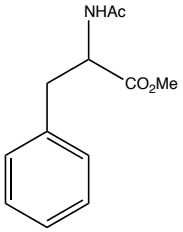
1.3 Reaction of dipeptides with nitrogen dioxide and ozone

1.3.1 Reaction of Phe-Phe (7) with NO_2• and O_3



*HPLC analysis of the reaction of NO_2• and O_3 with Phe-Phe (**7**)*

_N_-Acetyl-L-phenylalanine methyl ester (1a)



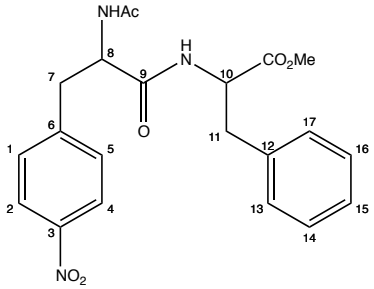
^{1}H NMR (500 MHz, DMSO-d_6) δ 8.33 (d, *J* = 7.7 Hz, 1H, N*H*), 7.36 – 7.25 (m, 2H, 3,5-*H*), 7.24 – 7.16 (m, 3H, 2,4,6-*H*), 4.44 (ddd, *J* = 9.3, 7.7, 5.6 Hz, 1H, C*H*), 3.59 (s, 3H, COOCH_3), 3.00 (dd, *J* = 13.7, 5.6 Hz, 1H, CH_2), 2.87 (dd, *J* = 13.7, 9.3 Hz, 1H, CH_2), 1.79 (s, 3H, NHCOCH_3).

^{13}C NMR (126 MHz, DMSO-d_6) δ 172.18 (C_q, NH*C*OCH$_3$), 169.29 (C_q, *C*OOCH$_3$), 137.25 (C_q, *C*-1), 128.98 (C_t, *C*-3,5), 128.22 (C_t, *C*-2,6), 126.52 (C_t, *C*-4), 53.60 (C_p, COO*C*H$_3$), 51.77 (C_t, *C*H), 36.72 (C_s, *C*H$_2$), 22.22 (C_q, NHCO*C*H$_3$).

HRMS (ESI) $C_{12}H_{15}NO_3$+H^+ (M+H^+) Calculated for 222.1125, found 222.1106.

$C_{12}H_{15}NO_3$+Na^+ (M+Na^+) Calculated for 244.0944, found 244.0921.

_N_-Acetyl-4-nitro-L-phenylalanyl-phenylalanine methyl ester (12a)

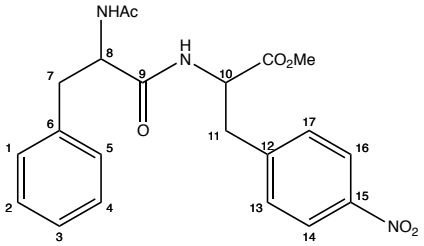


^{1}H NMR (500 MHz, DMSO-d_6) δ 8.51 (d, *J* = 8.0 Hz, 1H, N*H*), 8.17 – 8.07 (m, 2H, 2,4-*H*), 8.01 (d, *J* = 8.5 Hz, 1H, N*H*), 7.54 – 7.46 (m, 2H, 1,5-*H*), 7.32 – 7.06 (m, 5H, 13,14,15,16,17-*H*), 4.59 (ddd, *J* = 9.3, 7.9, 5.5 Hz, 1H, 8-*H*), 4.49 (ddd, *J* = 9.8, 8.8, 4.9 Hz, 1H, 10-*H*), 3.61 (s, 3H, COOCH_3), 3.21 (dd, *J* = 13.8, 5.3 Hz, 1H, CH_2), 3.06 (dd, *J* = 13.9, 9.2 Hz, 1H, CH_2), 2.92 (dd, *J* = 13.9, 4.7 Hz, 1H, CH_2), 2.70 – 2.58 (m, 1H, CH_2), 1.70 (s, 3H, NHCOCH_3).

HRMS (ESI) $C_{21}H_{23}N_3O_6$+H^+ (M+H^+) Calculated for 414.1660, found 414.1635.

$C_{21}H_{23}N_3O_6$+Na^+ (M+Na^+) Calculated for 436.1479, found 436.1452.

_N_-Acetyl-L-phenylalanyl-4-nitro-phenylalanine methyl ester (12b)

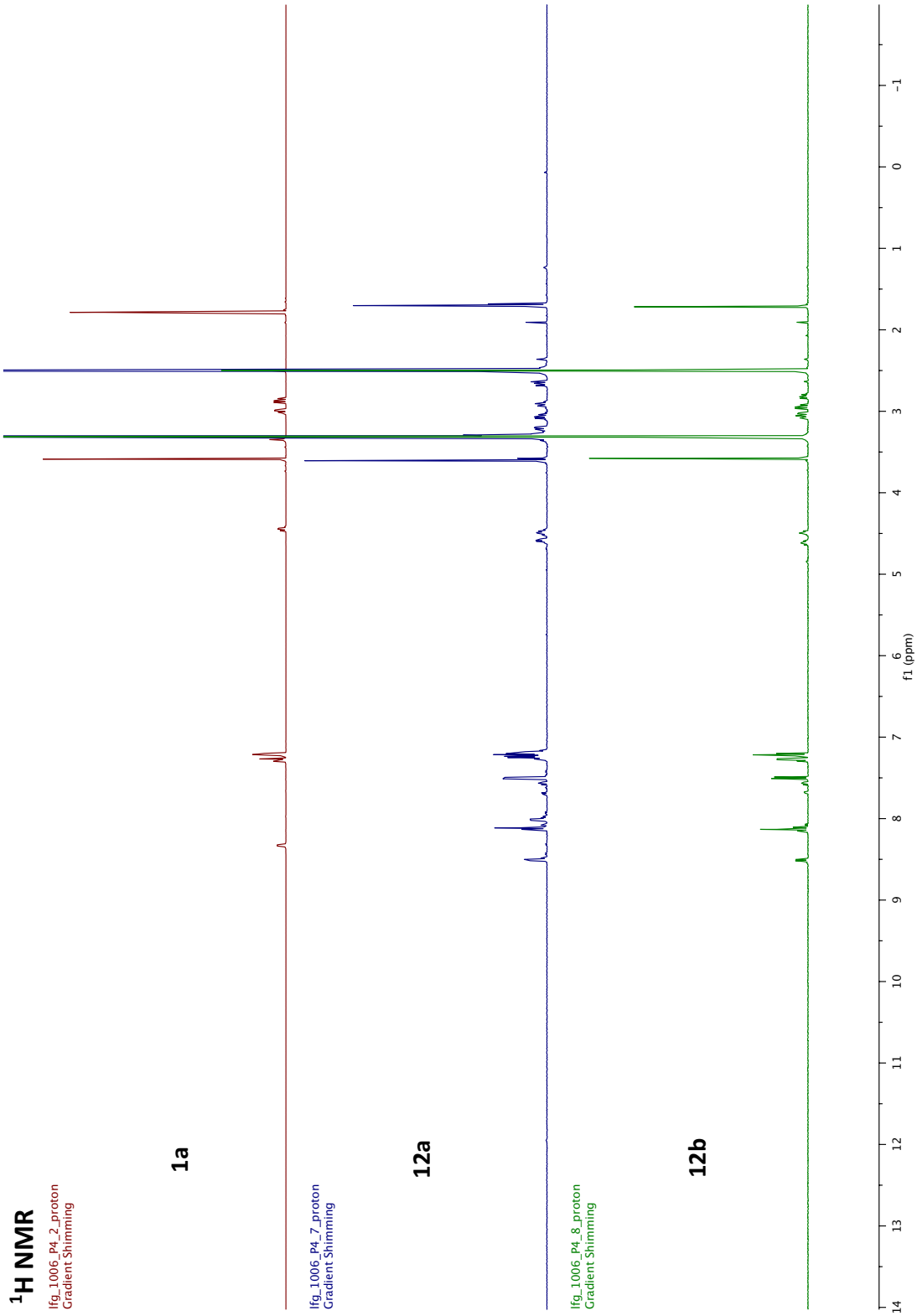


^{1}H NMR (500 MHz, DMSO-d_6) δ 8.51 (d, *J* = 7.5 Hz, 1H, N*H*), 8.16 – 8.13 (m, 2H, 14,16-*H*), 8.12 (d, *J* = 8.8 Hz, 1H, N*H*), 7.54 – 7.46 (m, 2H, 13,17-*H*), 7.31 – 7.24 (m, 2H, 2,4-*H*), 7.25 – 7.17 (m, 3H, 1,3,5-*H*), 4.62 (ddd, *J* = 10.1, 8.7, 4.7 Hz, 1H, 8-*H*), 4.49 (ddd, *J* = 8.6, 7.4, 5.8 Hz, 1H, 10-*H*), 3.58 (s, 3H, COOCH_3), 3.10 – 3.01 (m, 2H, CH_2), 2.94 (dd, *J* = 13.9, 8.6 Hz, 1H, CH_2), 2.82 (dd, *J* = 13.7, 9.9 Hz, 1H, CH_2), 1.72 (s, 3H, NHCOCH_3).

HRMS (ESI) $C_{21}H_{23}N_3O_6$+Na^+ (M+Na^+) Calculated for 436.1479, found 436.1450.

$C_{42}H_{46}N_6O_{12}$+H^+ (2M+Na^+) Calculated for 849.3066, found 849.3015.

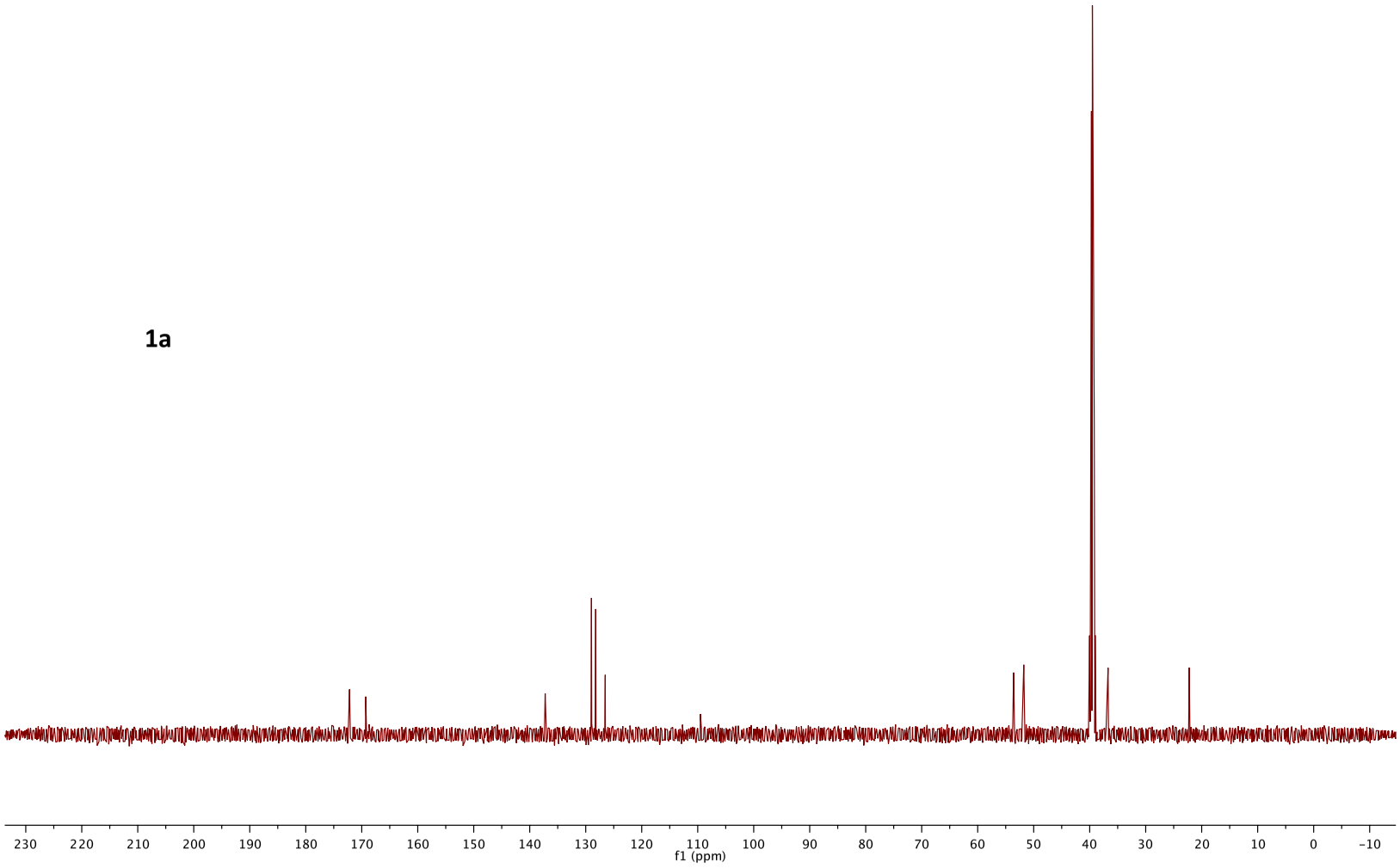
Note: The assignment of **12a** and **12b** could be exchanged.

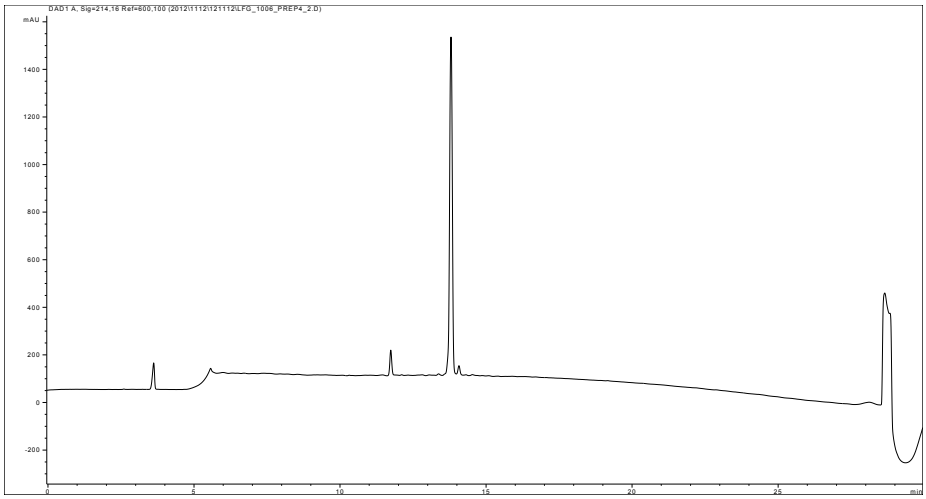

^{1}H NMR
lfg_1006_P4_2_proton
Gradient Shimming
1a
lfg_1006_P4_7_proton
Gradient Shimming
12a
lfg_1006_P4_8_proton
Gradient Shimming
12b
f1 (ppm)

^{13}C NMR

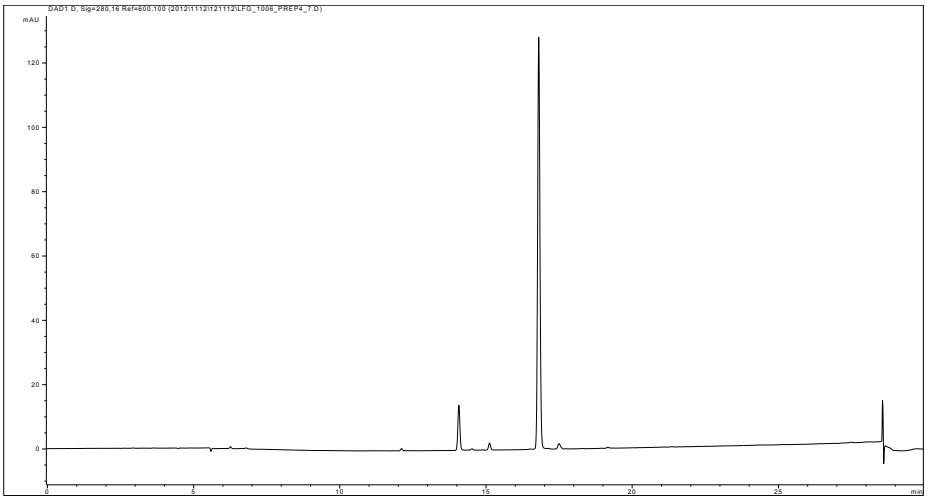
lfg_1006_P4_2_carbon

1a

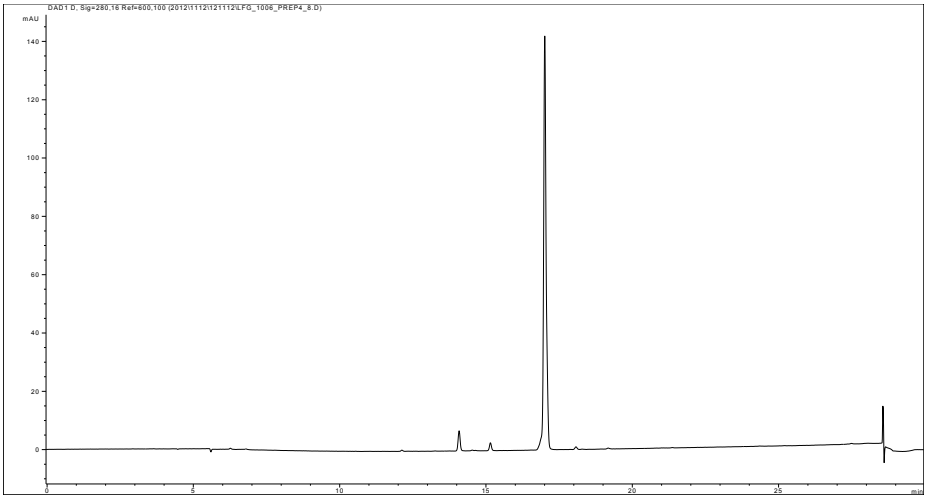




*HPLC analysis of **1a***

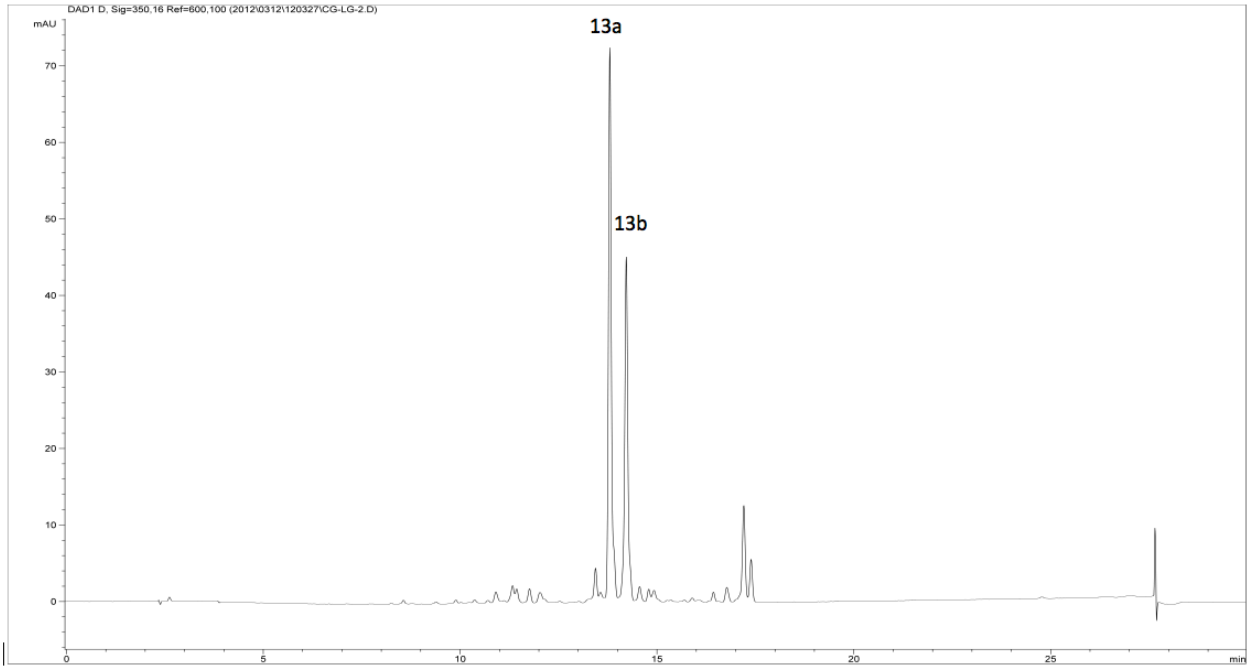


*HPLC analysis of **12a***



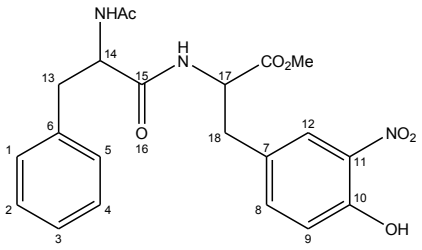
*HPLC analysis of **12b***

1.3.2 Reaction of Phe-Tyr (8) with NO_2• and O_3



*HPLC analysis of the reaction of NO_2• and O_3 with Phe-Tyr (**8**)*

***N*-Acetyl-L-phenylalanyl-3-nitro-L-tyrosine methyl ester (13a)**



^{1}H NMR (500 MHz, DMSO-d_6) δ 10.80 (s, 1H, O*H*), 8.43 (d, *J* = 7.6 Hz, 1H, N*H*), 8.01 (d, *J* = 8.5 Hz, 1H, N*H*), 7.73 (d, *J* = 2.2 Hz, 1H, 12-*H*), 7.39 (dd, *J* = 8.5, 2.2 Hz, 1H, 8-*H*), 7.32 – 7.13 (m, 5H, 1,2,3,4,5-*H*), 7.02 (d, *J* = 8.5 Hz, 1H, 9-*H*), 4.69 – 4.24 (m, 2H, C*H*), 3.60 (s, 3H, COOCH_3), 3.03 (dd, *J* = 13.9, 5.5 Hz, 1H, CH_2), 2.91 (ddd, *J* = 13.9, 6.8, 2.3 Hz, 2H, CH_2), 2.66 (dd, *J* = 13.9, 9.8 Hz, 1H, CH_2), 1.70 (s, 3H, NHCOCH_3).

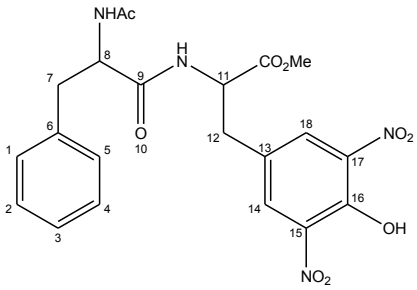
^{13}C NMR (126 MHz, DMSO-d_6) δ 171.51 (C_q, *C*-15), 171.44 (C_q, *C*OOCH$_3$), 168.91 (C_q, NH*C*OCH$_3$) 150.83 (C_q, *C*-10), 137.84 (C_q, *C*-6), 136.24 (C_q, *C*-11), 136.19 (C_t, *C*-8), 129.05 (C_q, *C*-7), 128.28 (C_t, Ar-*C*), 127.98 (C_t, Ar-*C*), 126.19 (C_t, Ar-*C*), 125.40 (C_t, Ar-*C*), 118.93 (C_t, *C*-9), 53.53 (C_t, *C*-14), 53.20 (C_t, *C*-17), 51.93 (C_p, COO*C*H$_3$), 37.45 (C_s, *C*-13), 35.05 (C_s, *C*-18), 22.33 (C_p, NHCO*C*H$_3$).

HRMS (ESI) $C_{21}H_{23}N_3O_7$+H^+ (M+H^+) Calculated for 430.1609, found 430.1635.

$C_{42}H_{46}N_6O_{14}$+H^+ (2M+H^+) Calculated for 859.3099, found 859.3107.

_N_-Acetyl-L-phenylalanyl-3,5-dinitro-L-tyrosine methyl ester (13b)

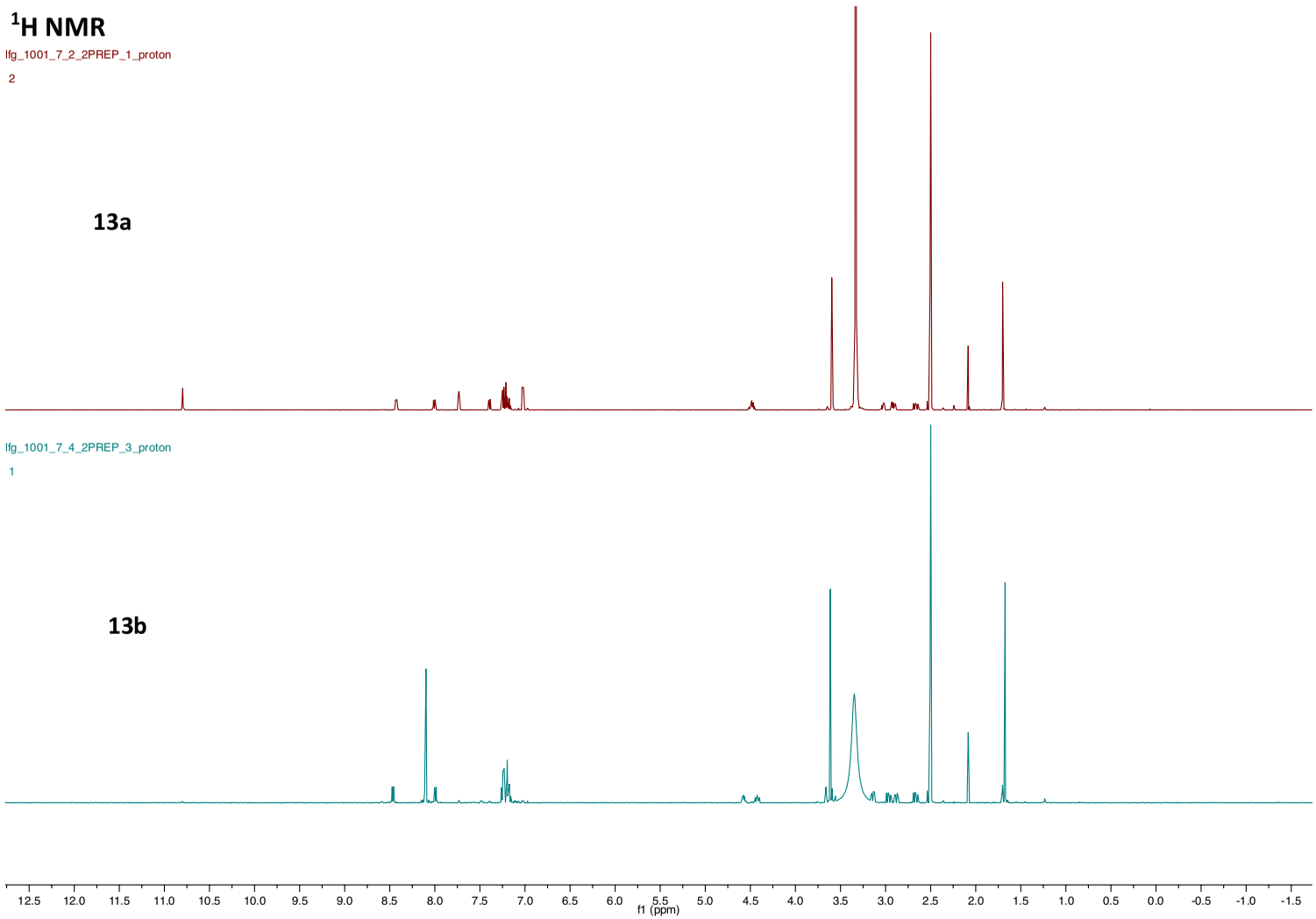
^{1}H NMR (500 MHz, DMSO-d_6) δ 8.47 (d, *J* = 8.1 Hz, 1H, N*H*), 8.10 (s, 2H, 14,18-*H*), 7.99 (d, *J* = 8.3 Hz, 1H, N*H*), 7.28 –7.17 (m, 5H, 1,2,3,4,5-*H*), 4.57 (ddd, *J* = 9.6, 7.9, 5.2 Hz, 1H, 8-*H*), 4.42 (ddd, *J* = 9.7, 8.3, 4.9 Hz, 1H, 11-*H*), 3.61 (s, 3H, COOCH_3), 3.14 (dd, *J* = 14.0, 5.2 Hz, 1H, CH_2), 2.97 (dd, *J* = 14.0, 9.6 Hz, 1H, CH_2), 2.88 (dd, *J* = 13.9, 5.0 Hz, 1H, CH_2), 2.67 (dd, *J* = 13.9, 9.7 Hz, 1H, CH_2), 1.67 (s, 3H, NHCOCH_3).

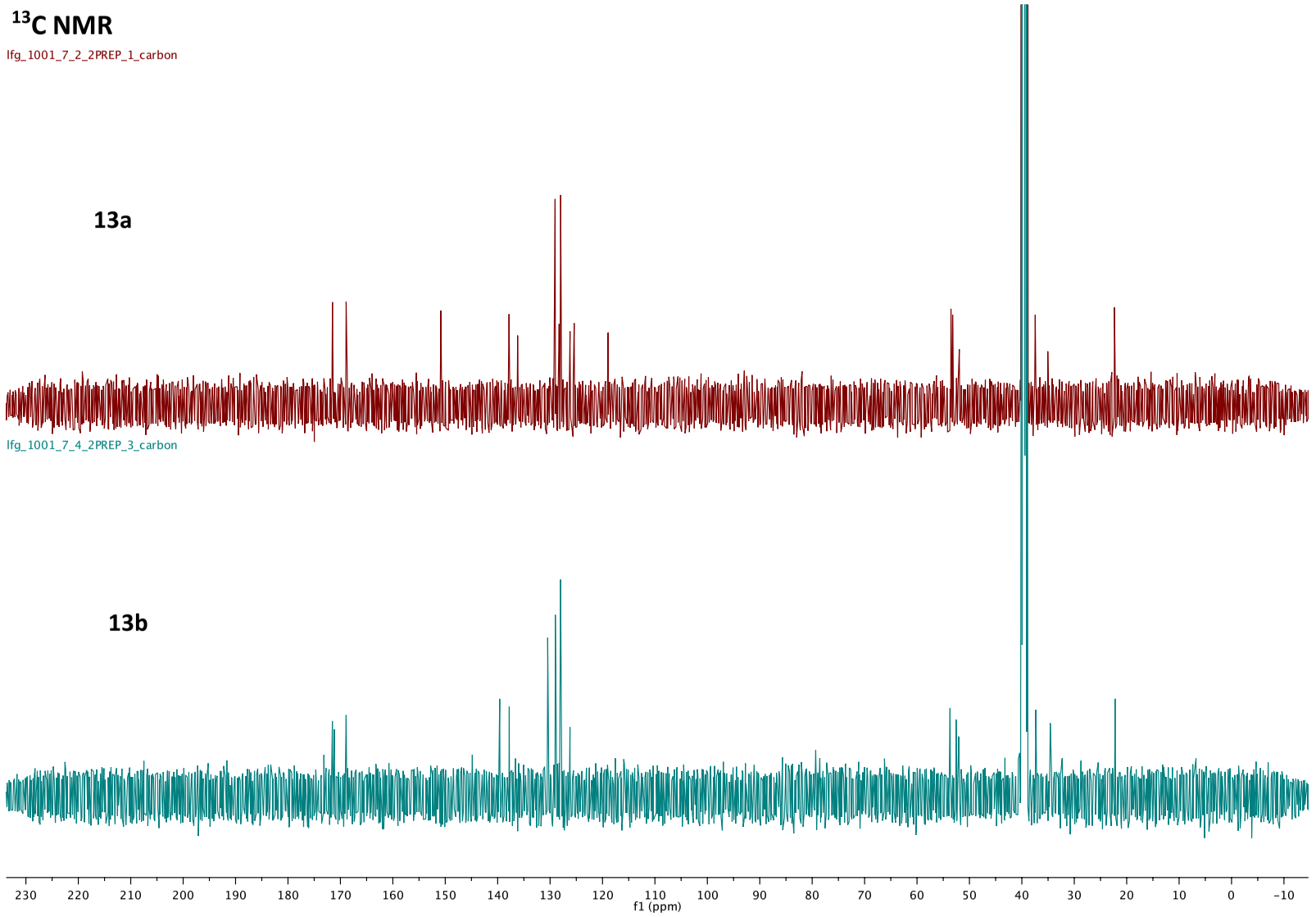


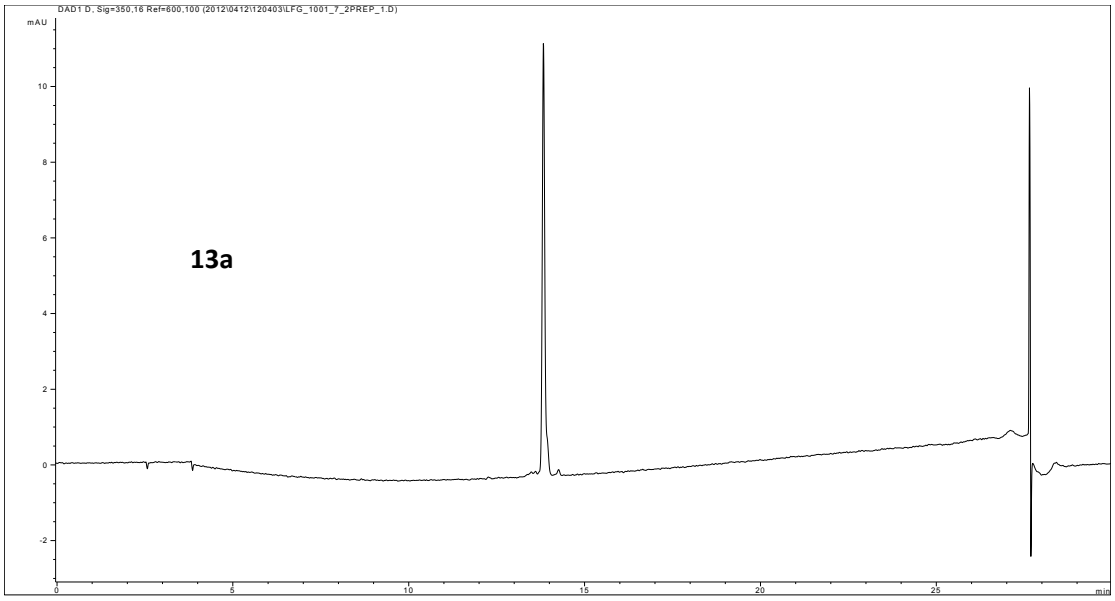
^{13}C NMR (126 MHz, DMSO-d_6) δ 171.53 (C_q, *C*-9), 171.15 (C_q, *C*OOCH$_3$), 168.91 (C_q,NH*C*OCH$_3$), 139.57 (C_q, *C*-6), 137.79 (C_q, *C*-15,17), 130.45 (C_t, *C*-14,18), 129.00 (C_t, *C*-2,4), 128.00 (C_t, *C*-1,5), 126.20 (C_t, *C*-3), 53.71 (C_t, *C*-8), 52.53 (C_t, *C*-11), 52.05 (C_p, COO*C*H$_3$), 37.34 (C_s, *C*-7), 34.57 (C_s, *C*-12), 22.21 (NHCO*C*H$_3$).

HRMS (ESI) $C_{21}H_{22}N_4O_9$+H^+ (M+H^+) Calculated for 475.1460, found 475.1455.

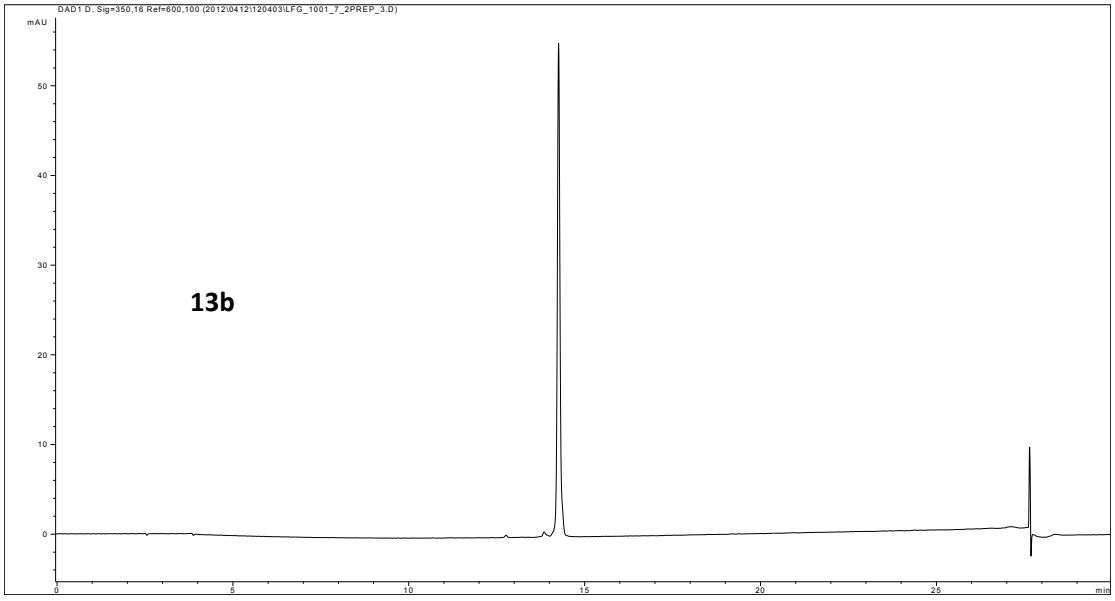
$C_{42}H_{44}N_8O_{18}$+H^+ (2M+H^+) Calculated for 949.2846, found 949.2828.


1H NMR
lfg_1001_7_2_2PREP_1_proton
2
13a
lfg_1001_7_4_2PREP_3_proton
1
13b
12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0 -1.5
f1 (ppm)


^{13}C NMR
lfg_1001_7_2_2PREP_1_carbon
13a
lfg_1001_7_4_2PREP_3_carbon
13b
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

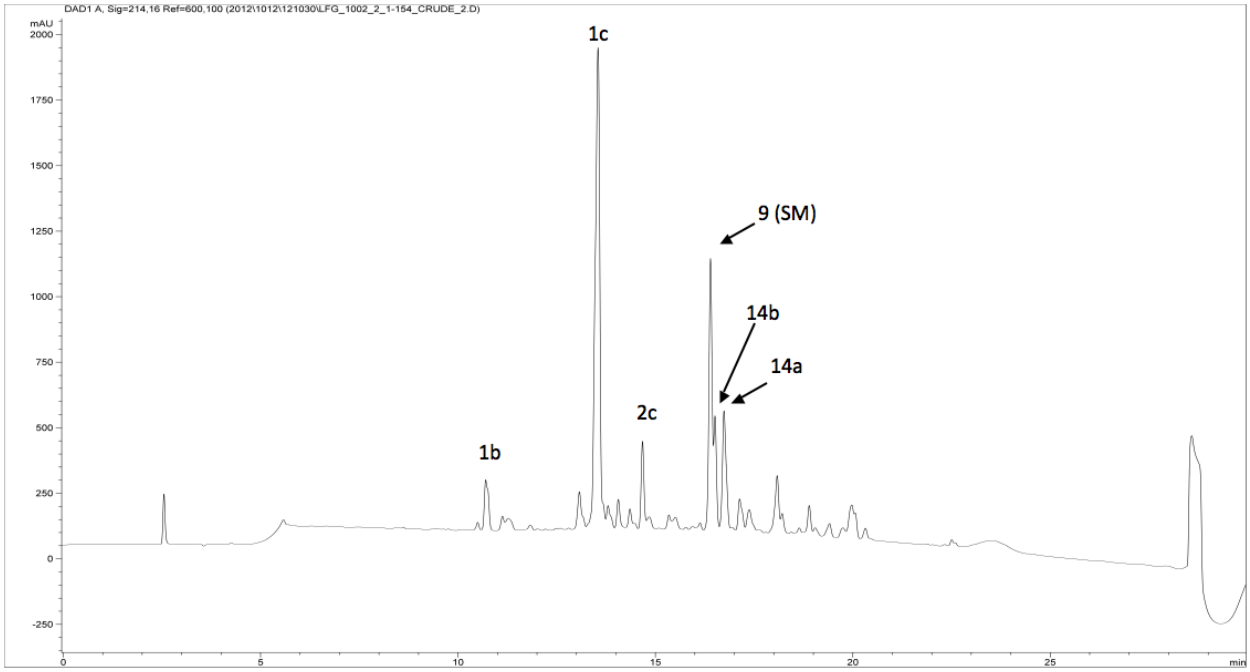


*HPLC analysis of **13a***



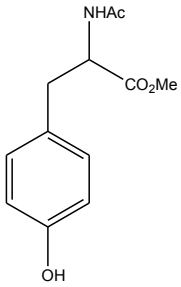
*HPLC analysis of **13b***

1.3.3 Reaction of Phe-OAcTyr (9) with NO_2• and O_3



*HPLC analysis of the reaction of NO_2• and O_3 with Phe-OAcTyr (**9**)*

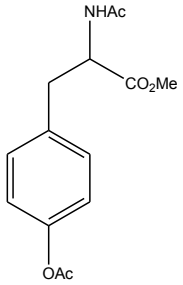
***N*-Acetyl-tyrosine methyl ester (1b)**



^{1}H NMR (500 MHz, DMSO-d_6) δ 9.21 (s, 1H, O*H*), 8.26 (d, *J* = 7.6 Hz, 1H, N*H*), 7.02 – 6.95 (m, 2H, Ar-*H*), 6.69 – 6.62 (m, 2H, Ar-*H*), 4.35 (ddd, *J* = 9.0, 7.7, 5.8 Hz, 1H, C*H*), 3.57 (s, 3H, COOCH_3), 2.86 (dd, *J* = 13.8, 5.8 Hz, 1H, CH_2), 2.74 (dd, *J* = 13.8, 9.0 Hz, 1H, CH_2), 1.79 (s, 3H, NHCOCH_3).

HRMS (ESI)	$C_{12}H_{15}NO_4$+H^+ (M+H^+)	Calculated for 238.1074, found 238.1119.
	$C_{12}H_{15}NO_4$+Na^+ (M+Na^+)	Calculated for 260.0893, found 260.0941.

_N_-Acetyl-_O_-acetyl-tyrosine methyl ester (1c)

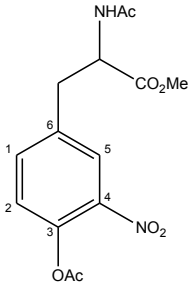


^{1}H NMR (500 MHz, DMSO-d_6) δ 8.35 (d, *J* = 7.8 Hz, 1H, N*H*), 7.51 – 7.10 (m, 2H, Ar-*H*), 7. 15 – 6.90 (m, 2H, Ar-*H*), 4.44 (ddd, *J* = 9.3, 7.7, 5.5 Hz, 1H, C*H*), 3.59 (s, 3H, COOCH_3), 3.01 (dd, *J* = 13.9, 5.6 Hz, 1H, CH_2), 2.88 (dd, *J* = 13.9, 9.4 Hz, 1H, CH_2), 2.25 (s, 3H, OCOCH_3), 1.79 (s, 3H, NHCOCH_3).

HRMS (ESI) $C_{14}H_{17}NO_5$+H^+ ($M+H^+$) Calculated for 280.1179, found 280.1280.

$C_{14}H_{17}NO_5$+Na^+ ($M+Na^+$) Calculated for 302.0999, found 302.1131.

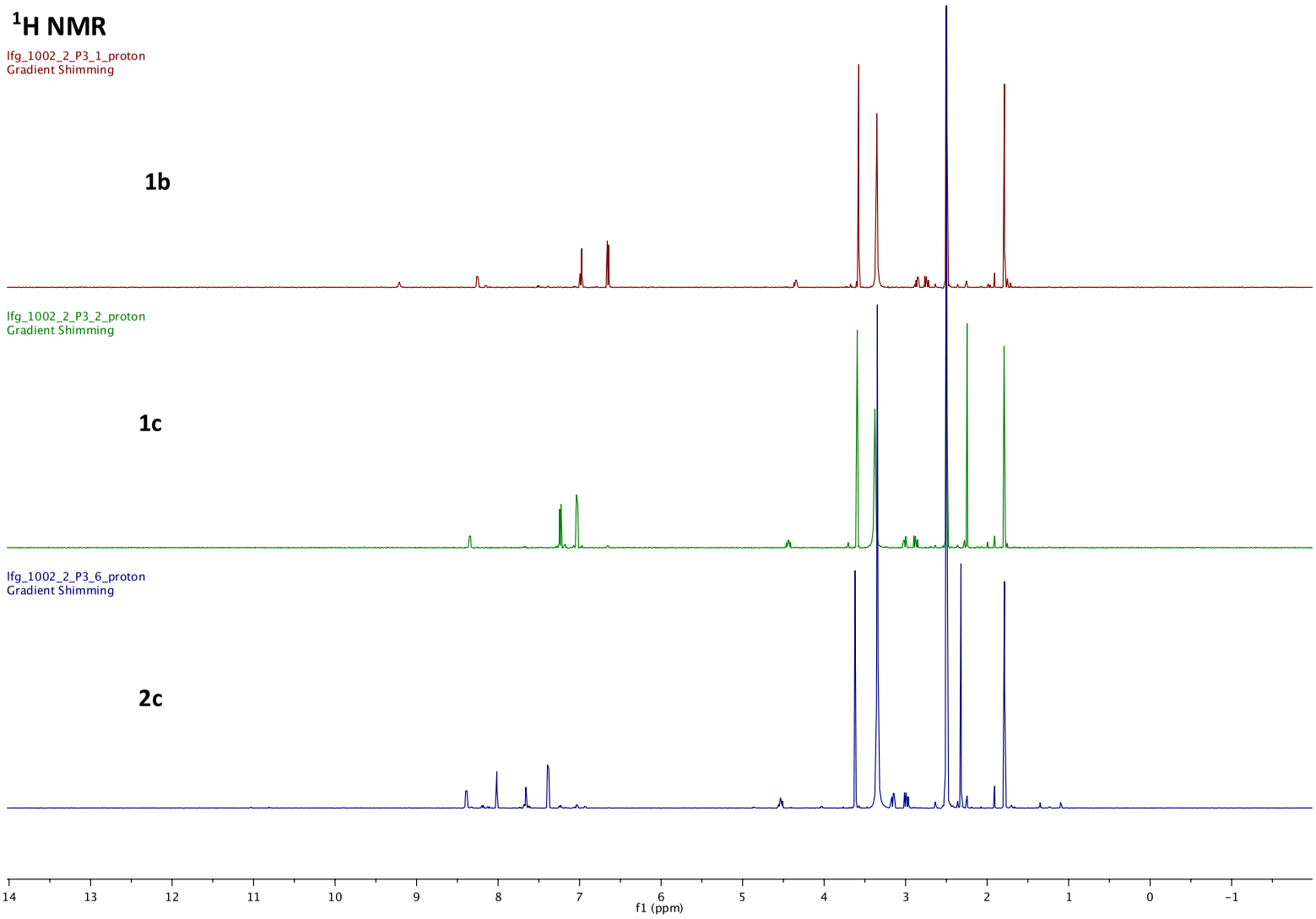
_N_-Acetyl-_O_-acetyl-3-nitro-tyrosine methyl ester (2c)

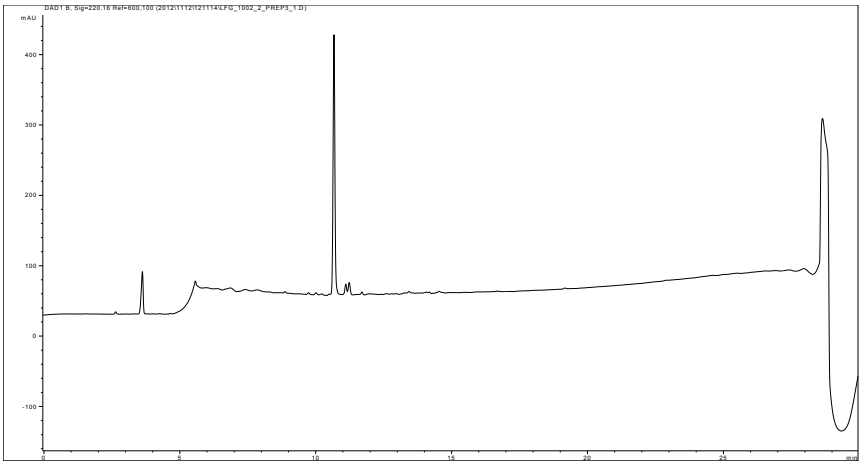


^{1}H NMR (500 MHz, DMSO-d6) δ 8.39 (d, *J* = 7.9 Hz, 1H, N*H*), 8.02 (d, *J* = 2.2 Hz, 1H, 5-*H*), 7.67 (dd, *J* = 8.4, 2.1 Hz, 1H, 1-*H*), 7.39 (d, *J* = 8.4 Hz, 1H, 2-*H*), 4.53 (ddd, *J* = 9.5, 7.8, 5.3 Hz, 1H, C*H*), 3.62 (s, 3H, COOCH_3), 3.16 (dd, *J* = 13.9, 5.2 Hz, 1H, CH_2), 2.99 (dd, *J* = 13.9, 9.6 Hz, 1H, CH_2), 2.32 (s, 3H, OCOCH_3), 1.79 (s, 3H, NHCOCH_3).

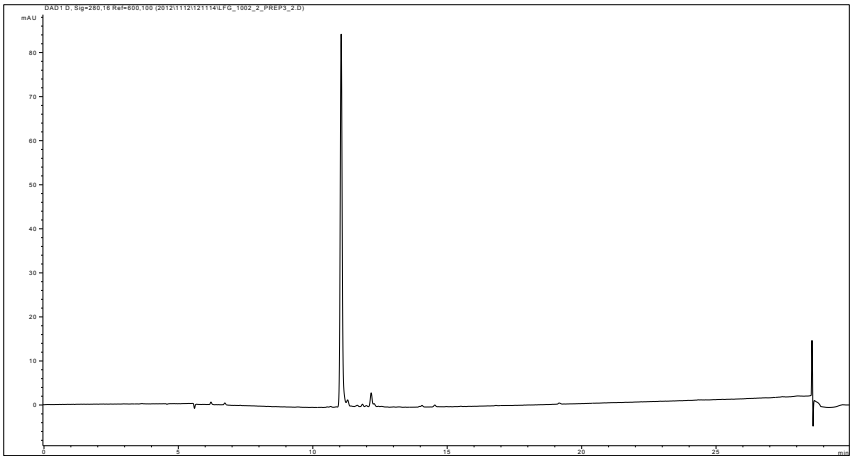
HRMS (ESI) $C_{14}H_{16}N_2O_7$+H^+ ($M+H^+$) Calculated for 325.1030, found 325.1038.

$C_{14}H_{16}N_2O_7$+Na^+ ($M+Na^+$) Calculated for 347.0850, found 347.0862.

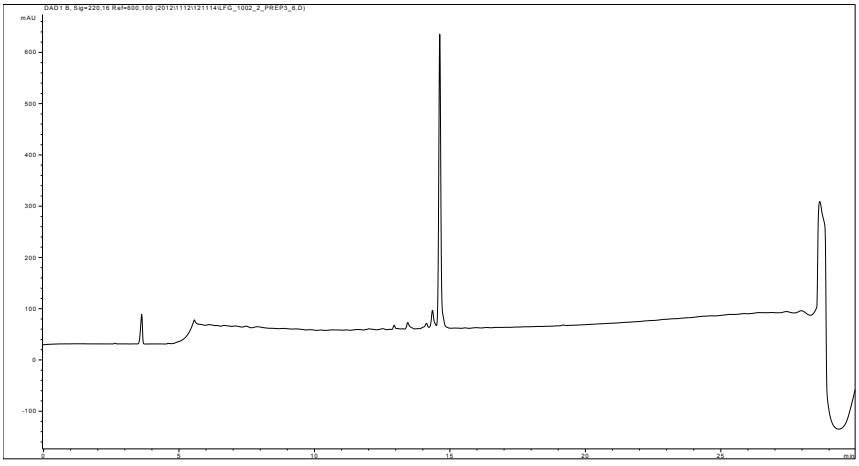

^{1}H NMR
lfg_1002_2_P3_1_proton
Gradient Shimming
1b
lfg_1002_2_P3_2_proton
Gradient Shimming
1c
lfg_1002_2_P3_6_proton
Gradient Shimming
2c
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
−1
f1 (ppm)



*HPLC analysis of **1b***

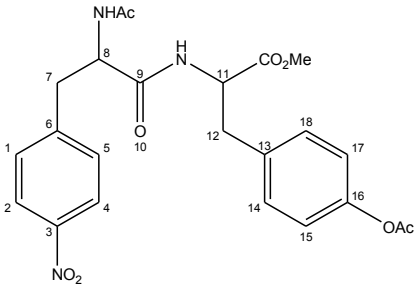


*HPLC analysis of **1c***



*HPLC analysis of **2c***

_N_-Acetyl-4-nitro-L-phenylalanyl-_O_-acetyl-L-tyrosine methyl ester (14a)*

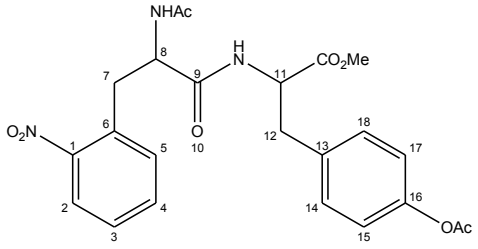


^{1}H NMR (500 MHz, DMSO-d_6) δ 8.53 (d, *J* = 7.5 Hz, 1H, N*H*), 8.16 – 8.13 (m, 2H, 2,4-*H*), 8.12 (d, *J* = 8.6 Hz, 1H, obs., N*H*), 7.51 – 7.40 (m, 2H, 1,5-*H*), 7.35 – 7.14 (m, 2H, 14,18-*H*), 7.20 – 6.61 (m, 2H, 15,17-*H*), 4.62 (ddd, *J* = 10.0, 8.6, 4.6 Hz, 1H, 8-*H*), 4.54 – 4.45 (m, 1H, 11-*H*), 3.58 (s, 3H, COOC*H*$_3$), 3.05 (ddd, *J* = 14.9, 10.0, 5.2 Hz, 2H, C*H*$_2$), 2.95 (dd, *J* = 14.0, 8.6 Hz, 1H, C*H*$_2$), 2.82 (dd, *J* = 13.6, 9.9 Hz, 1H, C*H*$_2$), 2.24 (s, 3H, OCOC*H*$_3$), 1.72 (s, 3H, NHCOC*H*$_3$).

HRMS (ESI) $C_{23}H_{25}N_3O_8$+Na^+ (M+Na^+) Calculated for 494.1534, found 494.1677.

$C_{46}H_{50}N_6O_{16}$+Na^+ (2M+Na^+) Calculated for 965.3176, found 965.3393.

_N_-Acetyl-2-nitro-L-phenylalanyl-_O_-acetyl-L-tyrosine methyl ester (14b)*

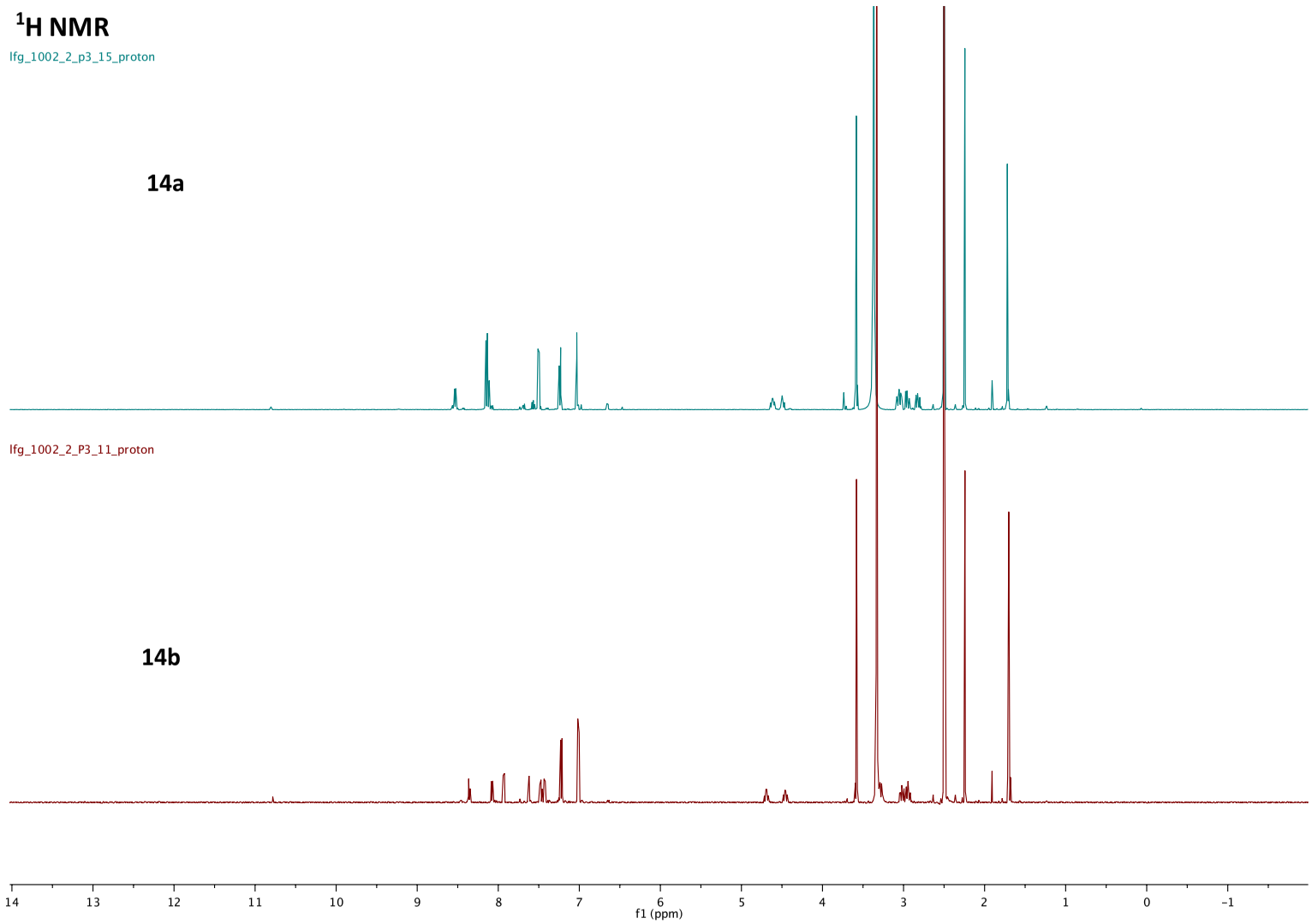


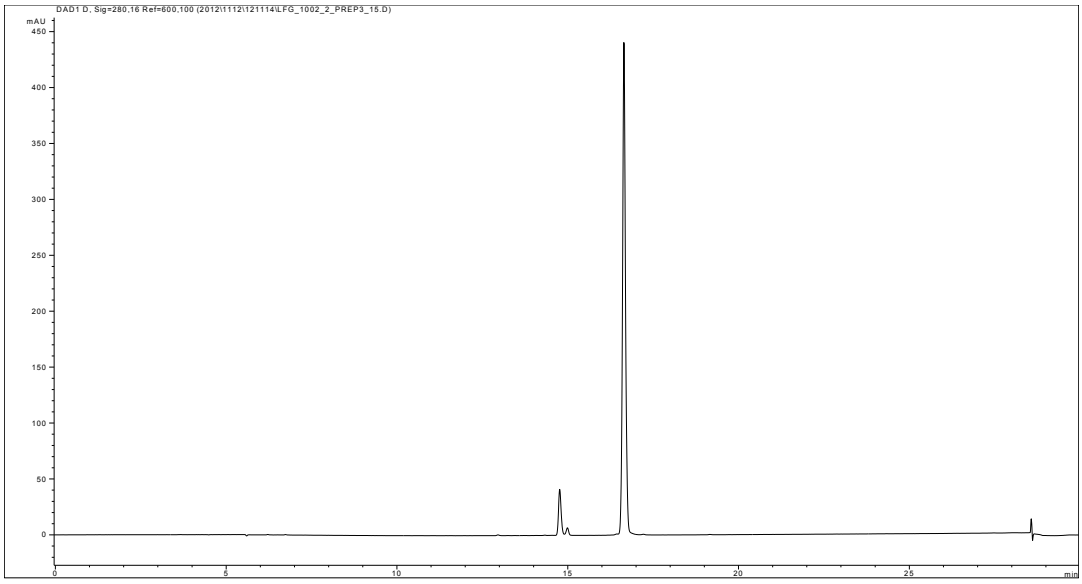
^{1}H NMR (500 MHz, DMSO-d6) δ 8.36 (d, *J* = 7.7 Hz, 1H, N*H*), 8.08 (d, *J* = 8.8 Hz, 1H, N*H*), 7.93 (dd, *J* = 8.1, 1.4 Hz, 1H, 2-*H*), 7.62 (td, *J* = 7.6, 1.4 Hz, 1H, 4-*H*), 7.50 – 7.45 (m, 1H, 3-*H*), 7.43 (dd, *J* = 7.8, 1.4 Hz, 1H, 5-*H*), 7.25 – 7.20 (m, 2H, 14,18-*H*), 7.04 – 6.99 (m, 2H, 15,17-*H*), 4.69 (td, *J* = 9.1, 5.2 Hz, 1H, 8-*H*), 4.46 (ddd, *J* = 8.8, 7.5, 5.7 Hz, 1H, 11-*H*), 3.58 (s, 3H, COOC*H*$_3$), 3.28 (dd, *J* = 14.4, 5.2 Hz, 1H, C*H*$_2$), 3.12 – 2.80 (m, 3H, C*H*$_2$), 2.24 (s, 3H, OCOC*H*$_3$), 1.70 (s, 3H, NHCOC*H*$_3$).

HRMS (ESI) $C_{23}H_{25}N_3O_8$+Na^+ (M+Na^+) Calculated for 494.1534, found 494.1761.

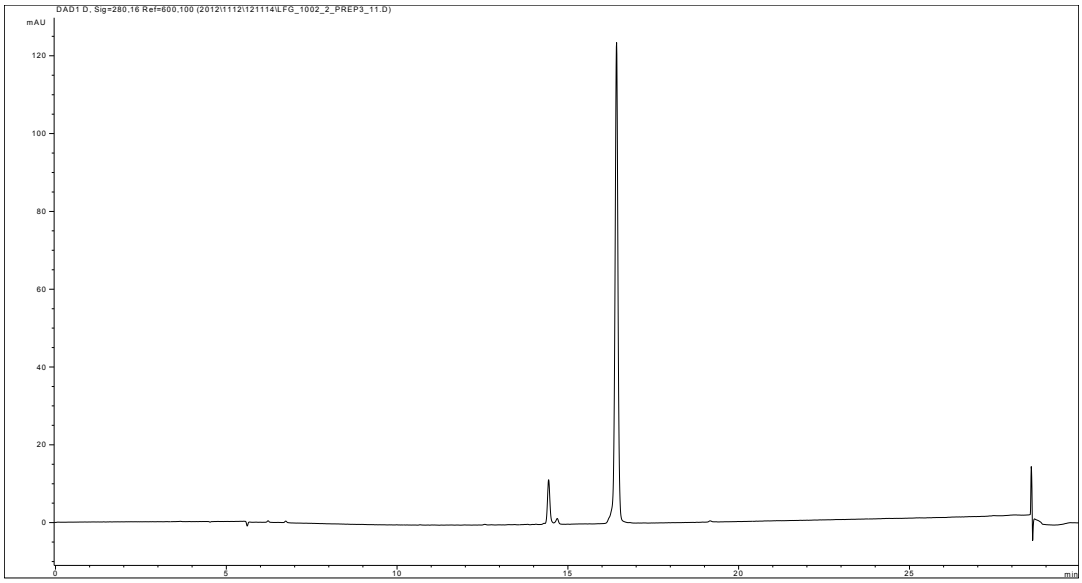
$C_{46}H_{50}N_6O_{16}$+Na^+ (2M+Na^+) Calculated for 965.3176, found 965.3612.

* Due to small amounts obtained following required repeated purifications by HPLC ^{13}C NMR spectra could not be obtained.


^{1}H NMR
lfg_1002_2_p3_15_proton
14a
lfg_1002_2_P3_11_proton
14b
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
−1
f1 (ppm)

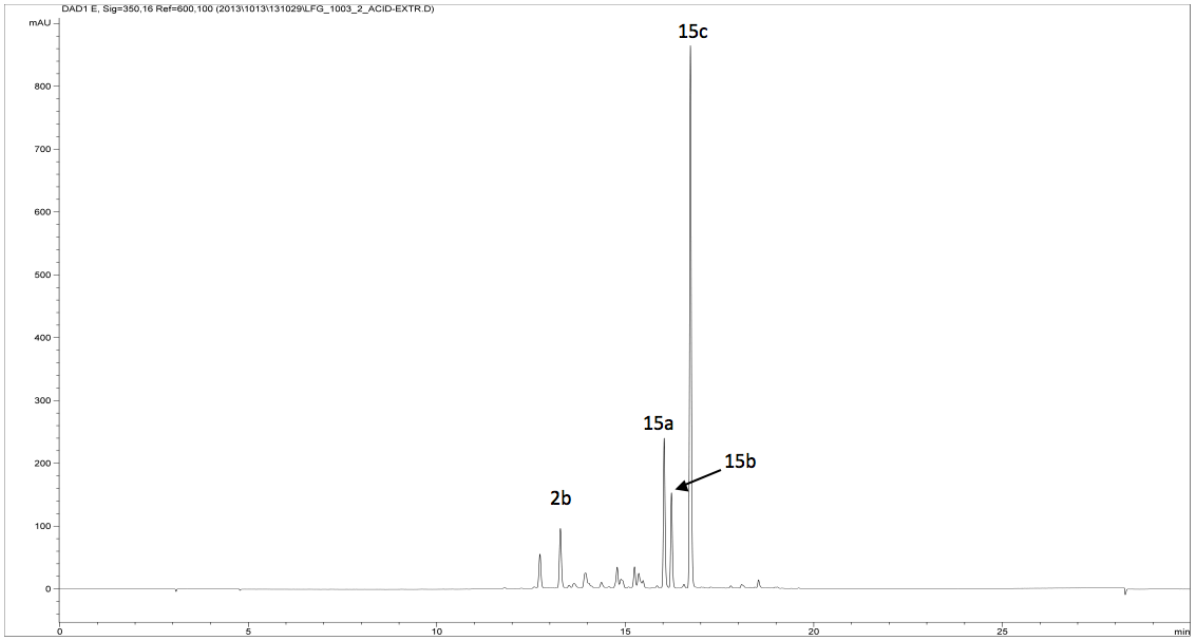


*HPLC analysis of **14a***



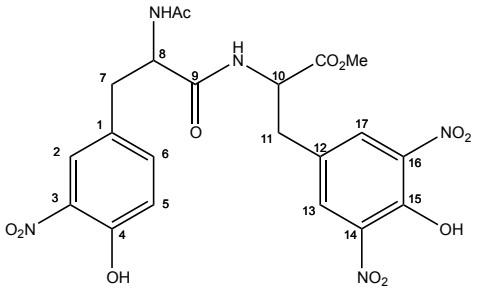
*HPLC analysis of **14b***

1.3.4 Reaction of Tyr-Tyr (10) with NO_2• and O_3



*HPLC analysis of the reaction of NO_2• and O_3 with Tyr-Tyr (**10**)*

***N*-Acetyl-3-nitro-L-tyrosyl-3,5-dinitro-L-tyrosine methyl ester (15a)**



^{1}H NMR (400 MHz, DMSO-d_6) δ 10.75 (s, 1H, 4-O*H*), 8.50 (d, J = 8.0 Hz, 1H, N*H*), 8.08 (s, 2H, 13,17-*H*), 8.02 (d, J = 8.4 Hz, 1H, N*H*), 7.74 (d, J = 2.2 Hz, 1H, 2-*H*), 7.36 (dd, J = 8.6, 2.2 Hz, 1H, 6-H), 7.02 (d, J = 8.5 Hz, 1H, 5-*H*), 4.57 (ddd, J = 9.4, 8.0, 5.3 Hz, 1H, 8-*H*), 4.40 (td, J = 9.0, 5.1 Hz, 1H, 10-*H*), 3.60 (s, 3H, COOCH_3), 3.12 (dd, J = 14.0, 5.3 Hz, 1H, CH_2), 2.95 (dd, J = 14.0, 9.4 Hz, 1H, CH_2), 2.85 (dd, J = 13.8, 5.1 Hz, 1H, CH_2), 2.64 (dd, J = 13.7, 9.5 Hz, 1H, CH_2), 1.68 (s, 3H, NHCOCH_3).

^{13}C NMR (101 MHz DMSO-d_6) δ 171.14 (C_q, *C*-9), 171.13 (C_q, *C*OOCH$_3$), 168.97 (C_q, NH*C*OCH$_3$), 150.80 (C_q, *C*-4), 145.09 (C_q, *C*-15), 139.69 (C_t, *C*-6), 136.34 (C_q, *C*-14,16), 136.07 (C_q, *C*-3), 130.46 (C_t, *C*-13,17), 128.97 (C_q, *C*-12), 127.64 (C_q, *C*-1), 125.28 (C_t, *C*-2), 118.80 (C_t, *C*-5), 53.56 (C_t, *C*-8), 52.57 (C_t, *C*-10), 52.03 (C_p, COO*C*H$_3$), 36.11 (C_s, *C*-7), 34.68 (C_s, *C*-11), 22.18 (C_p, NHCO*C*H$_3$).

HRMS (ESI) $C_{21}H_{21}N_5O_{12}+H^+$ (M+H$^+$) Calculated for 536.1259, found 536.1272.

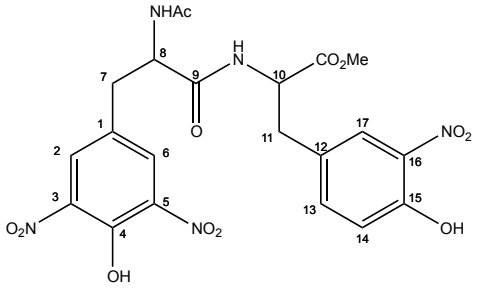
$C_{42}H_{42}N_{10}O_{24}+H^+$ (2M+H$^+$) Calculated for 1071.2446, found 1071.2424.

Collision Induced Dissociation (Low resolution tandem MS-MS, ESI)

	$C_{10}H_{11}N_3O_7$+H^+ (y_1+H^+)	Calculated for 286.07, found 286.08.
	$C_{11}H_{11}N_2O_5^+$ (b_1^+)	Calculated for 251.07, found 251.08.

_N_-Acetyl-3,5-dinitro-L-tyrosyl-3-nitro-L-tyrosine methyl ester (15b)

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.81 (s, 1H, 15-O*H*), 8.50 (d, J = 7.7 Hz, 1H, N*H*), 8.19 – 8.00 (m, 3H, obsc., N*H*, 2,6-*H*), 7.73 (d, J = 2.2 Hz, 1H, 17-*H*), 7.39 (dd, J = 8.6, 2.3 Hz, 1H, 13-*H*), 7.03 (d, J = 8.5 Hz, 1H, 14-*H*), 4.51 (dtd, J = 13.5, 8.6, 8.2, 5.3 Hz, 2H, 8,10-*H*), 3.59 (s, 3H, COOCH_3), 3.02 (dd, J = 13.9, 5.7 Hz, 1H, CH_2), 3.00 – 2.85 (m, 2H, CH_2), 2.72 (dd, J = 13.7, 9.4 Hz, 1H, CH_2), 1.73 (s, 3H, NHCOCH_3).

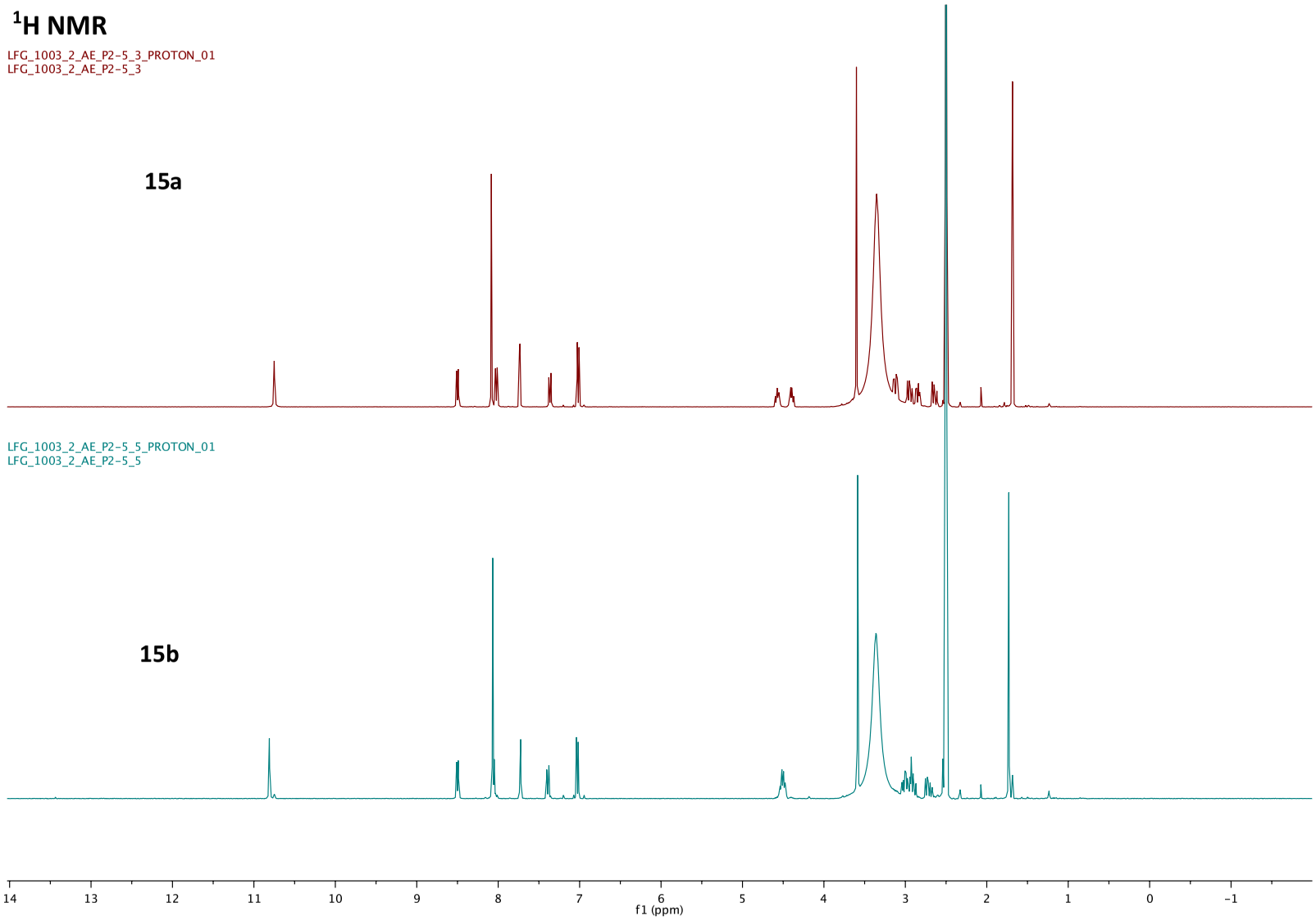


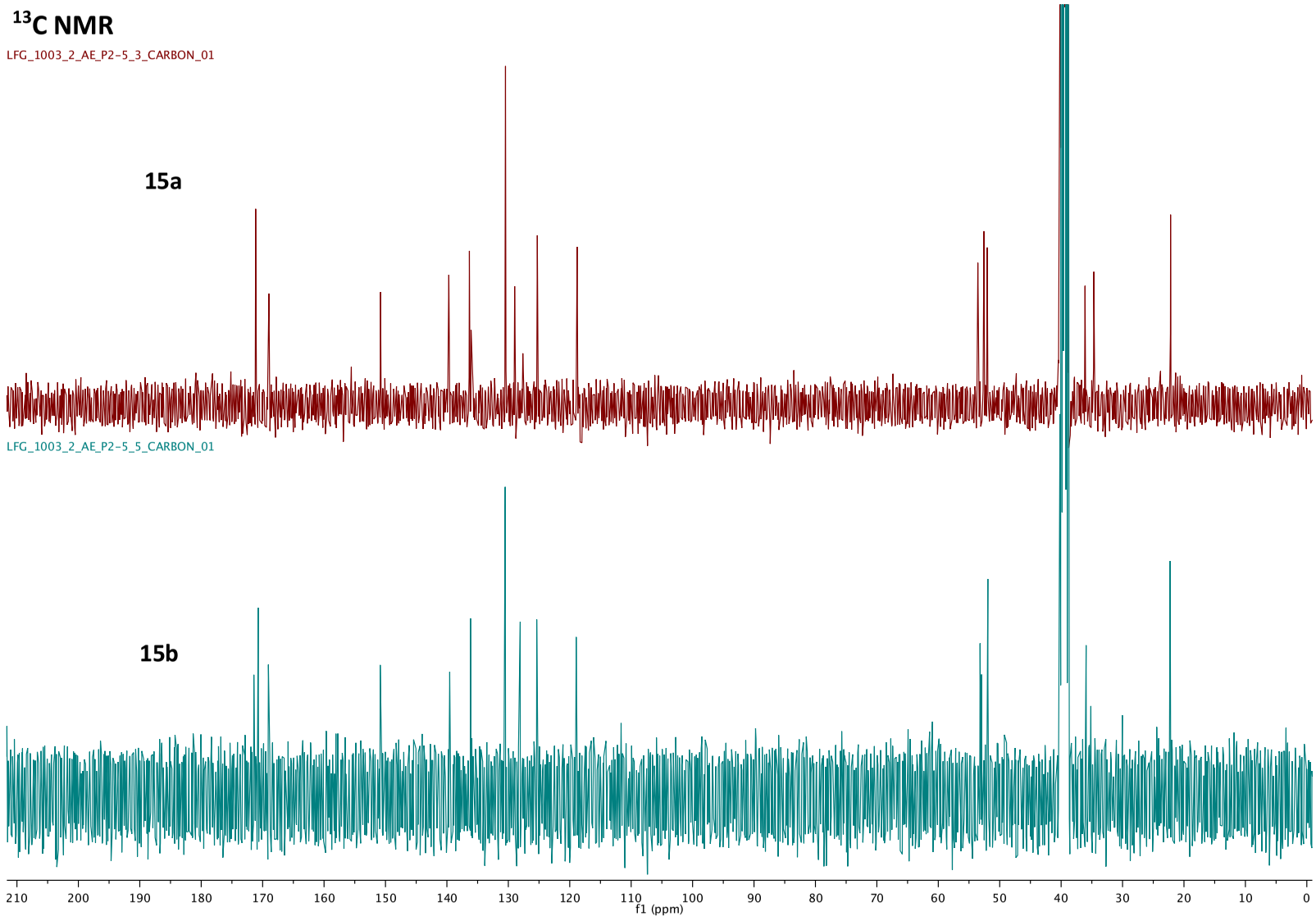
^{13}C NMR (101 MHz, DMSO-d_6) δ 171.43 (C_q, *C*-9), 170.72 (C_q, *C*OOCH$_3$), 169.06 (C_q, NH*C*OCH$_3$), 150.85 (C_q, *C*-15), 139.54 (C_t, *C*-13), 136.15 (C_q, *C*-3,5), 130.51 (C_t, *C*-2,6), 128.06 (C_q, *C*-1), 125.38 (C_q, *C*-12), 118.95 (C_q, *C*-14), 53.19 (C_t, *C*-8), 52.97 (C_t, *C*-10), 51.93 (C_p, COO*C*H$_3$), 35.93 (C_s, *C*-7), 35.16 (C_s, *C*-11), 22.25 (C_p, NHCO*C*H$_3$).

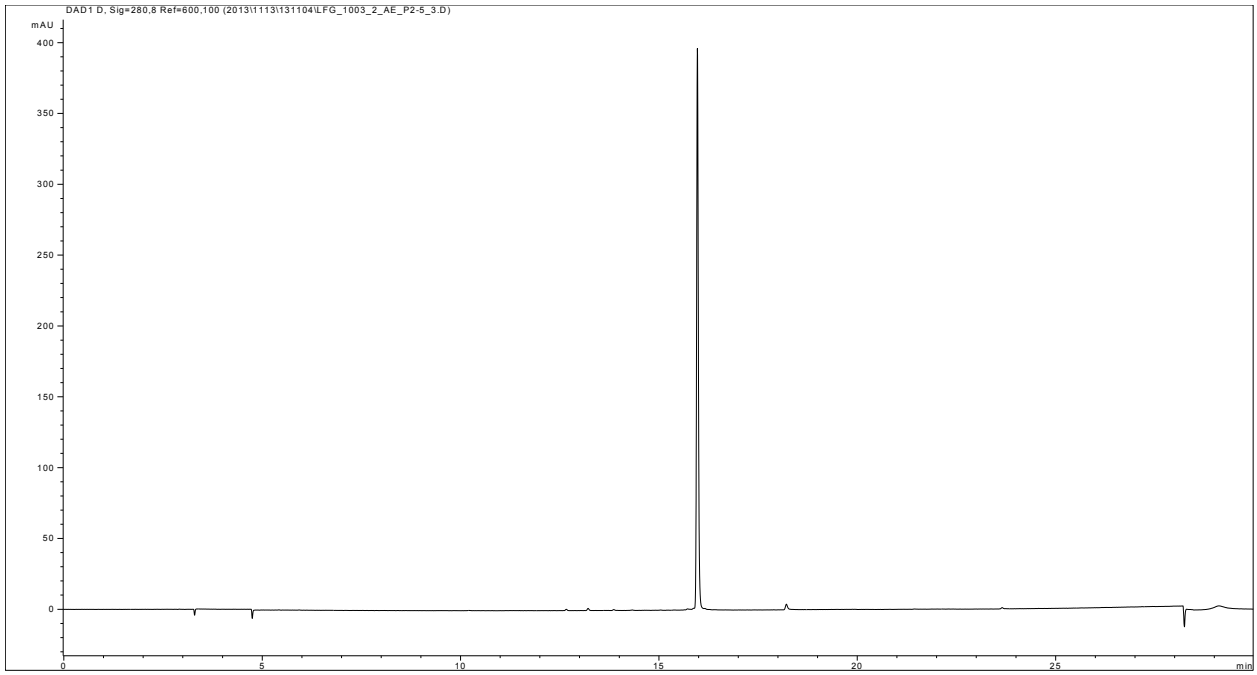
HRMS (ESI)	$C_{21}H_{21}N_5O_{12}$+H^+ (M+H^+)	Calculated for 536.1259, found 536.1270.
	$C_{42}H_{42}N_{10}O_{24}$+H^+ (2M+H^+)	Calculated for 1071.2446, found 1071.2417.

Collision Induced Dissociation (Low resolution tandem MS-MS, ESI)

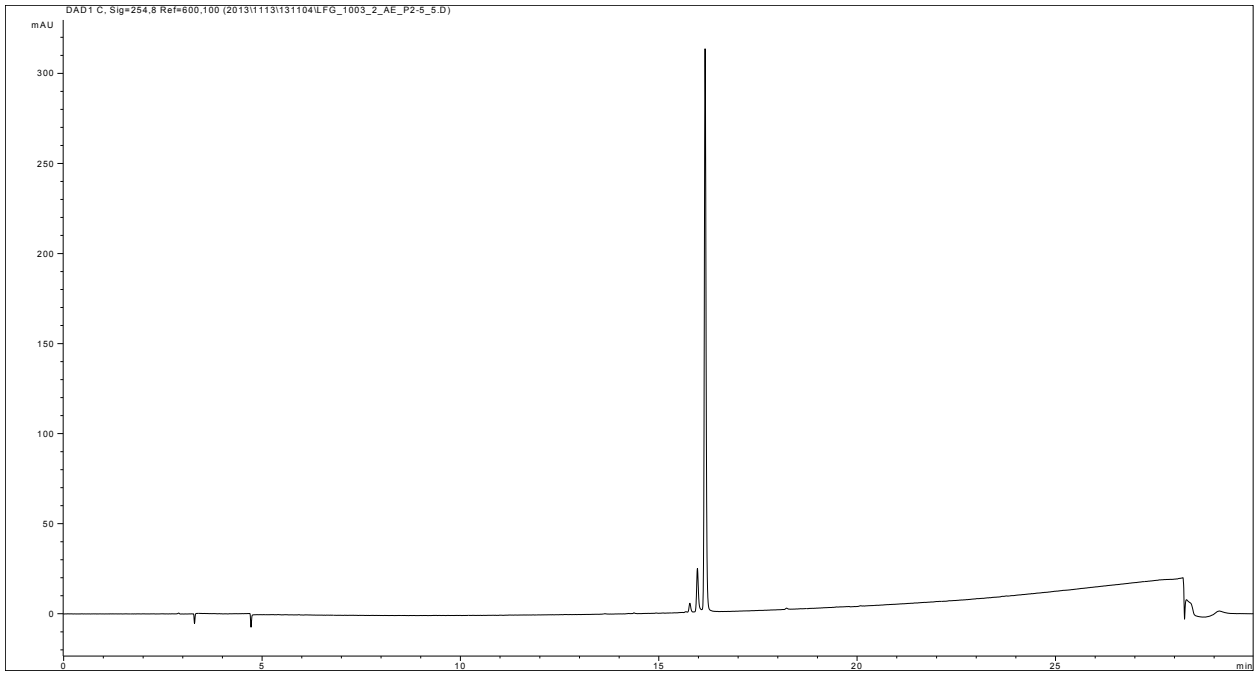
	$C_{10}H_{12}N_2O_5$+H^+ (y_1+H^+)	Calculated for 241.08, found 241.08.


1H NMR
LFG_1003_2_AE_P2-5_3_PROTON_01
LFG_1003_2_AE_P2-5_3
15a
LFG_1003_2_AE_P2-5_5_PROTON_01
LFG_1003_2_AE_P2-5_5
15b
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
f1 (ppm)


^{13}C NMR
LFG_1003_2_AE_P2-5_3_CARBON_01
15a
LFG_1003_2_AE_P2-5_5_CARBON_01
15b
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

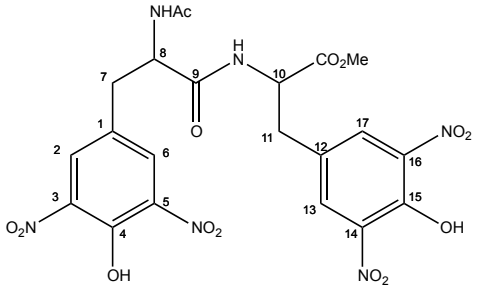


*HPLC analysis of **15a***



*HPLC analysis of **15b***

_N_-Acetyl-3,5-dinitro-L-tyrosyl-3,5-dinitro-L-tyrosine methyl ester (15c)



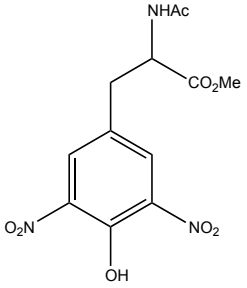
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.53 (d, J = 7.9 Hz, 1H, NH), 8.10 (s, 2H, 2,6-H), 8.06 (s, 2H, obs., 13,17-H), 8.06 (d, J = 8.4 Hz, 1H, obsc., NH), 4.58 (ddd, J = 9.6, 8.0, 5.3 Hz, 1H, 8-H), 4.45 (td, J = 8.8, 5.4 Hz, 1H, 10-H), 3.60 (s, 3H, COOCH_3), 3.13 (dd, J = 14.0, 5.3 Hz, 1H, CH_2), 3.03 – 2.86 (m, 2H, CH_2), 2.74 (dd, J = 13.7, 9.2 Hz, 1H, CH_2), 1.70 (s, 3H, NHCOCH_3).

^{13}C NMR (101 MHz, DMSO-d_6) δ 171.12 (C_q, C-9), 170.73 (C_q, COOCH$_3$), 169.06 (C_q, NHCOCH$_3$), 144.71 (C_q, C-OH), 144.69 (C_q, C-OH), 139.59 (C_q, C-NO$_2$), 139.47 (C_q, C-NO$_2$), 130.45 (C_t, C-2,6), 130.42 (C_t, C-13,17), 128.78 (C_q, C-12), 128.16 (C_q, C-1), 53.13 (C_t, C-10), 52.55 (C_t, C-8), 52.04 (C_p, COOCH$_3$), 35.75 (C_s, C-11), 34.70 (C_s, C-7), 22.13 (C_p, NHCOCH$_3$).

HRMS (ESI) $C_{21}H_{20}N_6O_{14}+H^+$ (M+H$^+$) Calculated for 581.1110, found 581.1122.

$C_{42}H_{40}N_{12}O_{28}+H^+$ (2M+H$^+$) Calculated for 1161.2148, found 1161.2126.

_N_-Acetyl-3,5-dinitro-L-tyrosine methyl ester (2b)

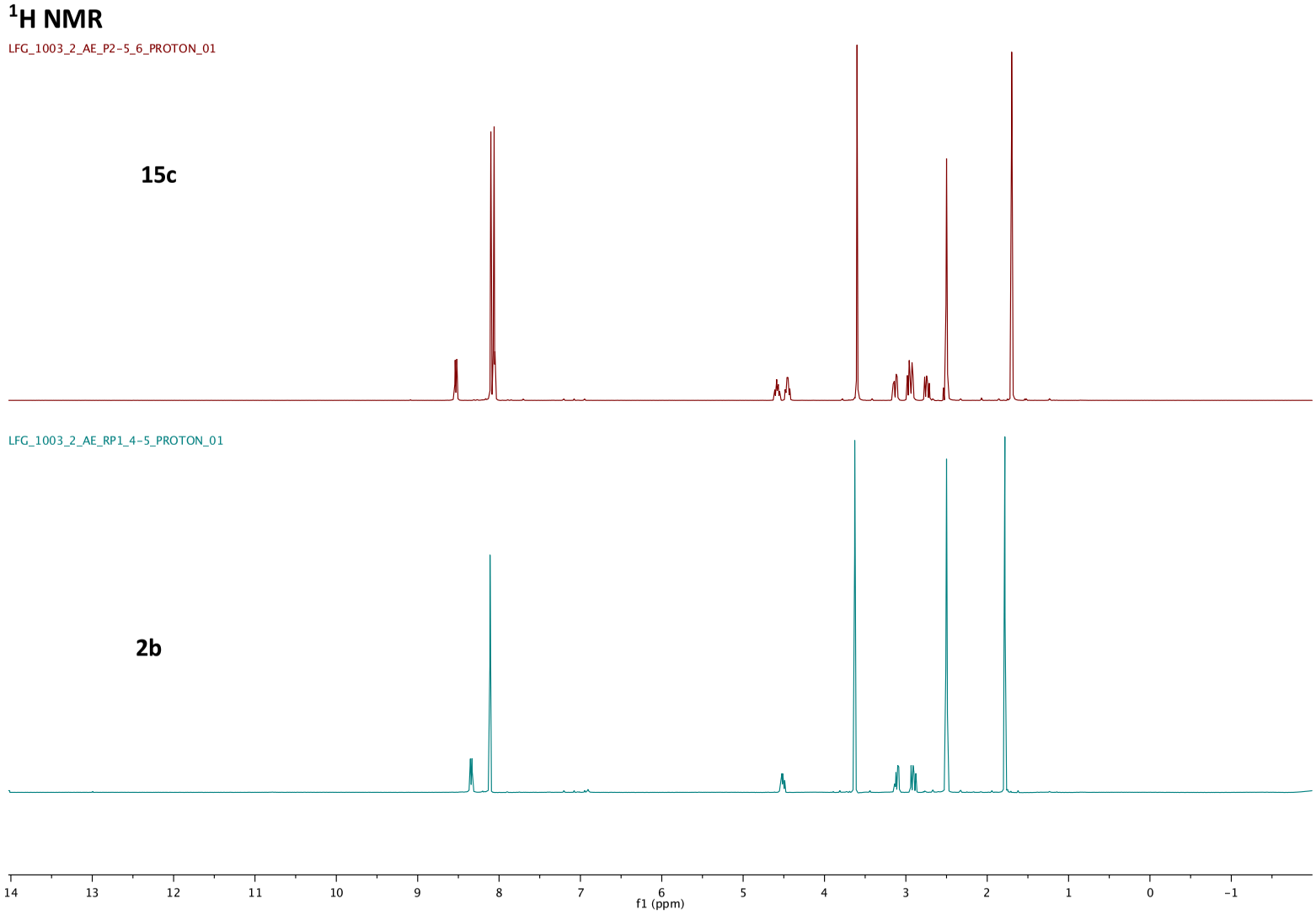


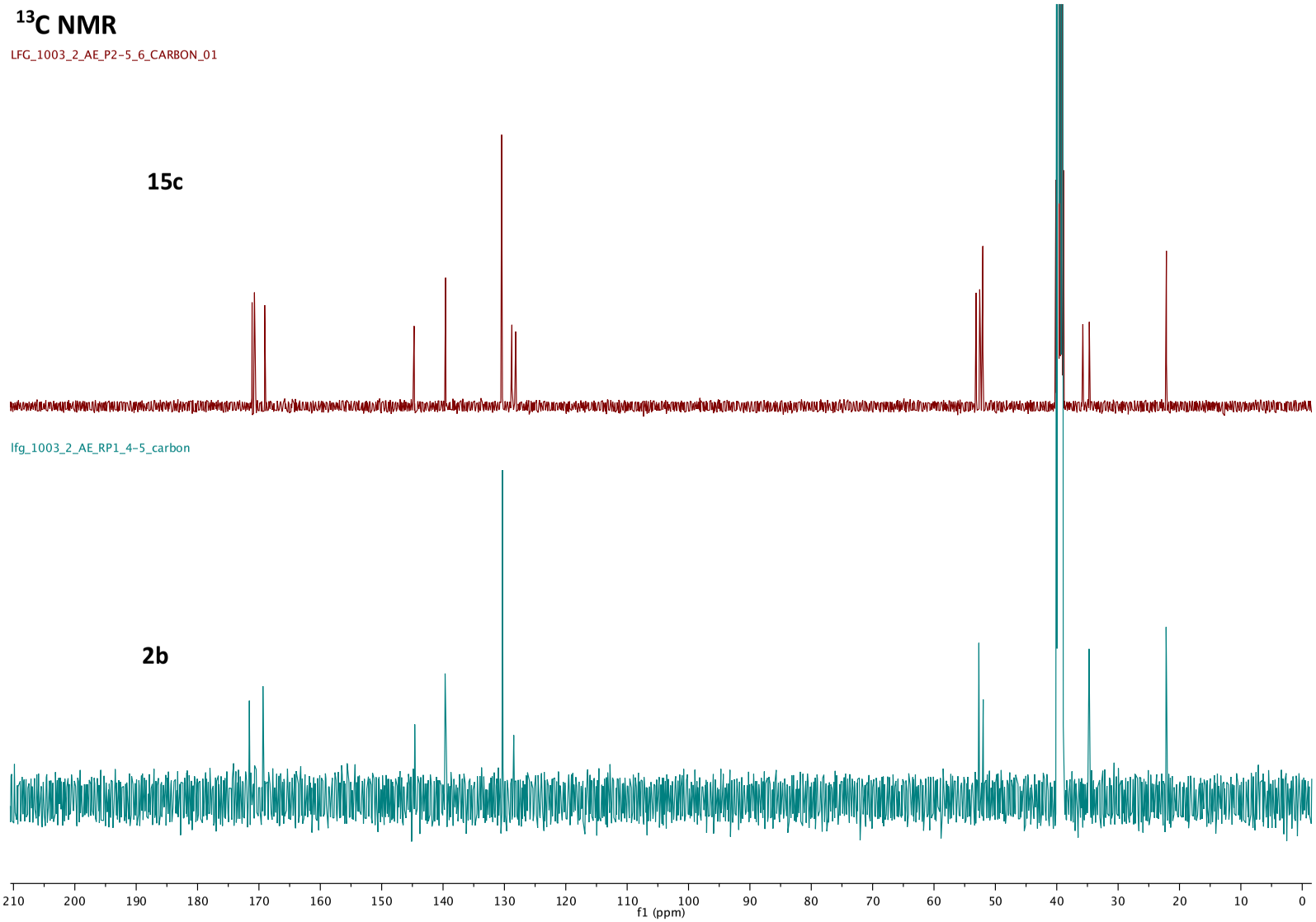
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.34 (d, J = 8.1 Hz, 1H, NH), 8.11 (s, 2H, 2,6-H), 4.52 (ddd, J = 9.6, 8.0, 5.2 Hz, 1H, CH), 3.63 (s, 3H, COOCH_3), 3.11 (dd, J = 13.9, 5.2 Hz, 1H, CH_2), 2.91 (dd, J = 13.9, 9.7 Hz, 1H, CH_2), 1.78 (s, 3H, NHCOCH_3).

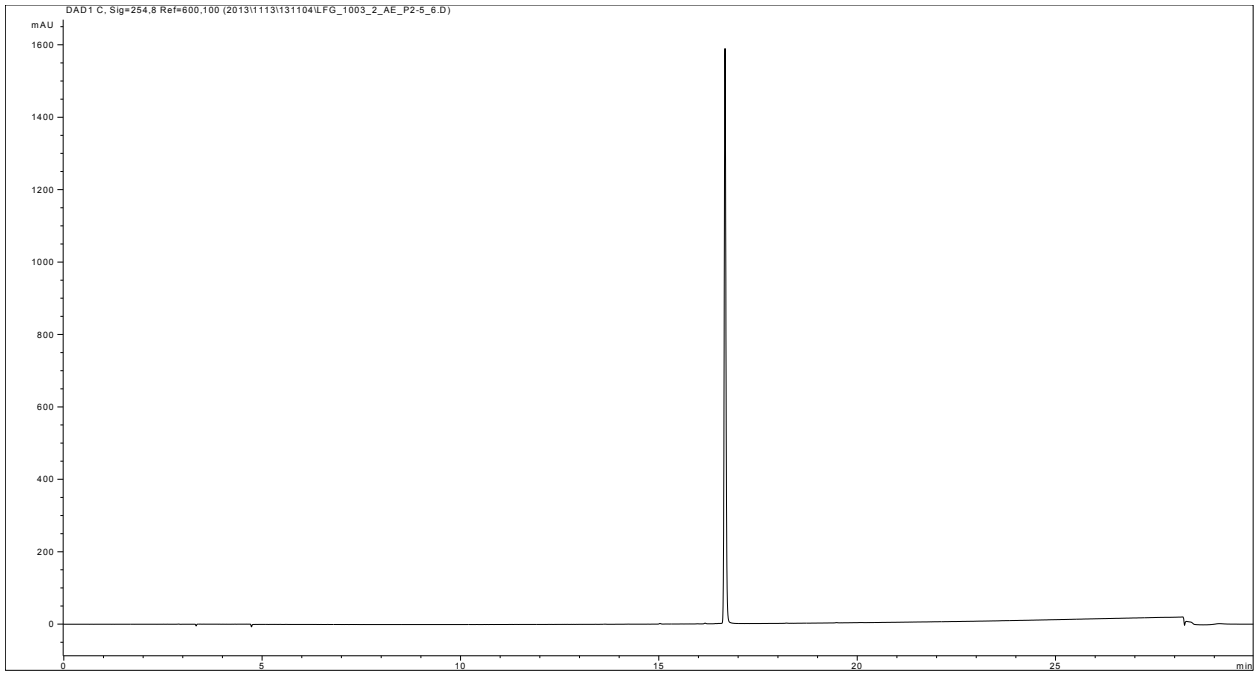
^{13}C NMR (126 MHz, DMSO-d_6) δ 171.56 (C_q, COOCH$_3$), 169.35 (C_q, NHCOCH$_3$), 144.61 (C_q, C-6), 139.66 (C_q, C-3,5), 130.31 (C_t, C-2,4), 128.48 (C_q, C-1), 52.69 (C_t, CH), 51.99 (C_p, COOCH$_3$), 34.73 (C_s, CH$_2$), 22.18 (C_p, NHCOCH$_3$).

HRMS (ESI) $C_{12}H_{13}N_3O_8+H^+$ (M+H$^+$) Calculated for 328.0775, found 328.0625.

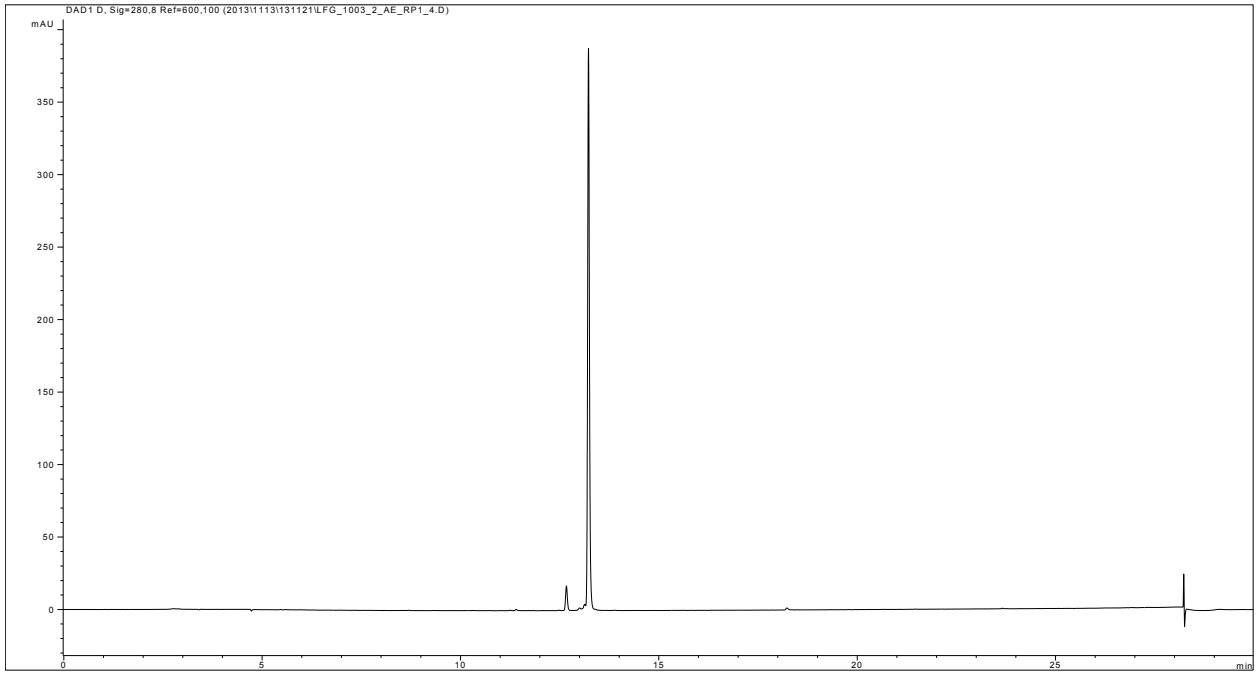
$C_{24}H_{26}N_6O_{16}+H^+$ (2M+H$^+$) Calculated for 655.1479, found 655.1366.


^{1}H NMR
LFG_1003_2_AE_P2-5_6_PROTON_01
15c
LFG_1003_2_AE_RP1_4-5_PROTON_01
2b
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
f1 (ppm)


^{13}C NMR
LFG_1003_2_AE_P2-5_6_CARBON_01
15c
lfg_1003_2_AE_RP1_4-5_carbon
2b
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

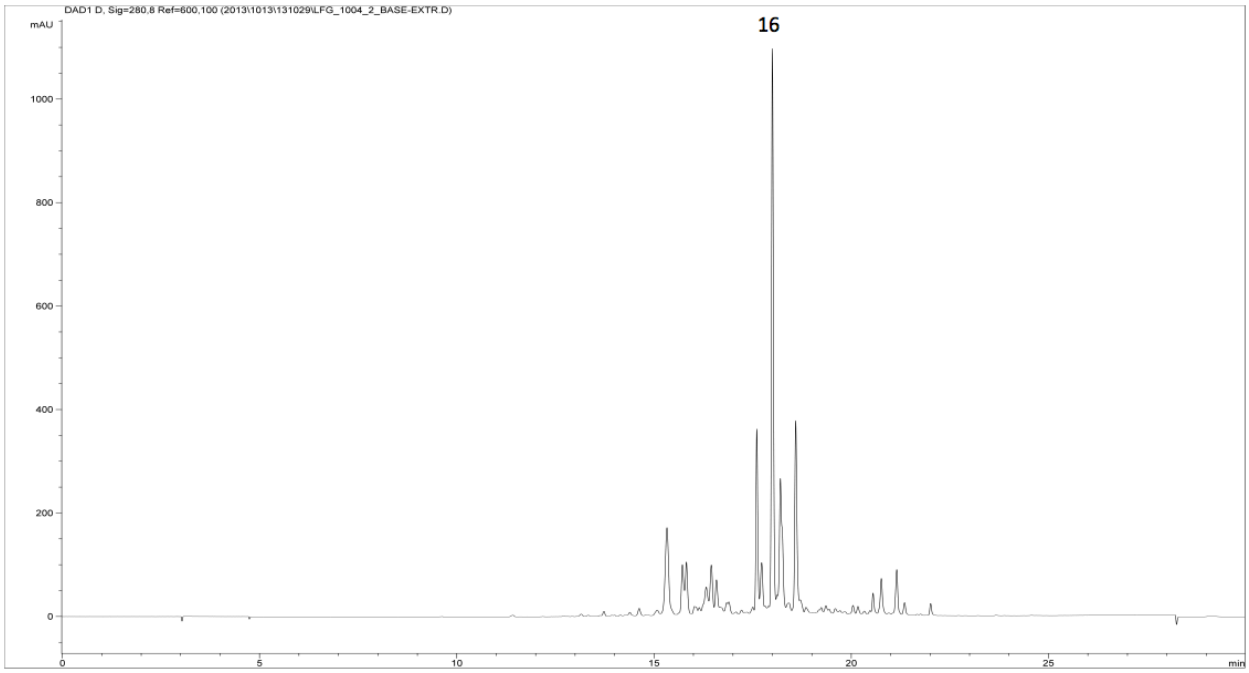


*HPLC analysis of **15c***



*HPLC analysis of **2b***

1.3.5 Reaction of Phe-Trp (11) with NO_2• and O_3



*HPLC analysis of the reaction of NO_2• and O_3 with Phe-Trp (**11**)**

*Crystal structure data for compound **16** can be found in section **2.1**.

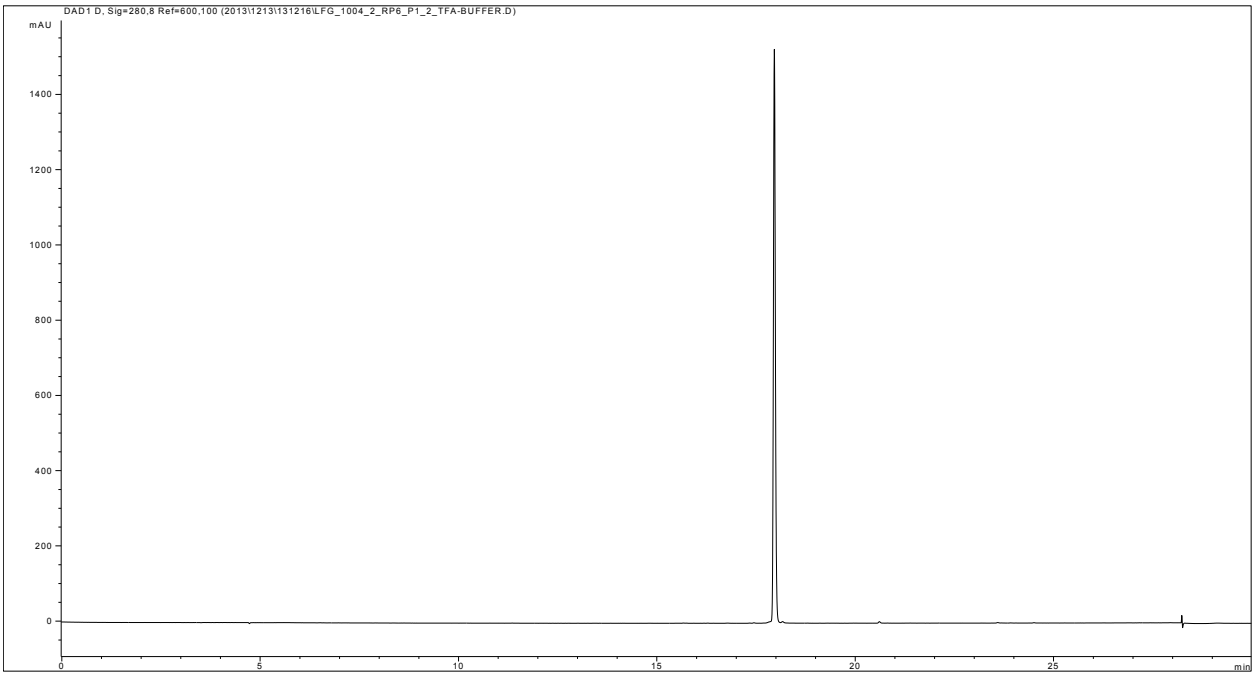
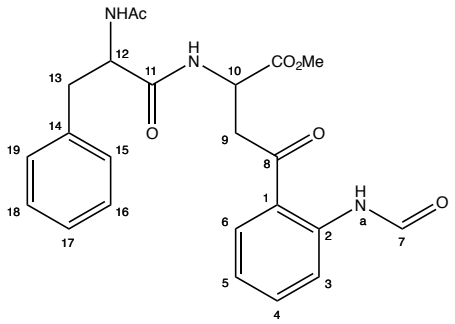


*Figure S33 : HPLC spectrum of **16***

_N_-Acetyl-phenylalanyl-N_a-formyl-kynurenine methyl ester (17)*

^{1}H NMR (500 MHz, Chloroform-*d*) δ 11.28 (s, 1H, 8-*H*), 8.74 (d, *J* = 8.4 Hz, 1H, 6-*H*), 8.48 (s, 1H, N_a-*H*), 7.87 (dd, *J* = 8.1, 1.5 Hz, 1H, 3-*H*), 7.60 (t, *J* = 8.2 Hz, 1H, 5-*H*), 7.37 – 7.11 (m, 6H, Ar-H), 6.88 (d, *J* = 7.8 Hz, 1H, N*H*), 6.37 (d, *J* = 7.6 Hz, 1H, N*H*), 4.88 (dt, *J* = 8.0, 4.1 Hz, 1H, C*H*), 4.71 (q, *J* = 7.0 Hz, 1H, C*H*), 3.76 (dd, *J* = 18.1, 4.2 Hz, 1H, CH_2), 3.73 (s, 3H, COOCH_3), 3.62 (dd, *J* = 18.3, 4.2 Hz, 1H, CH_2), 3.09 (d, *J* = 6.7 Hz, 2H, CH_2), 1.97 (s, 3H, NHCOCH_3).

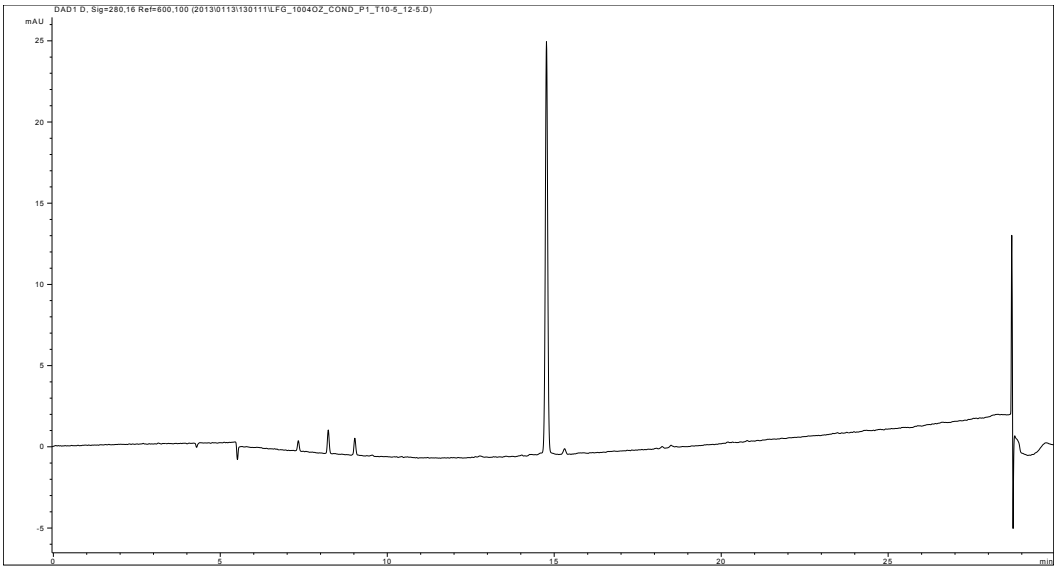


^{13}C NMR (126 MHz Chloroform-*d*) δ 201.51 (*C*-8), 171.24 (*C*OOCH$_3$), 170.91 (NH*C*OCH$_3$, *C*-11), 160.30 (*C*-7), 136.10 (*C*-2), 135.80 (*C*-14), 131.08 (*C*-6), 131.01 (*C*-4), 129.38 (*C*-16,18), 128.89 (*C*-15,19), 128.80 (*C*-1), 127.38 (*C*-17), 123.52 (*C*-5), 122.02 (*C*-3), 54.60 (*C*-12), 53.10 (*C*-10), 48.55 (*C*-12), 41.59 (*C*-9), 38.37 (*C*-13), 22.93 (NHCO*C*H$_3$).

HRMS (ESI) $C_{23}H_{26}N_3O_6$+H^+ (M+H^+) Calculated for 440.1816, found 440.1893.

$C_{23}H_{26}N_3O_6$+H^+ (M+Na^+) Calculated for 462.1636, found 462.1715.

* Data in accordance with literature: X. Fang, F. Jin, H. Jin, and C. v. Sonntag, *J. Chem. Soc., Perkin Trans. 2*, 1998, 259, and C. Goeschen, N. Wibowo, J. M. White, U. Wille, *Org. Biomol. Chem.*, 2011, **9**, 3380.

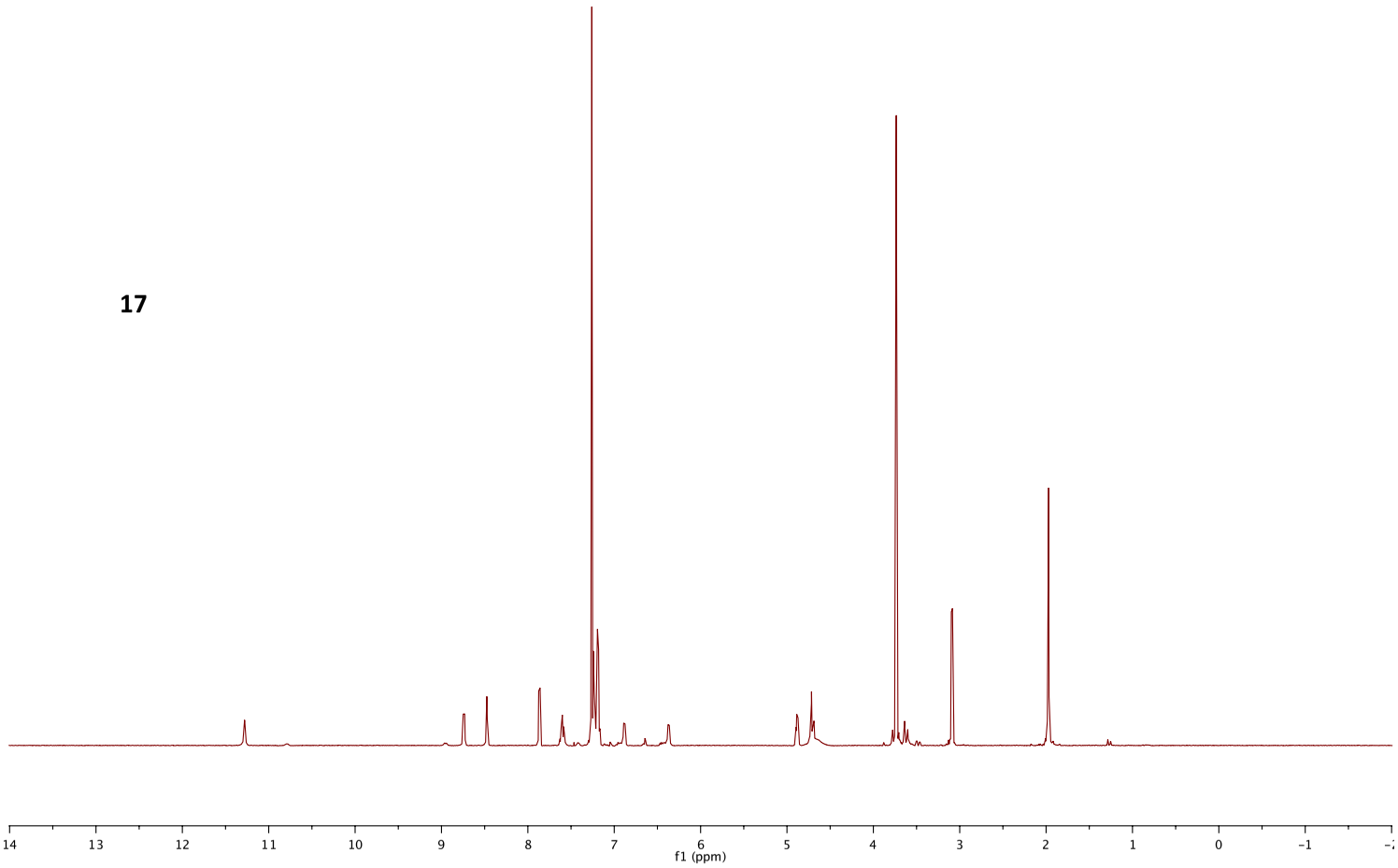


*HPLC analysis of **17***

^{1}H NMR

lfg_1004Oz_cond_p1_10-5_12-5_proton

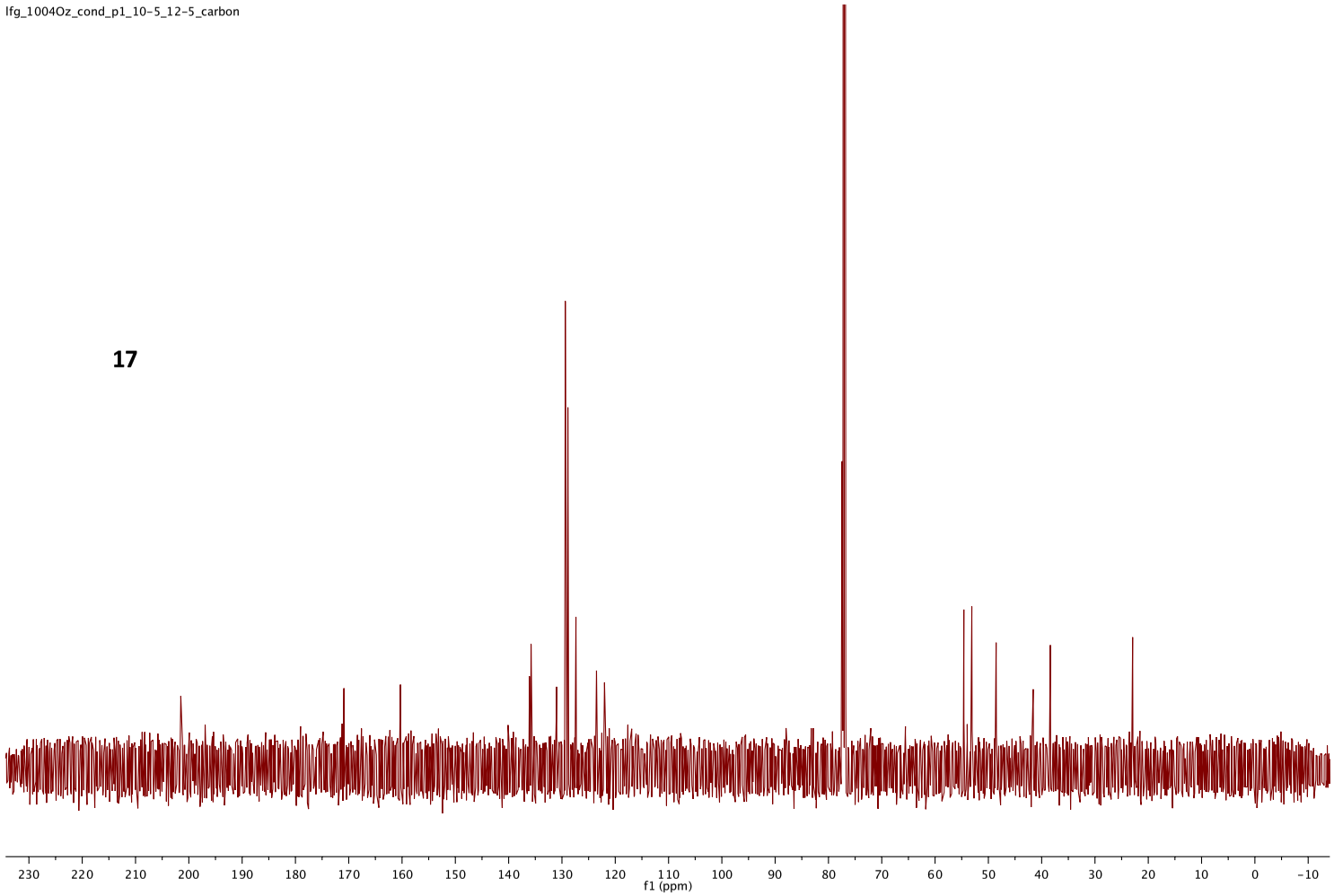
17



^{13}C NMR

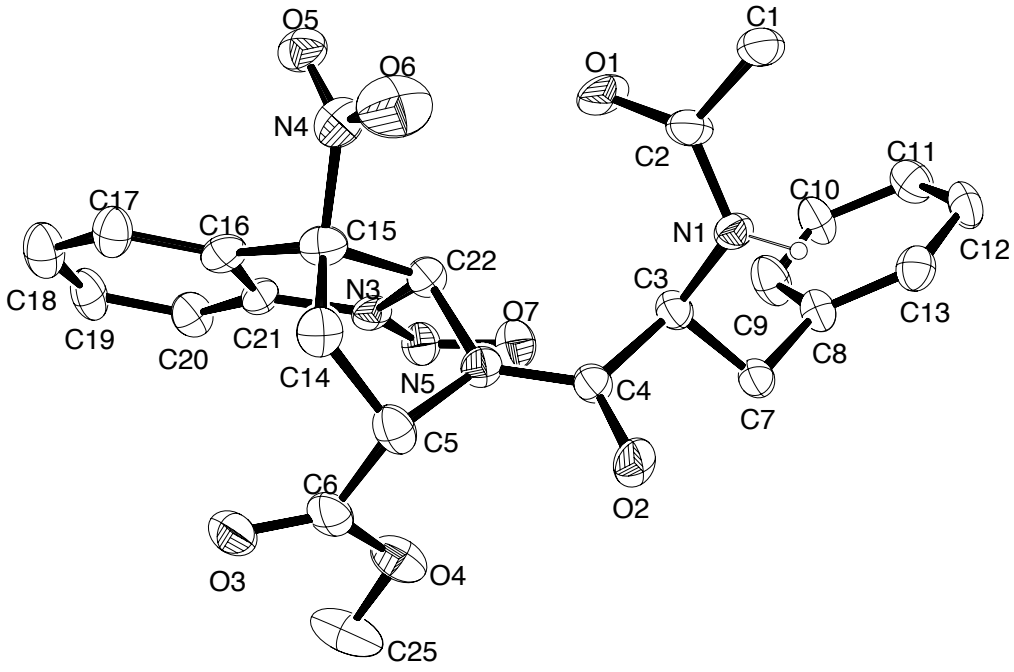
lfg_1004Oz_cond_p1_10-5_12-5_carbon

17



2. Crystallographic Data

2.1 Compound **16**



*Thermal ellipsoid plot for one of the two independent molecules of **16**. Ellipsoids are at the 20% probability level.*

Crystallography. Intensity data were collected with an Oxford Diffraction SuperNova CCD diffractometer using Cu-Kα microsource radiation (graphite crystal monochromator λ = 1.54184), The temperature during the data collections was maintained at 130.0(1). Structure solution,[i] and refinement were implemented within the WingX suite of programs.[ii] The structure contained ca. 1.5 molecules of acetonitrile per molecule of **16**, which was removed using the Squeeze procedure[iii]

Crystal data for **16** $C_{23}H_{23}N_5O_7$ 1.5(CH_3CN) M = 543.04, T = 130.0(2) K, λ = 1.5418 Å, Tetragonal, space group I4 a = 26.6311(9), b = 26.6311(9) c =15.8084(10), *Å* , V 11211.6(10) $Å^3$, Z = 16, Z' = 2, D_c = 1.287 Mg M^{-3} μ(Cu-Kα) 0.800 mm^{-1}, F(000) = 4560, crystal size 0.28 x 0.10 x 0.05 mm. 12681 reflections measured, 8318 independent reflections (R_{int} = 0. 077) the final R was 0.0855 [I > 2σ(I)] and wR(F^2) was 0.2326 (all data).

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[i] G.M. Sheldrick, *Acta Cryst.* 2008, **A64**, 112.
[ii] L. J. Farrugia, *J. Appl. Crystallogr.*, 1999, **32**, 837.
[iii] P. v.d. Sluis, and A. L. Spek, *Acta Cryst. Sect. A*, 1990, **46**, 194.