Supporting Information

"Combinatorial Synthesis of a Small Molecule Library Based on the Vinyl Sulfone Scaffold"

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1. General

All chemicals were purchased from commercial sources and used directly, unless indicated otherwise. THF was distilled from sodium/benzophenone before use. All chemical reactions were run under N_2, unless otherwise indicated. The ^1H and ^{13}C NMR spectra were taken on a Bruker 300 MHz NMR spectrometer. Chemical shifts are reported in parts per million downfield from $SiMe_4$ (δ 0.0) and relative to the signal of chloroform-d (δ 7.26). LC was performed on a Waters Delta 600 HPLC equipped with a Phenomenex RP-18 (4.6 x 250 mm) column for analytical and Phenomenex RP-18 (21.2 x 250 mm) column for preparative, using an acetontrile–water gradient (with 0.1% TFA). MS was run on an electrospray mass spectrometer (Finnigan, USA).

2. General Procedure for the Preparation of Vinyl Sulfone small molecules

2.1 4-[(diethoxyphosphoryl) thiomethyl] benzoic acid (2a)

To a dried flask flushed with N_2 was added 4-mercapto-benzoic acid (7.7 g, 50.0 mmol), 100 ml methanol (HPLC-grade), diethyl iodomethyl phosphonate (13.9 g, 50 mmol), and DBU (16 ml, 105 mmol). The reaction was stirred at room temperature for 6 h. The resulting mixture was neutralized by 5 M HCl solution, followed by addition of 400 ml ethyl acetate. The organic layer was washed with diluted HCl solution and brine, dried over $MgSO_4$ and concentrated to give an oily solid. The pure product was obtained by re-crystallization from hexane/ethyl acetate. Yield: 77.0% (11.7 g, 38.5 mmol). ^1H NMR (300 MHz, $CDCl_3$) δ 7.95 (d, J = 6.8 Hz, 2H), 7.41 (d, J = 8.4 Hz, 2H), 4.19 (p, 4H), 3.29 (d, J = 14.0 Hz, 2H), 1.32 (t, 6H). ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 22.5; ESI mass spectrum: $[M+H]^+$ calc: 305.2; found 305.0.

2.2 11-[(diethoxyphosphoryl) thiomethyl] undecanoic acid (2b)

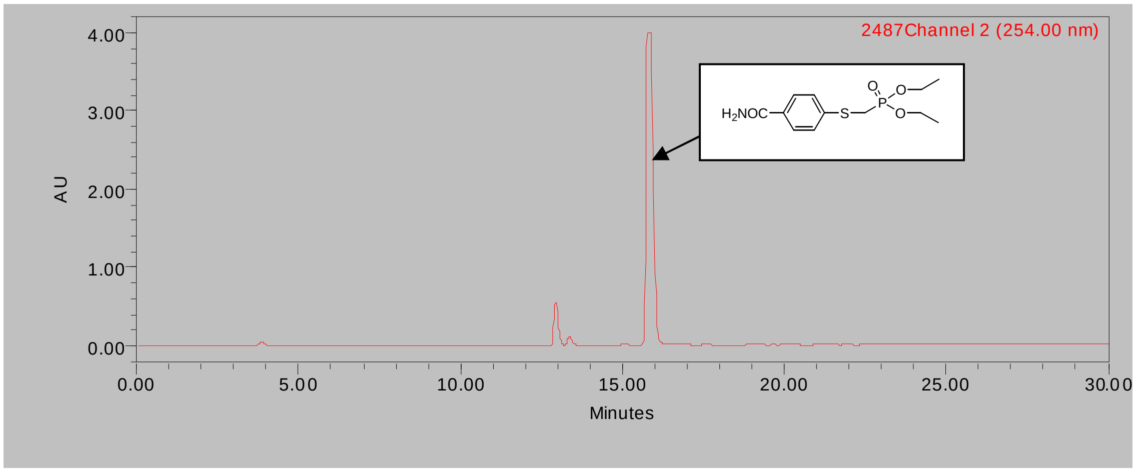
To a dried flask flushed with N_2 was added 11-mercapto-undecanoic acid (2.0 g, 9.2 mmol), 40 ml DMF (HPLC-grade), diethyl iodomethyl phosphonate (2.5 g, 9 mmol), and Cs_2CO_3 (5.9 g, 18.1 mmol). The reaction was stirred at room temperature overnight. The resulting mixture was neutralized by 5 M HCl solution, followed by addition of 200 ml ethyl acetate. The organic layer was washed with diluted HCl solution and brine, dried over $MgSO_4$ and concentrated to give an oily liquid. The pure product was obtained by flash chromatography. Yield: 85% (2.8 g, 7.6 mmol). ^1H NMR (300 MHz, $CDCl_3$) δ 4.1-4.3 (m, 4H), 2.73 (d, J = 13.8 Hz, 2H), 2.65-2.70 (m, 2H), 2.35 (t, J = 7.4 Hz, 2H), 1.55-1.75 (m, 4H), 1.20-1.45 (m, 18H); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 24.9; ESI mass spectrum: $[M+H]^+$ calc: 369.2; found 369.0.

2.3 2-[(diethoxyphosphoryl) thiomethyl] nicotinic acid (2c)

To a dried flask flushed with N_2 was added 2-mercapto-nicotinic acid (2.8 g, 18.0 mmol), 50 ml Methanol (HPLC-grade), diethyl iodomethyl phosphonate (5.56 g, 20 mmol), and DBU(7ml, 46mmol). The reaction was stirred at room temperature overnight. The resulting mixture was neutralized by 5 M HCl solution and concentrated to around 25 ml. 100 ml water was added and white solid precipitated from the solution. After filtration and washing the filtered solid with water and a small amount of ethyl acetate, the resulting white solid was dried *in vacuo* overnight. Yield: 69.2% (3.8 g, 12.4 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.57 (dd, J = 4.8, 2.0 Hz, 1H), 8.17 (dd, J = 7.6, 1.6 Hz, 1H), 7.09 (m, 1H), 4.20 (p, 4H), 3.77 (d, J = 13.6 Hz, 2H), 1.36 (t, 6H). ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 25.2; ESI mass spectrum: $[M+H]^+$ calc: 306.1; found 306.1.

2.4 Representative synthesis of Rink sulfide phosphonate resin (3a)

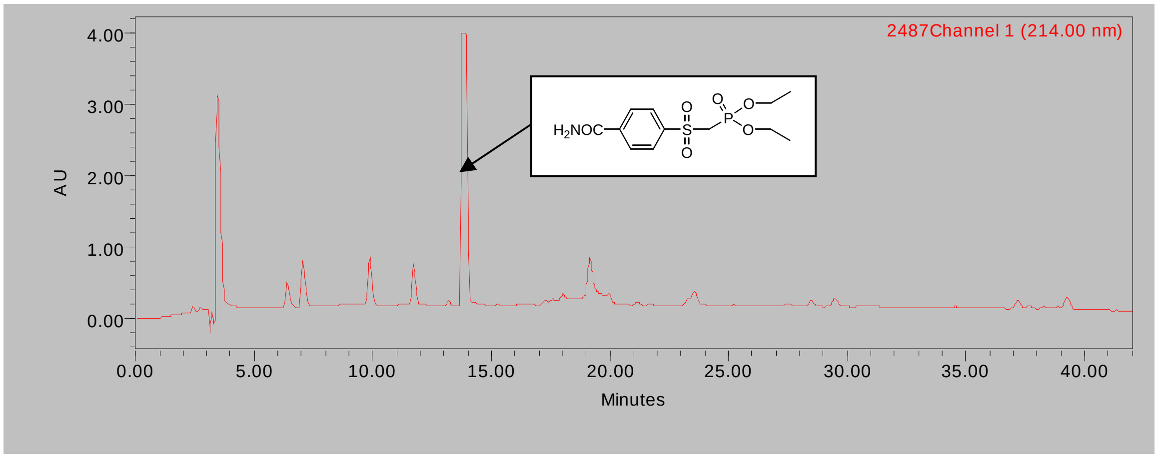
To 1.5 g (0.7 mmol/g, 1 mmol) rink amide resin in a 50-ml tube was added 15 ml 20% Piperidine (in DMF). After shaking for 30 min, the resin was filtered and washed with 5 x 15 ml DMF (HPLC-grade). The resin was transferred to a 15-ml tube. To the resin was added 4-[(diethoxyphosphoryl) thiomethyl] benzoic acid (1.0 g, 3.3 mmol, **2a**), HOBt (505 mg, 3.3 mmol), DIC (0.51 ml, 3.3 mmol), DIEA (1.0 ml, 6 mmol) and DMF (11 ml), the reaction mixture was shaken overnight, followed by addition of DIEA (1 ml) and acetic anhydride (0.5 ml). After another 20 min of reaction, the resulting resin was washed with DMF (AR grade, 5 x), DCM (HPLC, 3 x) and charged into a 15-ml tube for subsequent oxidation reaction. A small portion of the resin was cleaved with 50% TFA (in DCM; 30 min) and filtered glass pipette fitted with cotton wool. The filtration was evaporated and analyzed directly by HPLC-MS (below). ESI-MS for the cleaved product 4-[(diethoxyphosphoryl)-thiomethyl] benzamide, $[M+H]^+$ calc: 304.2; found 303.9.



HPLC condition: 10%-60% Acetonitrile in 30 min

2.5 Representative synthesis of rink sulfone phosphonate resin (4a)

To 1.5 g (0.7 mmol/g, 1 mmol) of rink sulfide phosphonate resin **3a**, prepared using above procedure, in a 15-ml tube was added DCM (12 ml, HPLC-grade) and *m*-CPBA (0.86 g, 3.5 mmol, 70%). The reaction mixture was shaken for 60-90 min, followed by washing with DMF (AR grade, 5 x), DCM (HPLC, 3 x) and MeOH (HPLC, 3 x), dried *in vacuo* for 24 h. The resulting resin was cleaved with 50% TFA (in DCM; 30 min) and the filtrate concentrated and directly analyzed by HPLC-MS (below). ESI-MS for the cleaved product 4-[(diethoxyphosphoryl-methyl) sulfonyl] benzamide, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.9-8.1 (m, 4H), 4.17 (p, 4H), 3.77 (d, *J* = 16.9 Hz, 2H), 1.31 (t, 6H), $[M+H]^+$ calc: 336.1; found 336.0.



HPLC condition: 10%-60% Acetonitrile in 30 min

2.6 Representative synthesis of Fmoc-AA-CHO (5)

To a dried flask flushed with N_2 was added Fmoc-AA-OH (3 mmol), N-hydroxybenzotriazole (0.51 g, 3.3 mmol), dicyclohexylcarbodiimide (0.68 g, 3.3 mmol) and 6 ml DMF. After stirring for 30 min at room temperature, a solid formed and was removed by filtration. N,O-dimethylhydroxylamine (0.35 g, 3.6 mmol), N,N'-diisopropylethylamine (0.46 g, 3.6 mmol) and another 4 ml DMF were added. After 3 h, the mixture was concentrated. The crude oil was dissolved in ethyl acetate and washed with saturated sodium bicarbonate, 0.1 M hydrochloric acid and brine. The organic layer, which contains Fmoc-AA-dimethylhydroxyl amide, was dried ($MgSO_4$), concentrated and further purified by flash chromatography. Yield: 80-99%. Subsequently, $LiAlH_4$ (137 mg, 3.6 mmol) was added to the amide (2.55 mmol) in THF (10 ml) at 0 °C. The reaction was stirred for 20 min and quenched by addition of 10 ml 5% $KHSO_4$. After aqueous work-ups, the pure product was obtained by purification with flash chromatography.

Fmoc-Asp(*t*Bu)-H: yield 86%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.64 (s, 1H), 7.20-7.90 (m, 8H), 4.10-4.50 (m, 4H), 2.86 (m, 2H), 1.31 (s, 9H). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 198.7, 170.1, 156.1, 143.6, 141.2, 127.7, 127.0, 125.0, 120.0, 82.1, 67.2, 56.5, 47.0, 35.6, 27.9. $[M]^+$ calc: 395.2; found 395.3.

Fmoc-Gln(Trt)-H: yield 76%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.43 (s, 1H), 7.10-7.90 (m, 23H), 4.10-4.60 (m, 4H), 2.30 (m, 2H), 1.70 (m, 2H). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ

198.6, 170.8, 156.4, 144.4, 143.7, 143.6, 141.3, 128.6, 127.9, 127.6, 127.0, 124.9, 119.9, 70.6, 66.6, 59.4, 47.2, 32.4, 24.5. $[M+H]^+$ calc: 595.3; found 595.7.

Fmoc-Leu-H: yield: 81.0%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.58 (s, 1H), 7.20-7.90 (m, 8H), 4.10-4.60 (m, 4H), 1.71 (br, 2H), 1.30 (br, 1H), 0.97 (d, *J* = 4.4 Hz, 6H). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 199.6, 156.1, 143.7, 141.2, 127.6, 127.0, 124.9, 119.9, 66.8, 58.7, 47.1, 38.0, 24.5, 23.0, 21.8. $[M]^+$ calc: 337.5; found 337.7.

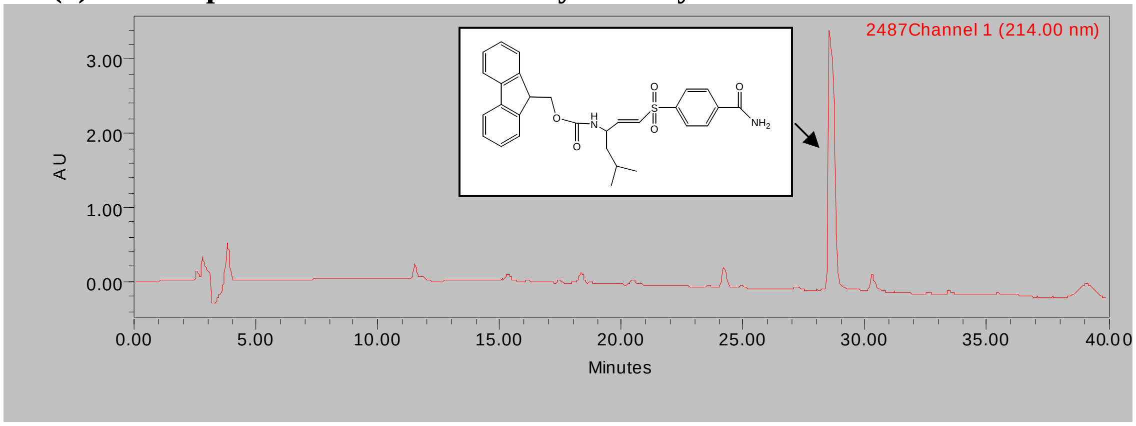
Fmoc-Lys(Boc)-H: yield 87%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.57 (s, 1H) 7.20-7.90 (m, 8H), 4.10-4.60 (m, 4H), 3.11 (br, 2H), 1.2-1.7 (m, 15H). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 199.2, 156.1, 143.6, 141.2, 127.6, 127.0, 124.9, 119.9, 79.2, 66.9, 60.0, 47.1, 39.7, 33.8, 29.7, 28.4, 22.0. $[M+H]^+$ calc: 453.2; found 452.7.

Fmoc-Tyr(*t*Bu)-H: yield 85%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.61 (s, 1H) 6.90-7.90 (m,12H), 4.10-4.50 (m, 4H), 3.10 (d, *J* = 6.4 Hz, 2H), 1.36 (s, 9H). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 198.8, 155.8, 154.4, 143.6, 141.3, 130.1, 129.7, 127.7, 127.0, 124.9, 124.2, 119.9, 78.4, 66.8, 61.1, 47.1, 34.6, 28.8. $[M+H]^+$ calc: 444.2; found 443.7.

2.7 Representative solid-phase synthesis of the resin-bound Fmoc-AA-viny sulfone (6).

To a dried 25 ml flask flushed with N_2 was added 200 mg rink sulfone phosphonate resin (**5**) and 5 ml anhydrous THF. After swelling for 1 h, 2 ml THF was removed by suction using a dropper or syringe. To the reaction mixture was added 1 M LHMDS solution in THF (0.7 ml, 0.7 mmol, 5 eq.) and stirred gently. After 30 min, the reaction solution was removed by suction. 15 ml anhydrous THF was added and stirred for 5mins, the THF was removed by the same method. This process was repeated. After the final THF wash step, Fmoc-AA-CHO (0.42 mmol, 3 eq) in 1 ml THF was added to the resin, which made up the final volume of THF in the reaction to be ~ 5 ml. The reaction was stirred for 2 h, and the resin was filtered and washed with 4 x 10 ml DMF. A small portion of the resulting resin was cleaved with 50% TFA (in DCM; 30 min) to give crude Fmoc-Leu-vinyl sulfonyl benzamide, which was further characterized by HPLC-MS (below) and NMR. ^{1}H NMR (300 MHz, DMSO-D_6) δ 8.21 (s, 1H, NH), 8.08 (d, 2H), 7.80-7.95 (m, 4H), 7.50-7.70 (m, 4H, NH_2 , CH, CH), 7,41 (t, *J* = 7.6 Hz, 2H), 7,29 (t, *J* = 7.2 Hz, 2H), 4.1-4.4 (m, 4H) 1.2-1.7(m, 3H), 0.7-0.9 (m, 6H), $[M+Na]^+$ calc: 541.2; found 541.1.

2.7 (a) HPLC spectrum of Fmoc-Leu-vinyl-sulfonyl-benzamide



HPLC condition: 10%-90% Acetonitrile in 30 min

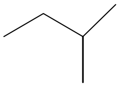
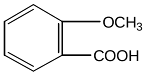
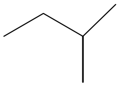
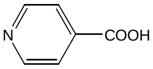
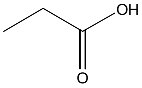
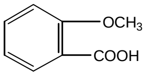
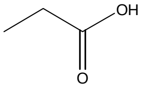
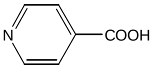
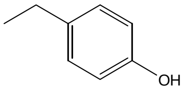
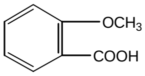
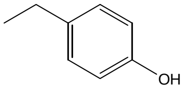
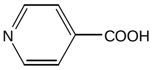
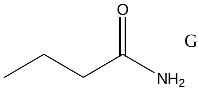
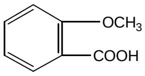
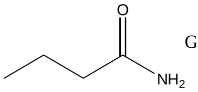
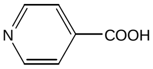
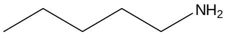
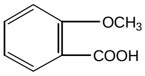
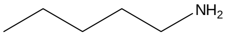
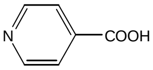
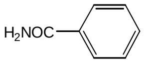
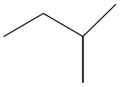
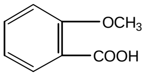
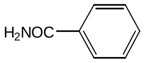
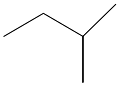
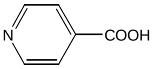
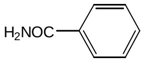
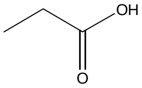
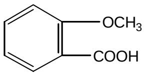
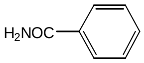
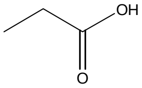
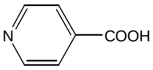
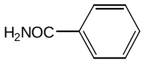
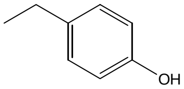
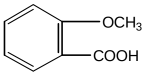
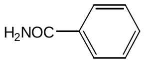
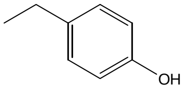
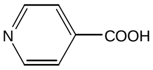
2.7 (b) Determination of resin loading efficiency by Fmoc Analysis

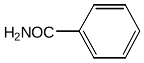
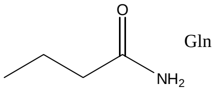
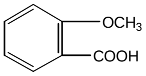
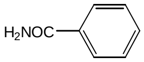
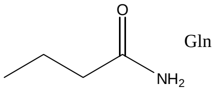
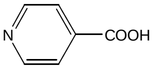
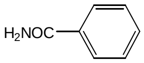
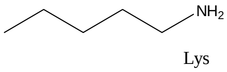
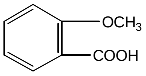
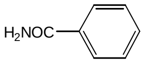
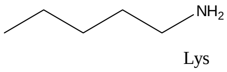
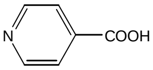
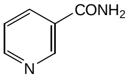
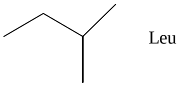
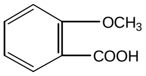
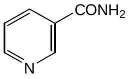
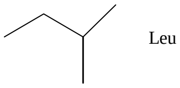
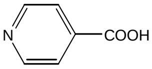
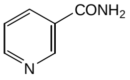
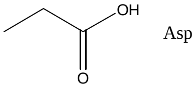
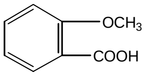
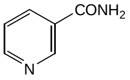
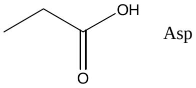
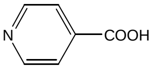
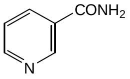
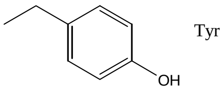
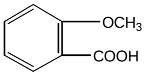
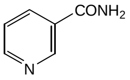
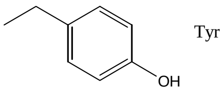
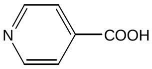
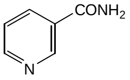
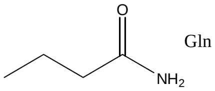
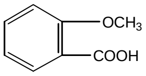
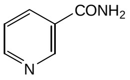
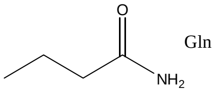
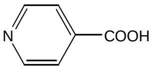
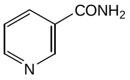
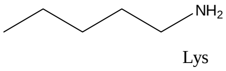
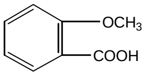
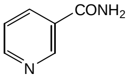
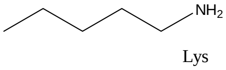
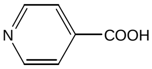
5 mg of resin **6** was weighed precisely into a 1.5 ml eppendorf tube. 1.0 ml of 20% piperidine (in DMF) was added and the tube was shaken for 30 min. 50 µl of the solution was transferred to a 5 ml volumetric flask and diluted to 5.0 ml with 20% piperidine in DMF. 5mg of rink resin with a previously determined loading efficiency (0.7 mmol/g) was treated the same way, in parallel, as a calibration standard. After the measurement of UV absorbance at 275 nm against a reference of 20% piperidine (in DMF), the results of the two resins were compared and adjusted to give the loading of Fmoc-AA-vinyl sulfone on resin **6**.

2.8 Representative synthesis of vinyl sulfone small molecules (8)

200 mg of rink vinyl sulfone resin obtained above was treated with 3 ml DBU/HOBt/DMF (2:1:97) solution to deprotect the Fmoc. After 10 min, the resin was filtered and washed. The resin was divided into two portions (150 mg and 50 mg, respectively). In a 2-ml eppendorf tube, 150 mg resin (around 0.6 mmol) was reacted with isonicotinic acid (70 mg, 0.48 mmol, **7b**), HOBt (69 mg, 0.45 mmol), DIC (69 µl, 0.45 mmol), DIEA (86 µl, 0.5 mmol) in 1.5 ml DMF solution for 5 hrs. After filtration and washing with DMF (AR grade, 5 x), DCM (HPLC-grade, 3 x), the resin was cleaved with 1.5 ml 50% TFA (in DCM with 1 drop of water as scavenger; 1 h). The resin was filtered through a glass pipette fitted with cotton wool, and the filtrate was collected in a 15-ml tube filled with 12 ml cold ether. The resin was washed with another 0.5 ml TFA. The precipitation was centrifuged and analyzed directly by HPLC-MS. Where ever necessary, the crude product (e.g. the precipitate) was further purified by preparative HPLC. Another 50 mg was reacted with anisic acid (33 mg, 0.22 mmol, **7a**) HOBt (31 mg, 0.20 mmol), DIC (31 µl, 0.20 mmol), DIEA (38 µl, 0.22 mmol) in 0.75 ml DMF for 5 h. After the same washing,cleavage and precipitation procedures, as stated above, the precipitate was analyzed by HPLC-MS. The data of all 30 compounds made are summarized in the table below:

2.8 (a) ESI-MS and HPLC data of vinyl sulfone small molecules (8)

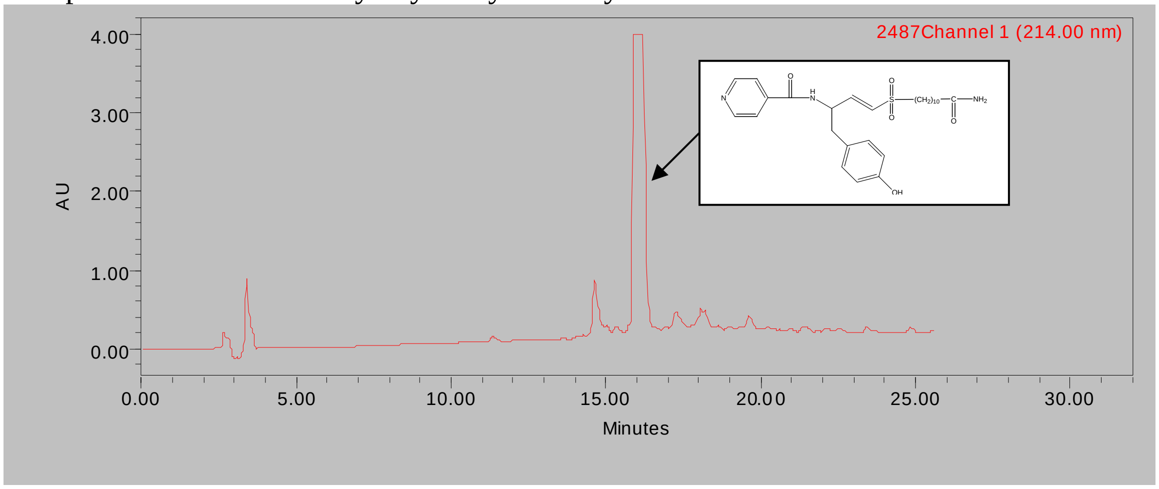
Compound	R1	R2	R3	(M+1)$^+$ Calcd.	Found	HPLC Purity^a
8.1	$H_2NOC-(CH_2)_{10}$	 Leu		495.3	495.3	94%
8.2	$H_2NOC-(CH_2)_{10}$	 Leu		466.3	466.3	93%
8.3	$H_2NOC-(CH_2)_{10}$	 Asp		497.2	497.2	90%
8.4	$H_2NOC-(CH_2)_{10}$	 Asp		468.2	468.2	96%
8.5	$H_2NOC-(CH_2)_{10}$	 Tyr		545.3	545.3	75%
8.6	$H_2NOC-(CH_2)_{10}$	 Tyr		516.3	516.2	95%
8.7	$H_2NOC-(CH_2)_{10}$	 Gln		510.3	510.3	80%
8.8	$H_2NOC-(CH_2)_{10}$	 Gln		481.3	481.3	88%
8.9	$H_2NOC-(CH_2)_{10}$	 Lys		510.3	510.3	82%
8.10	$H_2NOC-(CH_2)_{10}$	 Lys		481.3	481.3	93%
8.11		 Leu		431.2	431.2	77%
8.12		 Leu		402.2	402.2	90%
8.13		 Asp		433.2	433.2	84%
8.14		 Asp		404.2	404.2	91%
8.15		 Tyr		481.2	481.2	85%
8.16		 Tyr		451.2	451.2	92%

8.17				446.2	446.2	87%
8.18				417.2	417.2	82%
8.19				447.1	447.3	82%
8.20				417.2	417.4	89%
8.21				432.1	432.2	57%
8.22				403.1	403.1	67%
8.23				434.1	434.1	47%
8.24				405.1	405.1	74%
8.25				481.1	481.2	58%
8.26				452.1	452.2	68%
8.27				447.1	447.2	40%
8.28				418.1	418.2	43%
8.29				446.2	446.2	62%
8.30				418.2	418.2	52%

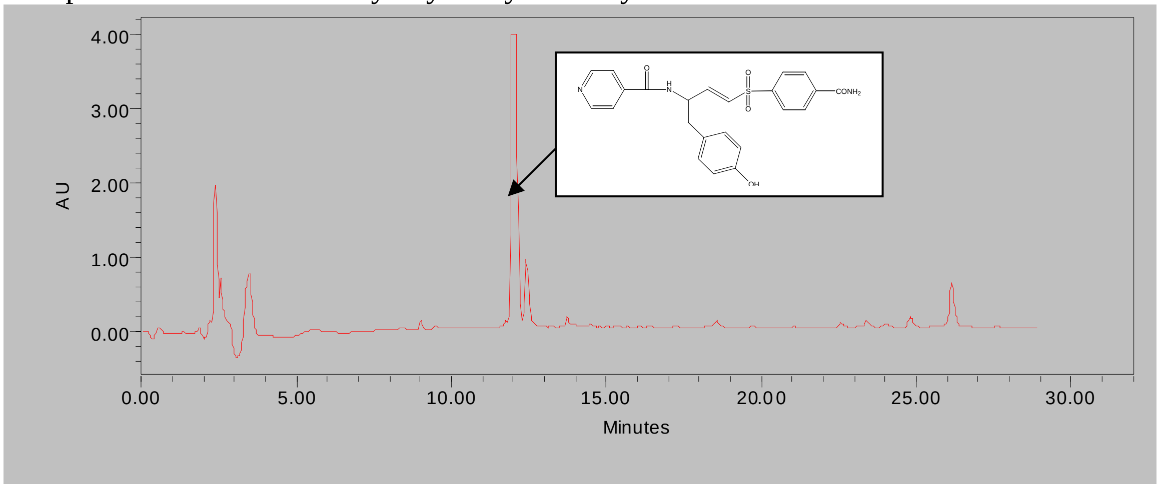
[a]The purity of the compounds were estimated from the HPLC profile of the crude product (e.g. the ether precipitate).

2.8 (b) Selected HPLC spectrum :

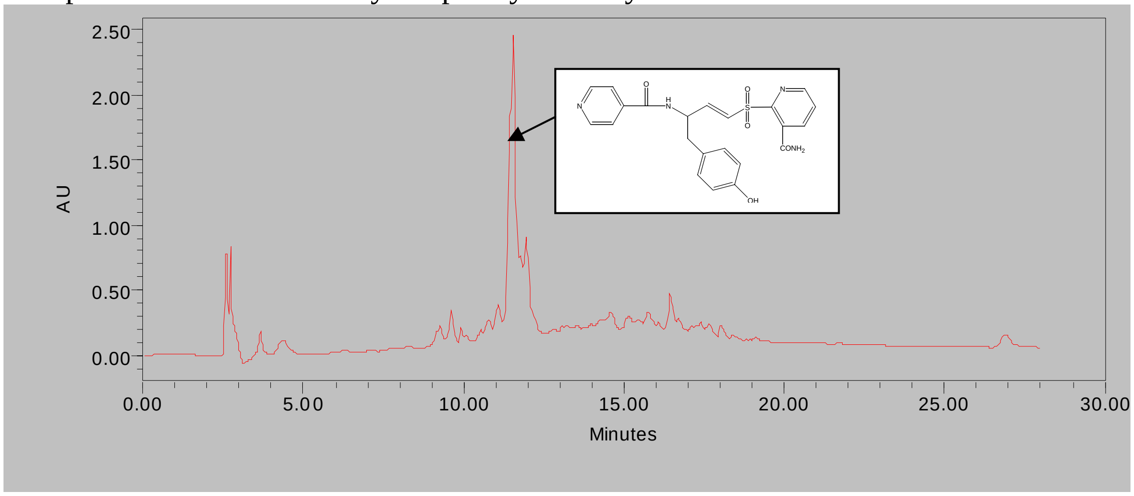
Compound **8**.6: Isonicotinoyl-Tyr-vinyl-sulfonyl-undecanamide



Compound **8**.16: Isonicotinoyl-Tyr-vinyl-sulfonyl-benzamide



Compound **8**.26: Isonicotinoyl-Asp-vinyl-sulfonyl-nicotinamide



2.9 (c) Selected NMR data of (8):

Compound **8**.1: Anisoyl-Leu-vinyl-sulfonyl-undecanamide
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 (dd, *J* = 7.5 Hz, 2.0 Hz, 1H), 7.82 (d, *J* = 8.0Hz, 1H), 7.48 (dt, *J* = 7.5 Hz, 2.0 Hz, 1H), 7.10 (t, *J* = 7.5 Hz, 1H), 7.01 (d, *J* = 8.0Hz, 1H), 6.88 (dd, *J* = 14.9 Hz, 4.8 Hz, 1H), 6.42 (dd, *J* = 15.2 Hz, 1.5 Hz, 1H), 5.45 (m, 2H), 4.98 (m, 1H), 3.99 (s, 3H), 2.93 (m, 2H), 2.21 (t, *J* = 7.6 Hz, 2H), 1.50-1.90 (m, 7H), 1.10-1.40 (m, 12H), 0.90-1.10 (m, 6H).

Compound **8**.2: Isonicotinoyl-Leu-vinyl-sulfonyl-undecanamide
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.78 (s, 2H), 7.88 (s, 2H), 7.54 (br, 1H), 6.85 (dd, *J* = 15.3 Hz, 5.2 Hz, 1H), 6.48 (d, *J* = 15.3 Hz, 1H), 5.77 (s, 2H), 4.96 (m, 1H), 2.90-3.15 (m, 2H), 2.10-2.30 (t, 2H), 1.50-1.95 (m, 7H), 1.10-1.40 (m, 12H), 0.90-1.10 (m, 6H). ^{13}C NMR (75.5 MHz, $CDCl_3$): 176.1, 164.2, 148.2, 148.0, 143.0, 128.2, 122.2, 54.6, 48.8, 42.6, 35.7, 28.9, 28.8, 28.7, 28.6, 27.9, 25.3, 24.8, 22.6, 22.1, 21.9.

Compound **8**.4: Isonicotinoyl-Asp-vinyl-sulfonyl-undecanamide
^{1}H NMR (300 MHz, CD_3OD) δ 8.84 (s, 2H), 8.07(d, *J* = 4.8 Hz, 2H), 6.92 (dd, *J* = 15.1 Hz, 6.0 Hz, 1H), 6.58 (dd, *J* = 15.3, 1H), 5.22 (m, 1H), 2.90-3.10 (m, 2H), 2.80-2.90 (m, 2H), 2.10-2.25 (m, 2H), 1.55-1.75 (m, 4H), 1.20-1.50 (m, 12H). ^{13}C NMR (75.5 MHz, CD_3OD): δ 179.3, 173.3, 166.7, 149.6, 147.3, 145.0, 130.7, 123.8, 55.2, 38.4, 36.5, 30.4, 30.3, 30.3, 30.3, 30.2, 30.1, 29.3, 26.8, 23.5.

Compound **8**.6: Isonicotinoyl-Tyr-vinyl-sulfonyl-undecanamide
^{1}H NMR (300 MHz, CD_3OD) δ 8.82 (s, 2H), 8.00(d, *J* = 6.0 Hz, 2H), 7.09 (d, *J* = 8.4 Hz, 2H), 6.88 (dd, *J* = 15.1 Hz, 6.0 Hz, 1H), 6.71 (d, *J* = 8.4 Hz, 2H), 6.58 (dd, *J* = 15.3, 1.6 Hz, 1H), 5.02 (m, 1H), 2.90-3.10 (m, 4H), 2.10-2.30 (t, 2H), 1.55-1.65 (m, 4H), 1.20-1.40 (m, 12H).

Compound **8**.7: Anisoyl-Gln-vinyl-sulfonyl-undecanamide
^{1}H NMR (300 MHz, DMSO-D_6) δ 8.33 (d, 1H), 7.60 (dd, *J* = 7.6 Hz, 1.8 Hz, 1H), 7.46(t, *J* = 7.2 Hz, 1H), 7.35 (s, 1H), 7.26 (s, 1H), 7.13 (d, , *J* = 7.8 Hz, 1H), 6.70-6.81 (m, 2H), 6.63 (m, 2H), 4.71 (m, 1H), 3.87 (s, 1H), 2.90-3.10 (m, 2H), 2.10-2.20 (m, 2H), 1.70-2.10 (m, 4H), 1.40-1.65 (m, 4H), 1.10-1.40 (m, 12H).

Compound **8**.9: Anisoyl-Lys-vinyl-sulfonyl-undecanamide
^{1}H NMR (300 MHz, CD_3OD) δ 7.76 (d, *J* = 7.6Hz, 1H), 7.48 (t, *J* = 7.5 Hz, 2.0 Hz, 1H), 7.15 (d, *J* = 8.4Hz, 1H), 7.06 (t, *J* = 7.6 Hz, 1H), 6.89 (dd, *J* = 14.9 Hz, 5.2 Hz, 1H), 6.42 (d, *J* = 15.2 Hz, 1H), 4.8-5.1 (m, 1H, overlapped by solvent peak), 3.97 (s, 3H), 2.90-3.20 (m, 4H), 2.10-2.30 (m, 2H), 1.45-1.95 (m, 10H), 1.10-1.40 (m, 12H). ^{13}C NMR (75.5 MHz, CD_3OD): δ 170.4, 168.8, 155.6, 148.6, 144.5, 140.0, 134.0, 131.7, 131.4, 129.8, 128.8, 123.9, 128.8, 123.9, 121.9, 112.8, 56.4, 51.2, 40.5, 33.9, 28.0, 24.0.

Compound **8**.10: Isonicotinoyl-Lys-vinyl-sulfonyl-undecanamide
^{1}H NMR (300 MHz, CD_3OD) δ 8.76 (s, 2H), 7.90 (d, *J* = 5.1 Hz, 2H), 6.87 (dd, *J* = 15.2 Hz, 5.2 Hz, 1H), 6.67 (d, *J* = 15.2 Hz, 1H), 4.8-5.1 (m, 1H, overlapped by solvent peak), 2.90-3.15 (m, 4H), 2.10-2.30 (t, 2H), 1.50-1.95 (m, 10H), 1.20-1.45 (m, 12H).

Compound **8**.12: Isonicotinoyl-Leu-vinyl-sulfonyl-benzamide
^{1}H NMR (300 MHz, CD_3OD) δ 8.85 (s, 2H), 7.90-8.12 (m, 6H), 7.03 (dd, *J* = 15.3 Hz, 5.2 Hz, 1H), 6.48 (d, *J* = 15.3 Hz, 1H), 4.8-5.1 (m, 1H, overlapped by solvent peak), 1.50-1.70 (m, 3H), 0.90-1.00 (m, 6H). ^{13}C NMR (75.5 MHz, CD_3OD): δ 170.4, 166.2, 148.3, 148.2, 146.6, 144.5, 140.0, 131.6, 129.7, 128.9, 124.5, 43.2, 26.1, 23.1, 22.0.

Compound **8**.14: Isonicotinoyl-Asp-vinyl-sulfonyl-benzamide
^{1}H NMR (300 MHz, CD_3OD) δ 8.84 (s, 2H), 7.90-8.12 (m, 6H), 7.11 (dd, *J* = 15.3 Hz, 5.2 Hz, 1H), 6.80 (dd, *J* = 15.3, 1.6 Hz, 1H), 5.20 (m, 1H), 2.70-2.90 (m, 2H).

Compound **8**.16: Isonicotinoyl-Tyr-vinyl-sulfonyl-benzamide
^{1}H NMR (300 MHz, CD_3OD) δ 8.97 (s, 2H), 7.80-8.10 (m, 6H), 6.90-7.10(m, 3H), 6.60-6.70 (m, 3H), 4.99 (m, 1H), 2.80-3.00 (m, 2H).

Compound **8**.18: Isonicotinoyl-Gln-vinyl-sulfonyl-benzamide
^{1}H NMR (300 MHz, CD_3OD) δ 8.89 (s, 2H), 8.22(d, *J* = 6.0 Hz, 2H), 7.90-8.10 (m, 4H), 7.04 (dd, *J* = 15.1 Hz, 6.0 Hz, 1H), 6.80 (dd, *J* = 15.3, 1H), 4.8-5.1 (m, 1H, overlapped by solvent peak), 2.00-2.40 (m, 4H).

Compound **8**.19: Anisoyl-Lys-vinyl-sulfonyl-benzamide
^{1}H NMR (300 MHz, CD_3OD) δ 7.90-8.10 (m, 4H), 7.67 (d, *J* = 7.6Hz, 1H), 7.48 (t, *J* = 7.5 Hz, 2.0 Hz, 1H), 6.90-7.15 (m, 3H), 6.76 (d, *J* = 15.2 Hz, 1H), 4.8-5.1 (m, 1H, overlapped by solvent peak), 3.89 (s, 3H), 2.85-3.00 (m, 2H), 1.40-1.90 (m, 6H).

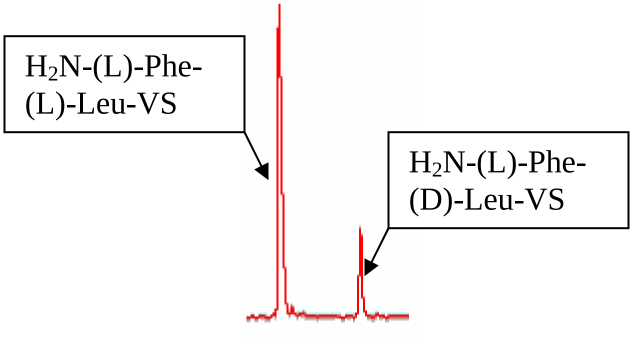
3. Determination of degree of epimerization in solid-phase Horner-Emmons reaction products (6)

The epimerization of the solid-phase Horner-Emmons reaction (step **d** in Scheme 1; maintext) was determined via procedures below. First, a pair of diastereomeric dipeptide vinyl sulfones was generated. Starting from the resin-bound Fmoc-Leu-vinyl sulfonyl benzamide (e.g. **6**), the Fmoc was deprotected, and the resulting resin was divided into two equal portions, then each coupled separately with enantiomerically pure Boc-(L)-phe-OH and Boc-(D)-phe-OH, respectively, using DIC/HOBT/DIEA methods. After 4 hrs of coupling reaction, the resins were washed, cleaved and their products analyzed by HPLC-MS.

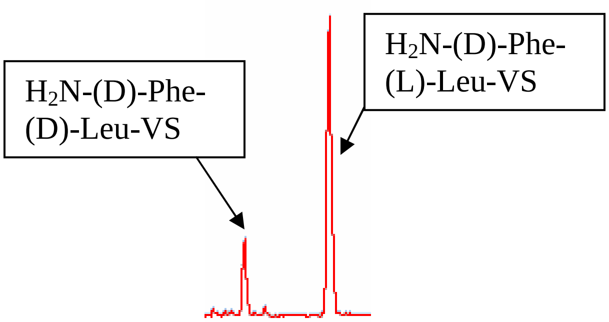
Figure 3(a) shows the spectrum resulting from the resin coupled with Boc-(L)-phe-OH, the peaks at 19.8 min and 22.6 min have the same MS (443), corresponding to the calculated MS of dipeptide vinyl sulfone. It's reasonable to assume the major peak at 19.8 min is the H_2N-(L)-Phe-(L)-Leu-VS since the epimerized product can't exceed 50%. Consequently, H_2N-(L)-Phe-(D)-Leu-VS should correspond to the smaller peak, and H_2N-(L)-Phe-(L)-Leu-VS the major peak. This assumption was unambiguously confirmed from **figure 3(b)** which was the HPLC profile of the product generated from the same resin coupled with Boc-(D)-phe-OH. Peaks at 19.8 min and 22.6 min correspond to the epimerized (e.g. H_2N-(D)-Phe-(D)-Leu-VS), and the desired dipeptide vinyl sulfone (e.g. H_2N-(D)-Phe-(L)-Leu-VS), respectively.

Figure 3(c) is the spectrum generated from resin using optimized Horner-Emmons reaction conditions, then coupled with Boc-(L)-phe-OH. The epimerization is less than 5%. $[M+H]^+$ for H_2N-Phe-Leu-VS (benzamide) calc: 443.2, found 444.2 .

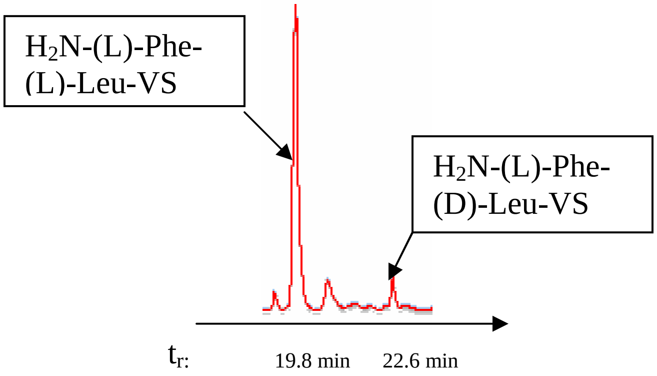
3(a)



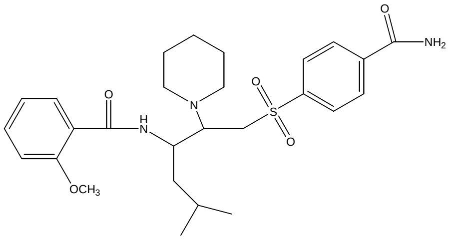
3(b)



3(c) Optimized Horner-Emmons Reaction (< 10% epimerization)

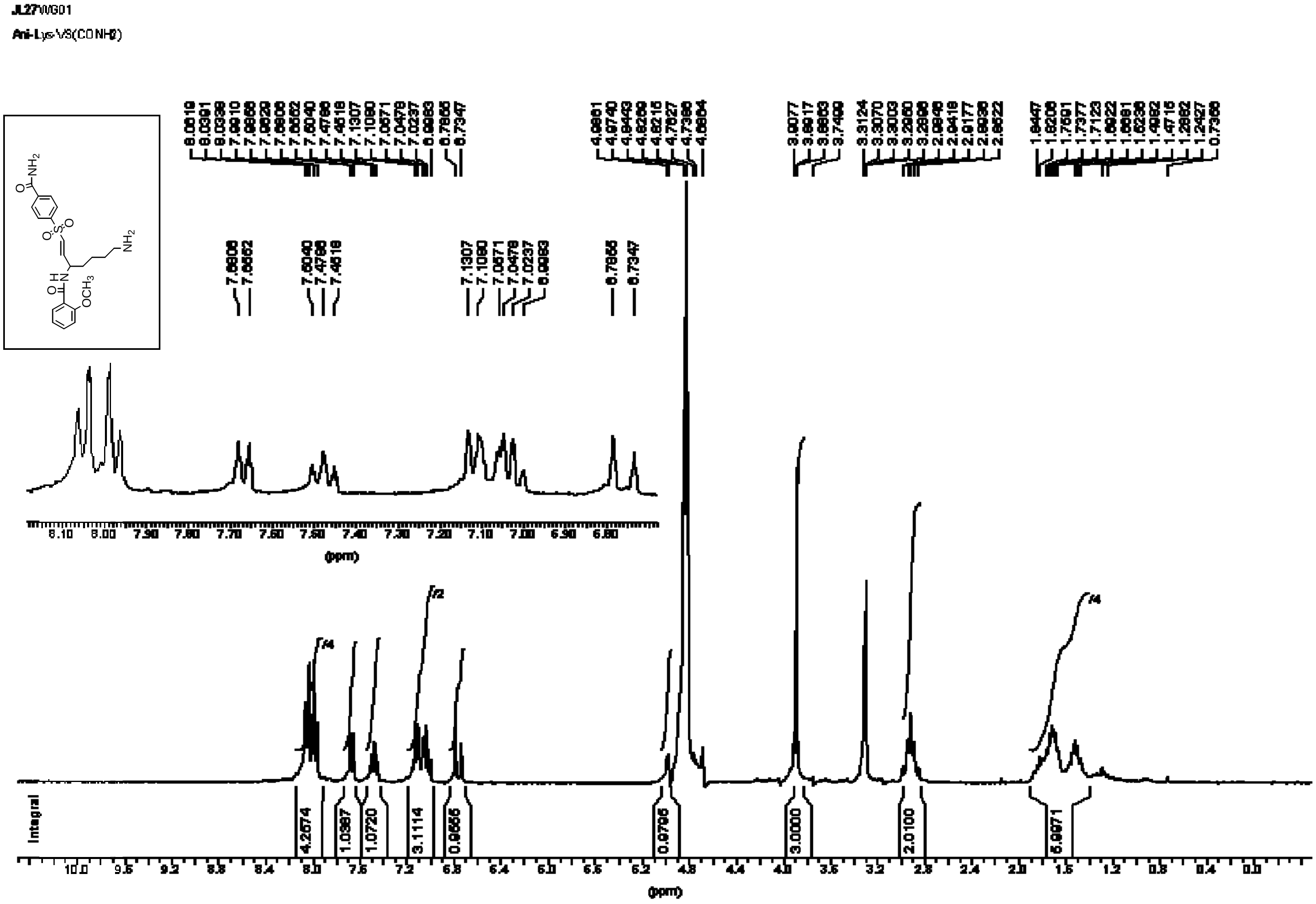


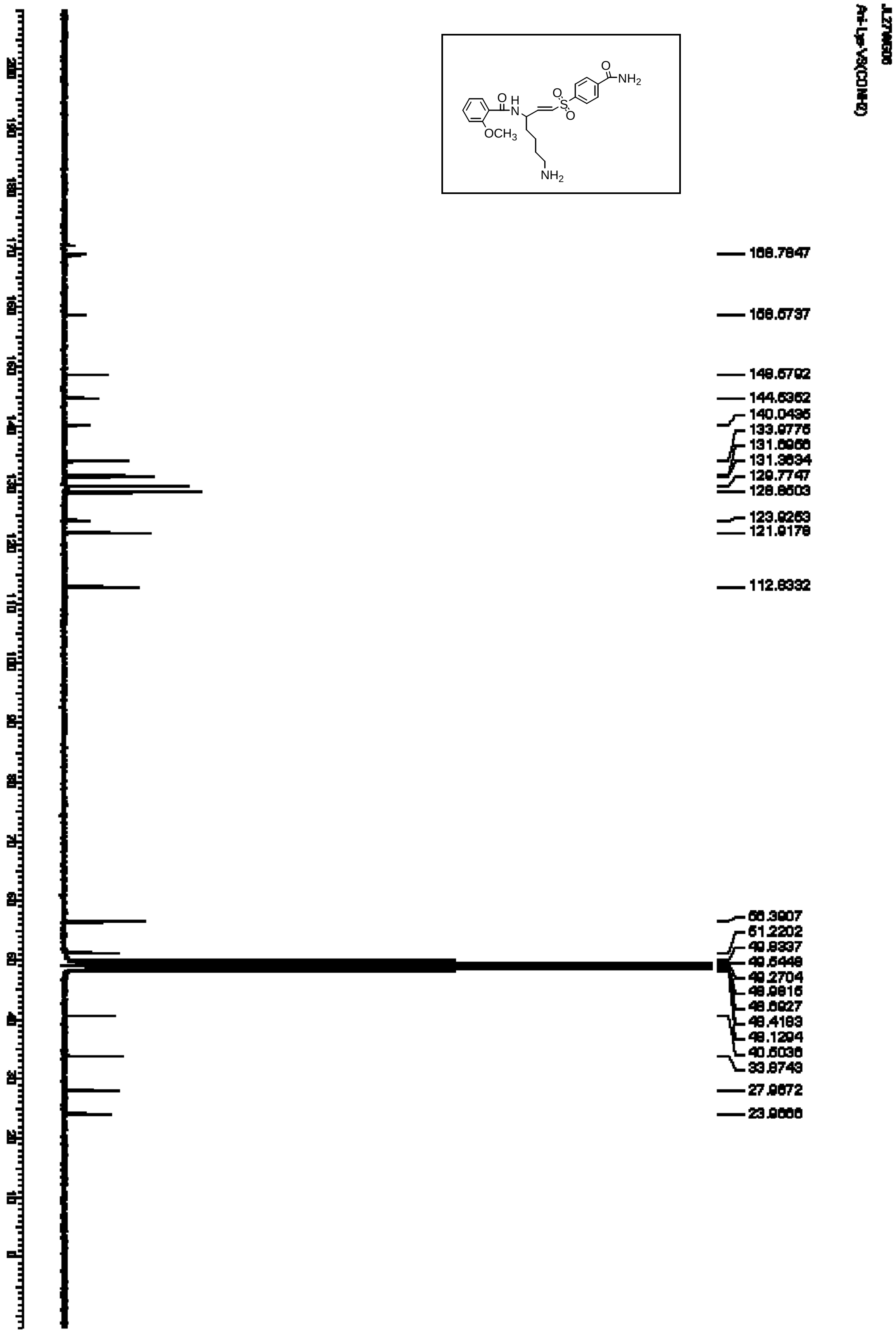
4. Impurity generated when 20% piperidine was used for Fmoc cleavage (step e) in the synthesis of Anisoyl-Leu-vinyl-sulfonyl-benzamide.

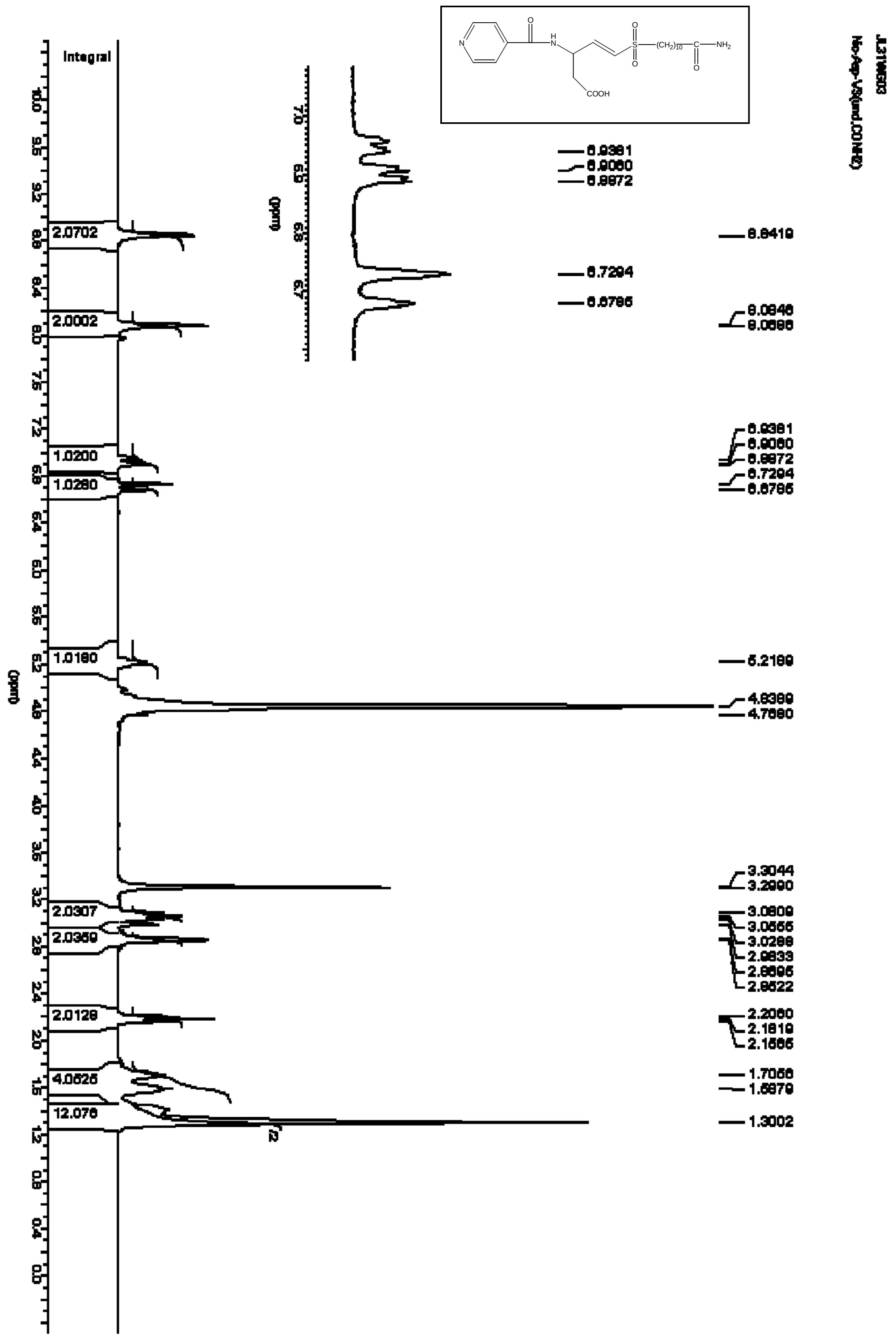


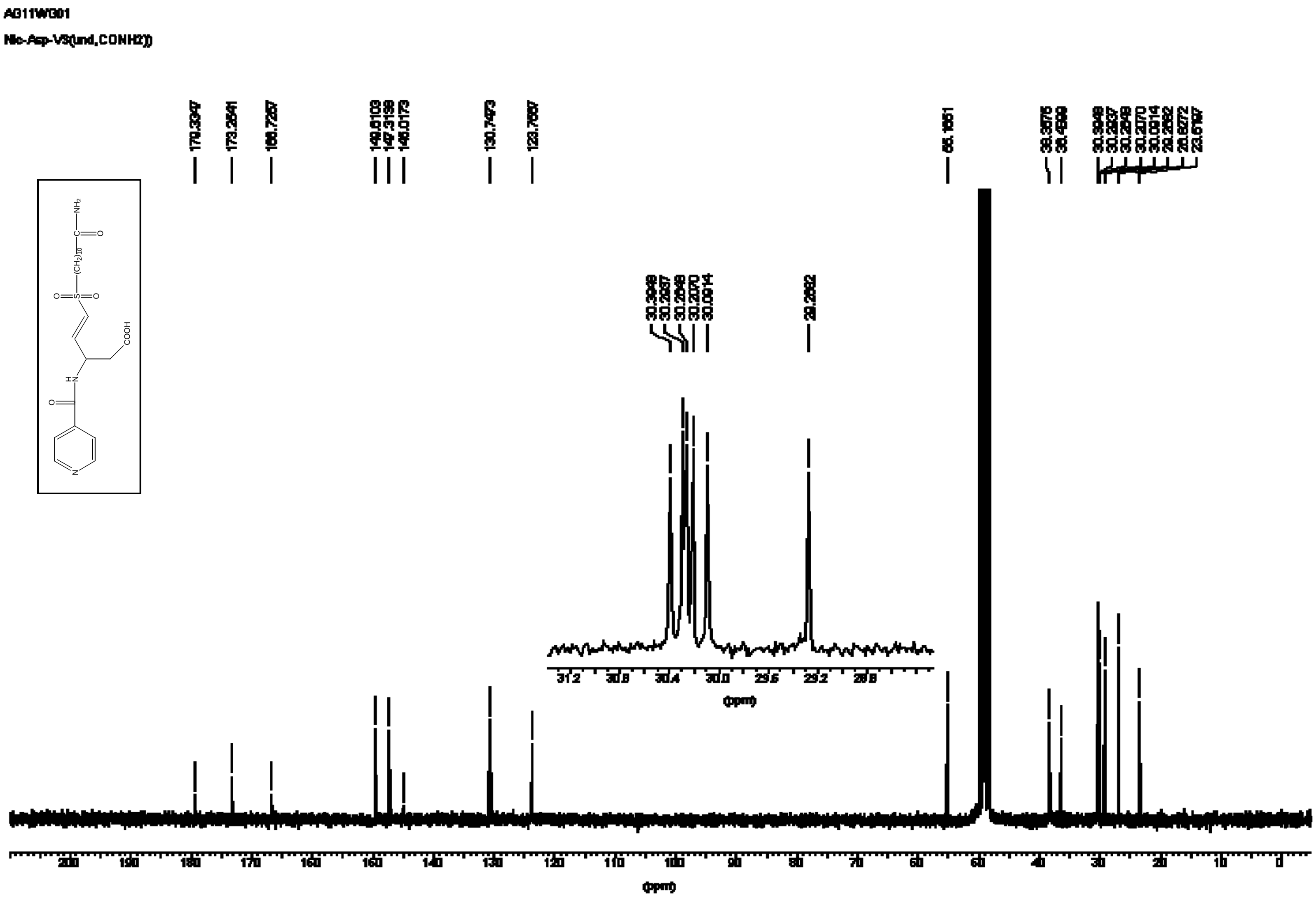
^{1}H NMR (300 MHz, CD_3OD) δ 7.9-8.2 (m, 4H), 7.88 (d, *J* = 8.1 Hz, 1H), 7.53 (t, *J* = 7.2 Hz, 1H), 7.16 (d, *J* = 8.4, 1H), 7.09 (t, *J* = 8.2, 1H), 4.40 (m, 1H), 3.98 (s, 3H), 3.45-3.75 (m, 2H), 2.3-3.2 (m, 5H), 1.2-1.8 (m, 9H), 0.8-1.1 (m, 6H), $[M+H]^+$ calc: 516.3; found 516.3.

5. Selected NMR spectrum


JL27WG01
Ani-Lys-VS(CONH2)
O
H
N
O
S
O
O
NH2
OCH3
NH2
8.0619
8.0391
8.0338
7.9910
7.9868
7.9629
7.6806
7.6562
7.5040
7.4796
7.4516
7.1307
7.1080
7.0671
7.0478
7.0237
6.9963
6.7866
6.7347
4.9961
4.9740
4.9443
4.9269
4.8216
4.7627
4.7386
4.6664
3.9077
3.8917
3.8663
3.7499
3.3124
3.3070
3.3003
3.2960
3.2896
2.9946
2.9419
2.9177
2.8936
2.8622
1.8447
1.8206
1.7591
1.7377
1.7123
1.6922
1.6691
1.6236
1.4962
1.4715
1.2862
1.2427
0.7366
8.10
8.00
7.90
7.80
7.70
7.60
7.50
7.40
7.30
7.20
7.10
7.00
6.90
6.80
(ppm)
Integral
4.2674
1.0367
1.0720
3.1114
0.9656
0.9796
3.0000
2.0100
5.9971
10.0
9.6
9.2
8.8
8.4
8.0
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0.0
(ppm)


JLZ76506
Ant-Lys-VS(CONH2)
O
NH2
O
S
O
O
H
N
OCH3
NH2
168.7847
158.5737
149.5792
144.6362
140.0436
133.9776
131.6966
131.3634
129.7747
128.6503
123.9263
121.9176
112.8332
56.3907
51.2202
49.8337
49.5448
49.2704
48.9816
48.6927
48.4183
48.1294
40.5036
33.8743
27.9672
23.9666
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)


JL316503
Nic-Asp-VS(amLCONH2)
N
O
H
N
S
O
O
(CH2)10
C
O
NH2
COOH
Integral
2.0702
2.0002
1.0200
1.0280
1.0160
2.0307
2.0359
2.0129
4.0625
12.076
(ppm)
7.0
6.9
6.8
6.7
8.6419
8.0846
8.0696
6.9381
6.9060
6.8972
6.7294
6.6795
5.2169
4.8399
4.7880
3.3044
3.2990
3.0809
3.0555
3.0289
2.9833
2.8695
2.8522
2.2060
2.1819
2.1566
1.7055
1.6879
1.3002



AG11WG01

Nic-Asp-VS(und,CONH2)