Supporting Information:

Stereoselective Synthesis of Photoreactive Peptidomimetic γ-Secretase Inhibitors

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1. General Information

^{1}H NMR and ^{13}C NMR spectra were referenced to TMS as internal standard (^{1}H) and the residual $CHCl_3$ at 77.00 ppm (^{13}C). $CDCl_3$ was the only solvent used for the NMR analyses unless otherwise indicated. Unless otherwise specified, concentrations and evaporations were performed under reduced pressure (oil pump).

2. Experimental Procedures

***tert*-Butyl (2*S*,3*S*)-4-chloro-3-hydroxy-1-phenylbutan-2-ylcarbamate (6)**. This compound was prepared following the literature method[1] with minor revision: to a solution of *N*-Boc-Phe-OMe, (**4**) (19.8 g, 70.9 mmol) and iodochloromethane (50 g) in dry THF (180 mL) LDA (177 mL, 2.0 M) was added slowly with stirring at –78 °C under N_2. After 30 min, a solution of 39 mL of HOAc in 39 mL of THF was added slowly to keep the internal temperature below –68 °C. The solution was stirred at –78 °C for an additional 15 min and allowed to warm. Toluene (177 mL) and 1% aqueous HCl (177 mL) were added. The organic layer was washed with 0.5 M $NaHCO_3$ (177 mL), and the aqueous layer was discarded. Anhydrous EtOH (177 mL) was added, then $NaBH_4$ (7.1 g) in anhydrous EtOH (236 mL) was added dropwise at –78 °C. The reaction mixture was stirred at –78 °C for 6 h, warmed to 0 °C, stirred for 2 h and quenched by addition of a solution of 88 mL saturated $KHSO_4$ and 88 mL of H_2O. The mixture was stirred at 0 °C for 30 min, and concentrated. The residual solid was rinsed with H_2O (100 mL), hexanes (3 × 40 mL), and dried. The resulting yellow solid was recrystallized from EtOAc to give chlorohydrin **6** (7.8 g, 38% from **4**) as brown crystals. The physical and NMR data are in full accord with the literature data.[1] LRMS [ESI, 322.0 (MNa^+)].

***tert*-Butyl (*S*)-1-((*S*)-oxiran-2-yl)-2-phenylethylcarbamate (7)**. This compound was prepared following the literature method:[1] a solution of KOH (0.5 g, 8.9 mmol) in anhydrous EtOH (8.9 mL) was added to a solution of chlorohydrin **6** (2.2 g, 7.35 mmol) in anhydrous EtOH (76 mL). The mixture was stirred at rt for 2 h. A solution of NaH_2PO_4 (0.44 g) in H_2O (8.7 mL) was added. After removing most EtOH, the slurry was partitioned between EtOAc (50 mL) and H_2O (15 mL). The aqueous layer was

extracted with EtOAc (2 × 20 mL). The combined extracts were dried ($MgSO_4$), filtered and concentrated. The resulting solid was recrystallized from hexane to give epoxide **7** as colorless needles (1.59 g, 82%). The physical and NMR data are in full accord with the literature data.[1] LRMS [ESI, 285.7 (MNa^+)].

***tert*-Butyl (*S*)-1-((*R*)-4-(ethoxycarbonyl)-tetrahydro-5-oxofuran-2-yl)-2-phenylethylcarbamate (8)**. This compound was prepared following the literature method[2] with minor revision: sodium (0.26 g, 11.3 mmol) was placed to a 100 mL flask under N_2 and 20 mL of absolute EtOH was added. After all of the Na dissolved, diethyl malonate (2.0 mL, 2.11 g, 13.2 mmol) in 5.0 mL of absolute EtOH was added dropwise. After stirring for 10 min, a solution of epoxide **7** (1.0 g, 3.8 mmol) in 10 mL of absolute EtOH was added. The mixture was stirred overnight and then acidified to pH 4 with 10% citric acid. After removing most of the EtOH under reduced pressure, the mixture was extracted with EtOAc (3 × 30 mL). The combined EtOAc extracts were washed with brine, dried (Na_2SO_4), filtered, and evaporated. The residue was purified by chromatography on silica gel (hexane/EtOAc 3:1) to give lactone **8** (1.29 g, 90%) as a white solid. The physical and NMR data are in full accord with the literature data.[2] LRMS [ESI, 400.1 (MNa^+)].

***tert*-Butyl (*S*)-1-((*R*)-tetrahydro-5-oxofuran-2-yl)-2-phenylethylcarbamate (9). Method A:** this compound was prepared in 81% yield following the literature method.[3] **Method B:** unsaturated ester **13** (377 mg, 1.0 mmol) was dissolved in ethyl acetate (5 mL) and 10% palladium on carbon (30 mg) was added. Hydrogen was passed through the stirred suspension in a hydrogenation flask for 4 h. The catalyst was filtered off and the filtrate was evaporated. The saturated ester was obtained as a white solid; ^1H

NMR δ 1.35 (s, 9H), 1.45 (s, 9H), 1.70-1.77 (m, 2H), 2.43 (m, 2H), 2.78 (m, 1H), 2.94 (m, 1H), 3.57 (br s, 1H), 3.64 (br s, 1H), 3.85 (br s, 1H), 4.70 (br s 1H), 7.20-7.30 (m, 5H); ^{13}C NMR δ 28.0, 28.2, 32.3, 35.8, 56.6, 73.5, 79.5, 80.6, 126.3, 128.4, 129.3, 138.0, 156.3, 173.8. This saturated ester was dissolved in toluene (50 ml) and acetic acid (0.5 ml) and refluxed for 3 h. The solution was evaporated to dryness. Purification of the residue by column chromatography (hexane/EtOAc 1:1) gave 245 mg (80%) of lactone **9** as a white solid. The physical and NMR data are in full accord with the literature data.[3] LRMS [ESI, 328.1 (MNa^+)].

Dimethyl [(3*S*)-3-[*N*-(*tert*-Butoxycarbonyl)amino]-2-oxo-4-phenylbutyl] phosphonate (10). This compound was prepared in 72% yield following the literature method.[4] The physical and NMR data are in full accord with the literature data.[4] LRMS [ESI, 372.2 (MH^+), 394.0 (MNa^+)].

***tert*-Butyl (*S*)-3-oxo-1-phenyl-4-(phenylsulfinyl)butan-2-ylcarbamate (11).** To a solution of diisopropylamine (930 μL, 6.6 mmol) in 8 mL of THF was added 2.64 mL of *n*-butyllithium (a 2.5 M solution in hexane, 6.6 mmol) at -78 °C under N_2. After the mixture was stirred at this temperature for 30 min, a solution of methyl phenyl sulfoxide (924 mg, 6.6 mmol) in 5 mL of THF was added dropwise. After the mixture was stirred at -78 °C for 30 min, a solution of ester **4** (587 mg, 2.1 mmol) in 5 mL of THF was added dropwise. The solution was stirred at -78 °C for 2 h and allowed to warm to rt overnight. The mixture was diluted with saturated aqueous NH_4Cl (10 mL), the product was extracted with EtOAc, washed with brine, dried (Na_2SO_4), and concentrated. Purification of the residue by column chromatography (hexane/EtOAc 1:4) gave 569 mg (70%) of **11** as a yellow solid: mp 115.0-116.5 °C; ^{1}H NMR δ 1.30 (s, 9H), 2.88 (m, 1H), 3.08 (m,

1H), 3.73-4.34 (m, 3H), 5.30-5.43 (m, 1H), 7.10-7.63 (m, 10H); ^{13}C NMR δ 28.1, 35.8, 36.1, 61.2, 61.3, 80.1, 124.0, 126.8, 128.5, 129.1, 129.2, 131.5, 136.0, 136.1, 142.8, 143.1, 155.2, 200.7, 200.8. HR-MS (FAB, MNa^+) calcd for *m/z* $C_{21}H_{25}NNaO_4S$ 410.1402, found 410.1409.

***tert*-Butyl (5*S*)-5-[*N*-(*tert*-butoxycarbonyl)amino]-4-oxo-6-phenyl-(*E*)-2-hexenoate (12). Method A:** the HWE reaction with phosphonate **10**, see ref. 4. **Method B:** to a solution of β-ketosulfoxide **11** (387 mg, 1.0 mmol) in 4 mL of DMF was added K_2CO_3 (290 mg, 2.1 mmol) at rt. After the mixture was stirred at rt for 1 h under N_2, a solution of *tert*-butyl bromoacetate (215 mg, 1.1 mmol) in 2 mL of DMF was added. The mixture was stirred at rt for 5 h. Water (5 mL) was added, and the product was extracted with EtOAc (3 x 20 mL), washed with brine, and dried (Na_2SO_4). Concentration under reduced pressure (water bath at 50 °C for 30 min) and purification of the residue by flash column chromatography (hexane/EtOAc 4:1) gave 150 mg (40%) of **12** as a white solid. The physical data are in full accord with the literature data.[4] ^{1}H NMR δ 1.25 (s, 9H), 1.49 (s, 9H), 3.99 (dd, 1H, *J* = 6.3, 13.9 Hz), 3.14 (dd, 1H, *J* = 6.0, 13.7 Hz), 4.80 (q, 1H, *J* = 6.0 Hz), 5.22 (d, 1H, *J* = 7.0 Hz), 6.67 (d, 1H, *J* = 15.8 Hz), 7.04 (d, 1H, *J* = 15.8 Hz), 7.10-7.30 (m, 5H); ^{13}C NMR δ 27.8, 28.2, 37.3, 59.4, 79.9, 81.9, 127.0, 128.5, 129.3, 134.2, 135.4, 135.5, 155.0, 164.1, 197.5. LRMS [ESI, 376.2 (MH^+), 398.2 (MNa^+)].

***tert*-Butyl (4*R*,5*S*)-5-[*N*-(*tert*-butoxycarbonyl)amino]-4-hydroxy-6-phenyl-(*E*)-2-hexenoate (13).** This compound was prepared in 81% yield following the literature method.[4] The physical and NMR data are in full accord with the literature data.[4]

2-(4-Bromophenyl)-2-phenyl-1,3-dioxolane (15). This compound was prepared following the literature method[5] with minor revision: a stirred solution of 4-

bromobenzophenone **14** (11 g, 42.1 mmol), ethylene glycol (12 mL, 211 mmol), and *p*-TsOH (0.25 g) in benzene (200 mL) was refluxed for 44 h. During the reflux the resulting water was removed by azeotropic distillation with a Dean-Stark trap. The resulting reaction mixture was allowed to cool to rt and then washed with aqueous 1 M NaOH (100 mL) and H_2O (100 mL). The mixture was dried (Na_2SO_4), concentrated, and the crude product was purified by chromatography on silica gel (hexane/CH_2Cl_2 3:1) to afford 11.8 g (92%) of **15** as white solid. The physical and NMR data are in full accord with the literature data.[5]

4-(2-Phenyl-1,3-dioxolan-2-yl)benzaldehyde (16). This compound was prepared in 90% yield following the literature method[5] and was used in the next step without further purification.

***tert*-Butyl (*S*) (1-((*R*)-4-(4-(2-phenyl-1,3-dioxolan-2-yl)benzylidene)-tetrahydro-5-oxofuran-2-yl)-2-phenylethylcarbamate (17).** This compound was prepared following the literature method[3] with minor revision: to a solution of lactone **9** (305 mg, 1.0 mmol) in THF (4.0 mL) was added a solution of LDA (2.0 M solution, 2.2 mmol, 1.1 mL) at –78 °C under N_2. The resulting mixture was stirred for 30 min, a solution of aldehyde **16** (660 mg, 2.6 mmol) in THF (3.0 mL) was slowly added, and the mixture was stirred at –78 °C for 30 min. The reaction was quenched with aqueous NH_4Cl solution, the mixture was extracted with EtOAc (3 × 30 mL) and the combined EtOAc extracts were washed with 10% aqueous $NaHCO_3$, brine, dried (Na_2SO_4), filtered, and evaporated. The crude product was treated with Ac_2O (0.5 mL) and Et_3N (0.5 mL) and heated at 120 °C for 1 h. The mixture was allowed to cool to rt, then extracted with EtOAc and washed with aqueous NH_4Cl solution, 10% $NaHCO_3$, and brine. The extracts

were dried (Na_2SO_4), filtered, and evaporated. Chromatography of the residue on silica gel (EtOAc/hexane 1:2) afforded 433 mg (80%) of **17** as a white solid; mp 74.0-78.0 °C; ^{1}H NMR δ 1.26 (s, 9H), 2.86 (m, 1H), 3.01 (m, 2H), 3.22 (dd, 1H, *J* = 8.2, 17.9 Hz), 4.11 (m, 5H,), 4.56 (br s, 2H), 6.94-7.60 (m, 15H); ^{13}C NMR δ 28.2, 30.5, 34.6, 65.0, 109.0, 123.8, 126.0, 126.7, 126.8, 128.3, 128.6, 129.5, 130.0, 134.2, 136.5, 136.8, 141.6, 152.4, 171.4; HR-MS (FAB, MNa^+) calcd for *m/z* $C_{33}H_{35}NNaO_6$ 564.2362, found 564.2350.

***tert*-Butyl (*S*) (1-((2*R*, 4*R*)-4-(4-(2-phenyl-1,3-dioxolan-2-yl)benzyl)-tetrahydro-5-oxofuran-2-yl)-2-phenylethylcarbamate (19).** Anhydrous EtOAc (3 mL) and PtO_2 (25 mg) were placed in a 25 mL flask fitted with a dropping funnel and a hydrogen filled balloon. A solution of compound **17** (200 mg, 0.37 mmol) in 5 mL of anhydrous EtOAc was placed in the dropping funnel. The apparatus was evacuated, then flushed with hydrogen from the balloon. The solution of **17** was then added to the flask and the mixture was stirred under H_2 at rt until the complete consumption of **17** was shown by TLC (~ 2 h). The catalyst was removed by filtration and the filtrate was evaporated. Column chromatography of the residue (EtOAc/hexane 1:2) gave 181 mg (90%) of **19** as a yellow solid; mp 65.5-67.0 °C; $[\alpha]^{25}_D$ –69.6 (*c* 0.5, $CHCl_3$); ^{1}H NMR δ 1.29 (s, 9H), 1.74 (m, 1H), 2.20 (m, 1H), 2.66 (dd, 1H, *J* = 10.0, 13.8 Hz), 2.82 (m, 3H,), 3.25 (dd, 1H, *J* = 3.9, 13.9 Hz), 3.89 (br s, 1H), 4.00 (s, 4H), 4.24 (br s, 1H), 4.42 (d, 1H, *J* = 7.6 Hz), 7.11-7.49 (m,14H); ^{13}C NMR δ 28.6, 31.9, 36.3, 36.7, 42.8, 54.8, 65.3, 79.5, 80.3, 109.7, 126.5, 126.9, 127.1, 128.5, 129.0, 130.0, 137.1, 138.7, 141.1, 142.4, 155.7, 178.0; HR-MS (FAB, MNa^+) calcd for *m/z* $C_{33}H_{37}NNaO_5$ 566.2519, found 566.2497.

(1*S*-(4-(4*R*-Benzoyl-benzyl)-5-oxo-tetrahydrofuran-2-yl)-2*R*-phenylethyl)-carbamic acid *tert*-butyl ester (24). Compound **19** (130 mg, 0.24 mmol) was dissolved

in CF_3CO_2H (1.0 mL) at 0 °C, and the mixture was stirred overnight at rt. Concentration under reduced pressure gave an oily residue, which was dissolved in $CHCl_3$-DMF-H_2O (50:9:1, 3.0 mL). Et_3N was added to the mixture until the solution was basic, and then $(Boc)_2O$ (259 mg, 1.2 mmol) was added at 0 °C. The mixture was stirred for 3 h at rt, then concentrated under reduced pressure to give an oily residue which was acidified with saturated aqueous citric acid and extracted with EtOAc. The extract was washed with brine and dried over $MgSO_4$. Concentration followed by flash chromatography of the residue on silica gel (hexane/EtOAc 2:1) gave **24** (108 mg, 90%) as a white solid; mp 74.5-76.5 °C; $[\alpha]^{25}_D$ –70.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR δ 1.27 (s, 9H), 1.82 (m, 1H), 2.27 (m, 1H), 2.80 (m, 2H), 2.95 (m, 2H,), 3.35 (dd, 1H, *J* = 4.0, 13.8 Hz), 3.92 (br s, 1H), 4.30 (br s, 1H), 4.50 (d, 1H, *J* = 8.1 Hz), 7.16-7.77 (m, 14H); ^{13}C NMR δ 28.6, 31.9, 36.5, 36.9, 42.6, 54.8, 79.4, 80.3, 127.2, 128.7, 129.0, 129.2, 130.4, 131.0, 132.8, 136.5, 137.0, 138.0, 143.8, 155.7, 177.7, 196.7; HR-MS (FAB, MNa^+) calcd for *m/z* $C_{31}H_{33}NNaO_5$ 522.2256, found 522.2244.

2*R*-(4-Benzoyl-benzyl)-5*S*-*tert*-butoxycarbonylamino-4*R*-(*tert*-butyldimethyl-silanyloxy)-6-phenyl-hexanoic acid (25). A solution of lactone **24** (138 mg, 0.276 mmol) in 1,2-dimethoxyethane (2.0 mL) was treated with a solution of aqueous LiOH (1.0 M, 2.0 ml, 2.0 mmol) and stirred at rt for 5 h. After TLC indicated complete consumption of lactone **24**, the reaction mixture was acidified to pH 4 with citric acid, and then extracted with EtOAc. The combined extracts were washed with brine and dried (Na_2SO_4), the solvent was evaporated and the residue was dried *in vacuo*. The resulting hydroxyacid was dissolved in DMF (2.0 mL) and treated with *tert*-butyldimethylsilyl chloride (417 mg, 2.76 mmol) and imidazole (204 mg, 3.0 mmol) and

stirred at rt overnight. This reaction mixture was treated with MeOH (1.5 mL), stirred for 2 h, and evaporated *in vacuo*. The residue was partitioned between aqueous citric acid and EtOAc. The organic layer was washed with brine, dried (Na_2SO_4), and the solvent was evaporated. Chromatography of the residue on silica gel (CH_2Cl_2/MeOH 20:1) afforded acid **25** (129 mg, 74%); ^{1}H NMR (DMSO) δ -0.08. (s, 3H), -0.04 (s, 3H), 0.78 (s, 9H), 1.07-1.20 (s, 9H), 1.59 (m, 1H), 1.76 (m, 1H), 2.56 (s, 1H), 2.60 (s, 1H), 2.76 (m, 1H), 2.86 (m, 1H), 3.60 (s, 1H), 3.72 (s, 1H), 6.46 (s, 1H), 7.07-7.60 (m, 14H); ^{13}C NMR δ -4.8, -4.4, 17.9, 25.6, 25.8, 28.1, 31.5, 36.6, 38.4, 58.4, 71.9, 78.1, 80.1, 126.2, 128.1, 128.3, 129.0, 129.8, 130.3, 132.1, 135.6, 137.6, 138.2, 155.5, 162.9, 179.0, 196.2; HR-MS (FAB, MNa^+) calcd for *m/z* $C_{37}H_{49}NNaO_6Si$ 654.3227, found 654.3220.

(5-(4-Benzoyl-phenyl)-1*S*-benzyl-2*R*-(*tert*-butyldimethylsilanyloxy)-4*R*-(1-(1*S*-carbamoyl-2-phenyl-ethylcarbamoyl)-3(1*S*)-methyl-butylcarbamoyl)-pentyl)-carbamic acid *tert*-butyl ester (22). Method A: a solution of **25** (105 mg, 0.167 mmol), Leu-Phe-NH_2 ·HCl (52.4 mg, 0.167 mmol), 1-(3-dimethyaminopropyl)-3-ethyl-carbodiimide hydrochloride (35.3 mg, 0.184 mmol), 1-hydroxybenzotriazole (25 mg, 0.184 mmol) and Hunig's base (48 mg, 0.368 mmol) in DMF (2 mL) was stirred at rt overnight. The reaction mixture was diluted with EtOAc, and the organic phase was washed with aqueous citric acid, aqueous $NaHCO_3$, and brine, then dried (Na_2SO_4), filtered and evaporated. Purification of the residue by flash column chromatography (CH_2Cl_2/MeOH 20:1) gave the product **22** (129 mg, 87%) as a white solid. **Method B:** compound **21** (35 mg, 0.037 mmol) was dissolved in CF_3CO_2H (0.6 mL) at 0 °C, and the mixture was stirred overnight at rt. Concentration under reduced pressure gave an oily residue, which was dissolved in $CHCl_3$-DMF-H_2O (50:9:1, 3 mL). Et_3N was added until

the solution was basic, followed by $(Boc)_2O$ (78 mg, 0.36 mmol) at 0 °C, and the mixture was stirred for 3 h at rt. The mixture was then concentrated under reduced pressure to give an oily residue, which was acidified with saturated citric acid and extracted with EtOAc. The extract was washed with brine and dried (Na_2SO_4). Concentration followed by flash chromatography of the residue over silica gel (CH_2Cl_2/MeOH 20:1) gave **22** (13 mg, 38%) as a white solid; mp 129.0-131.0 °C; $[\alpha]^{25}_D$ –2.0 (*c* 0.5, $CHCl_3$); ^{1}H NMR δ -0.03 (s, 3H), 0.03 (s, 3H), 0.60 (m, 6H), 0.77 (s, 9H), 1.18 (s, 9H), 1.48 (m, 3H), 1.80 (m, 1H), 2.34 (m, 1H), 2.49 (m, 3H), 2.73 (m, 2H), 2.96 (d, 2H, *J* = 6.4 Hz), 3.73 (m, 2H), 4.37 (m, 1H), 4.56 (d, 1H, *J* = 7.9 Hz), 4.64 (m, 1H), 6.09 (s, 1H), 6.31 (s, 1H), 6.86-7.66 (m, 19H); ^{13}C NMR δ -5.1, -4.6, 14.1, 17.9, 21.0, 22.1, 22.5, 28.3, 33.6, 35.9, 37.7, 38.7, 40.9, 44.5, 52.4, 53.7, 54.3, 72.2, 79.4, 126.2, 126.8, 128.1, 128.3, 128.5, 128.9, 130.0, 130.4, 132.2, 135.7, 136.5, 137.5, 138.4, 144.3, 155.5, 171.6, 173.3, 174.9, 196.1; HR-MS (FAB, MH^+) calcd for *m/z* $C_{52}H_{71}N_4O_7Si$ 891.5092, found 891.5102.

{5-(4-Benzoyl-phenyl)-1*S*-benzyl-4*R*-[1-(1*S*-carbamoyl-2-phenyl-ethylcarbamoyl)-3(1*S*)-methyl-butylcarbamoyl]-2*R*-hydroxy-pentyl}-carbamic acid, *tert*-butyl ester (2). Method A: a solution of compound **22** (53 mg, 0.059 mmol) in a solution of tetrabutylammonium fluoride in THF (1.0 M, 0.5 mL) was stirred at rt overnight. The reaction mixture was diluted with aqueous citric acid solution and EtOAc. The organic phase was washed with aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), filtered and evaporated. Purification of the residue by flash column chromatography (CH_2Cl_2/MeOH 20:1) gave product **2** (33 mg, 72%) as a white solid. **Method B**: compound **23** (30 mg, 0.037 mmol) was dissolved in CF_3CO_2H (0.3 mL) at 0 °C, and the mixture was stirred overnight at rt. Concentration under reduced pressure

gave an oily residue, which was dissolved in $CHCl_3$-DMF-H_2O (50:9:1, 3 mL). Et_3N was added to the mixture until the solution is basic and then $(Boc)_2O$ (39 mg, 0.18 mmol) was added at 0 °C. This mixture was stirred for 3 h at rt, then concentrated under reduced pressure to give an oily residue, which was acidified with saturated citric acid and extracted with EtOAc. The extract was washed with brine and dried over Na_2SO_4. Concentration followed by flash chromatography of the residue over silica gel (CH_2Cl_2/MeOH 20:1) gave **2** (11 mg, 37%) as a white solid; mp 192.2-193.6 °C; $[\alpha]^{25}_D$ – 6.2 (*c* 0.5, $CHCl_3$/MeOH 4:1); ^{1}H NMR ($CDCl_3$/CD_3OD) δ 0.53 (d, 3H, *J* = 6.1 Hz), 0.67 (d, 3H, *J* = 6.0 Hz), 1.25 (s, 9H), 1.44 (d, 3H, *J* = 6.4 Hz), 1.71 (m, 1H), 1.86 (m, 1H), 2.59 (m, 1H), 2.78 (m, 3H), 2.92 (m, 2H), 3.14 (dd, 1H, *J* = 6.0, 13.9 Hz), 3.58 (m, 1H), 3.73 (br s, 1H), 4.18 (t, 1H, *J* = 6.4 Hz,), 4.55 (t, 1H, *J* = 6.5 Hz), 5.68 (d, 0.5H, unexchanged H, *J* = 9.2 Hz), 7.06-7.49 (m, 19H); ^{13}C NMR δ 20.9, 22.4, 27.8, 35.0, 37.0, 38.2, 39.7, 45.4, 49.3, 53.6, 55.8, 72.0, 79.2, 125.9, 126.5, 128.0, 128.1, 128.7, 128.8, 129.6, 130.1, 132.2, 135.3, 136.4, 137.2, 138.0, 144.3, 156.2, 172.4, 173.7, 176.3, 196.9; HR-MS (FAB, MNa^+) calcd for *m/z* $C_{46}H_{56}N_4NaO_7$ 799.4047, found 799.4044.

5 (*S*)-*tert*-Butoxycarbonylamino-4 (*R*)-(*tert*-butyldimethylsilanyloxy)-6-phenyl-2-(4-(2-phenyl-1,3-dioxolan-2-yl)-benzyl)-hexanoic acid (20). This compound was prepared in 72% yield by using the same procedure as described for **25.** ^{1}H NMR δ 0.05 (s, 6H), 0.88 (m, 9H), 1.23 (s, 9H), 1.66 (m, 1H), 2.50 (s, 2H,), 2.70 (m, 1H), 2.84-3.00 (m, 5H), 3.80 (s, 2H), 3.98 (br s, 4H), 5.26 (s, 1H), 7.00-7.42 (m, 14H); ^{13}C NMR δ -4.1, 18.4, 26.3, 28.4, 28.7, 32.0, 35.4, 37.0, 38.4, 43.6, 55.5, 65.2, 72.1, 109.7, 126.5, 126.8, 128.4, 128.5, 128.7, 129.3, 129.6, 139.2, 140.8, 142.5, 155.6, 163.3, 179.0.

{1*S*-Benzyl-2*R*-(*tert*-butyldimethylsilanyloxy)-4*R*-[1-(1*S*-carbamoyl-2-phenyl-ethylcarbamoyl)-3(1*S*)-methyl-butylcarbamoyl]-5-[4-(2-phenyl-[1,3]dioxolan-2-yl)-phenyl]-pentyl}-carbamic acid, *tert*-butyl ester (21). A solution of **20** (113 mg, 0.167 mmol), Leu-Phe-NH_2 ·HCl (52.4 mg, 0.167 mmol), 1-(3-dimethyaminopropyl)-3-ethyl-carbodiimide hydrochloride (35.3 mg, 0.184 mmol), 1-hydroxybenzotriazole (25 mg, 0.184 mmol) and Hunig's base (48 mg, 0.368 mmol) in DMF (2 mL) was stirred at rt overnight. The reaction mixture was diluted with EtOAc, and the organic phase was washed with aqueous citric acid, aqueous $NaHCO_3$, and brine, dried (Na_2SO_4), filtered and evaporated. Purification of the residue by flash column chromatography (CH_2Cl_2/MeOH 20:1) gave the product **21** (137 mg, 88%) as a white solid; mp 65.5-67.0 °C; $[\alpha]^{25}_D$ –7.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR δ 0.03 (s, 3H), 0.05 (s, 3H), 0.74 (d, 3H, *J* = 4.6 Hz), 0.78 (d, 3H, *J* = 4.9 Hz), 0.86 (s, 9H), 1.12 (s, 9H), 1.44 (m, 3H), 1.54 (m, 1H), 1.76 (t, 3H, *J* = 11.8 Hz), 2.38 (m, 4H), 2.68 (m, 1H), 2.96 (dd, 1H, *J* = 7.3, 13.8 Hz), 3.06 (dd, 1H, *J* = 6.3, 13.6 Hz), 3.73 (t, 2H, *J* = 11.1 Hz), 3.87 (br s, 4H), 4.29 (d, 1H, *J* = 6.0 Hz), 4.56 (d, 1H, *J* = 7.5 Hz), 4.64 (m, 1H), 5.89 (s, 1H), 6.31 (s, 1H), 6.54 (d, 1H, *J* = 7.7 Hz), 6.94-7.40 (m, 19H); ^{13}C NMR δ -4.6, -4.1, 18.4, 22.6, 22.9, 25.0, 26.3, 28.8, 34.0, 36.6, 37.8, 38.8, 41.1, 45.1, 53.3, 54.1, 54.8, 65.2, 72.6, 79.9, 109.6, 126.5, 126.7, 127.0, 127.3, 128.4, 128.5, 128.8, 129.0, 129.2, 129.4, 129.7, 137.2, 138.9, 139.4, 141.0, 142.4, 156.1, 172.0, 173.7, 176.0; HR-MS (FAB, MH^+) calcd for *m/z* $C_{54}H_{75}N_4O_8Si$ 935.5354, found 935.5352.

{1*S*-Benzyl-4*R*-[1-(1*S*-carbamoyl-2-phenyl-ethylcarbamoyl)-3-(1*S*)-methyl-butylcarbamoyl]-2*R*-hydroxy-5-[4-(2-phenyl-[1,3]dioxolan-2-yl)-phenyl]-pentyl}-carbamic acid, *tert*-butyl ester (23). Compound **21** (55 mg, 0.059 mmol) was dissolved

in a solution of tetrabutylammonium fluoride in THF (1.0 M, 0.5 mL) and stirred at rt overnight. The reaction mixture was diluted with citric acid and EtOAc. The organic phase was washed with aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), filtered and evaporated. Purification of the residue by flash column chromatography (CH_2Cl_2/MeOH 9:1) gave product **23** (34 mg, 71%) as a white solid; mp 176.4-177.6 °C; $[\alpha]^{25}_D$ –32.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR δ 0.73 (d, 3H, *J* = 6.0 Hz), 0.80 (d, 3H, *J* = 6.0 Hz), 1.16 (s, 3H), 1.25 (s, 9H), 1.66 (m, 2H), 2.04 (s, 1H), 2.61 (m, 3H), 2.79-2.95 (m, 3H), 3.20 (d, 1H, *J* = 8.8 Hz), 3.60 (br s, 1H), 3.75 (br s, 1H), 4.02 (m, 5H), 4.64 (m, 1H), 4.72 (s, 1H), 4.89 (d, 1H, *J* = 7.4 Hz), 5.98 (s, 1H), 6.51 (s, 1H), 6.60 (s, 1H), 7.06-7.49 (m, 19H); ^{13}C NMR δ 21.9, 23.2, 25.0, 28.7, 35.9, 36.2, 37.6, 39.3, 40.4, 47.5, 54.0, 54.3, 57.1, 65.2, 73.9, 80.1, 109.7, 126.5, 126.9, 127.2, 128.4, 128.6, 128.9, 129.2, 129.6, 137.4, 138.4, 139.1, 141.0, 142.4, 156.8, 172.9, 174.4, 177.1; HR-MS (FAB, MNa^+) calcd for *m/z* $C_{48}H_{60}N_4NaO_8$ 843.4309, found 843.4283.

{5-(4-Benzoyl-phenyl)-1*S*-benzyl-2*R*-(*tert*-butyldimethylsilanyloxy)-4*R*-[3-methyl-1*S*-(1-{5-[5-(2-oxo-(3a*R*,6a*S*)hexahydro-thieno[3,4-*d*]imidazol-6*S*-yl)-pentanoylamino]-pentylcarbamoyl}-2*S*-phenyl-ethylcarbamoyl)-butylcarbamoyl]-pentyl}-carbamic acid *tert*-butyl ester (27). A solution of amine **26** (40 mg, 0.068 mmol), acid **25** (43 mg, 0.068 mmol), 1-(3-dimethyaminopropyl)-3-ethyl-carbodiimide hydrochloride (14.3 mg, 0.075 mmol) and 1-hydroxybenzotriazole (10.1 mg, 0.075 mmol) in dry DMF (2 mL) was stirred at rt overnight. The reaction mixture was diluted with ethyl acetate, and the organic phase was washed with aqueous citric acid, aqueous $NaHCO_3$, and brine, dried (Na_2SO_4), filtered and evaporated. Purification of the residue by flash column chromatography (CH_2Cl_2/MeOH 9:1) gave the product **27** (67 mg, 82%);

mp 106.0-107.0 °C; $[\alpha]^{25}_{D}$ +7.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR δ 0.03 (s, 3H), 0.06 (s, 3H), 0.78 (m, 6H), 0.87 (s, 9H), 1.15-1.85 (m, 27H), 2.19 (t, 2H, *J* = 6.8 Hz), 2.41 (m, 1H), 2.51 (m, 1H), 2.56 (m, 1H), 2.61 (m, 2H), 3.03 (d, 2H, *J* = 7.0 Hz), 3.17 (m, 5H), 3.75 (m, 2H), 4.19 (s, 1H), 4.35 (m, 1H), 4.44 (m, 1H), 4.57 (q, 1H, *J* = 6.8 Hz), 4.71 (d, 1H, *J* = 8.4 Hz), 6.12 (s, 1H), 6.56 (s, 1H), 6.62 (s, 1H), 6.80 (s, 1H), 6.97-7.81 (m, 19H); ^{13}C NMR δ -5.1, -4.5, 14.0, 17.9, 22.0, 22.8, 23.5, 23.7, 24.6, 25.7, 28.0, 28.3, 28.8, 30.3, 33.9, 35.8, 38.2, 38.7, 38.9, 39.1, 40.6, 41.2, 43.8, 52.6, 54.0, 54.5, 55.7, 60.1, 61.9, 72.4, 79.2, 126.1, 126.6, 128.2, 128.25, 128.29, 128.7, 129.1, 129.3, 129.9, 130.4, 132.2, 135.5, 136.8, 137.5, 138.6, 144.6, 155.4, 163.9, 170.8, 172.2, 173.3, 174.6, 196.5; HR-MS (FAB, MH^+) calcd for *m/z* $C_{67}H_{96}N_7O_9SSi$ 1202.6759, found 1202.6785.

{5-(4-Benzoyl-phenyl)-1*S*-benzyl-2*R*-hydroxy-4*R*-[3-methyl-1*S*-(1-{5-[5-(2-oxo-(3a*R*,6a*S*)hexahydro-thieno[3,4-*d*]imidazol-6*S*-yl)-pentanoylamino]-pentylcarbamoyl}-2*S*-phenyl-ethylcarbamoyl)-butylcarbamoyl]-pentyl}-carbamic acid *tert*-butyl ester (3). A solution of compound **27** (36 mg, 0.03 mmol) in a solution of tetrabutylammonium fluoride in THF (1.0 M, 0.5 ml) was stirred at rt overnight. The reaction mixture was diluted with citric acid and EtOAc. The organic phase was washed with aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), filtered and evaporated. Purification of the residue by flash column chromatography (CH_2Cl_2/MeOH 9:1) gave product **3** (24 mg, 72%) as a white solid; mp 172.2-174.0 °C; $[\alpha]^{25}_{D}$ +13.0; (*c* 1.0, $CHCl_3$/MeOH 4:1) ^{1}H NMR ($CDCl_3$/CD_3OD) δ 0.81 (d, 3H, *J* = 4.3 Hz), 0.87 (d, 3H, *J* = 4.3 Hz), 1.02-1.22 (m, 9H), 1.44 (m, 10H), 1.68 (m, 6H), 1.74 (m, 1H), 2.19 (t, 2H, *J* = 7.0 Hz), 2.71-3.21 (m, 16H), 3.36 (s, 1H), 3.61 (m, 1H), 3.73 (s, 1H), 4.31 (s, 1H), 4.50 (br s, 2H), 5.63 (d, 0.7H, unexchanged H, *J* = 9.0 Hz), 7.12-7.85 (m, 19H); ^{13}C NMR δ 13.4, 20.0, 21.3,

22.8, 23.9, 24.7, 25.7, 28.1, 28.2, 28.5, 28.8, 35.5, 35.8, 37.9, 38.6, 39.1, 39.3, 40.2, 40.4, 45.7, 52.7, 53.0, 54.6, 55.8, 56.3, 60.3, 62.1, 72.4, 79.5, 126.3, 126.9, 128.4, 128.5, 129.1, 129.3, 130.0, 130.6, 132.7, 135.7, 136.8, 137.6, 138.6, 145.0, 156.7, 164.5, 171.5, 172.9, 174.5, 176.6, 197.4; HR-MS (FAB, MH^+) calcd for *m/z* $C_{61}H_{82}N_7O_9S$ 1088.5895, found 1088.5842.

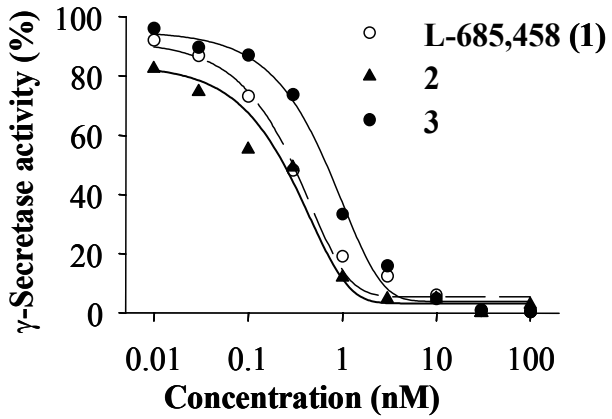

Figure 1'. Inhibition of γ-secretase activity by **1**, **2** and **3**. γ-Secretase activity was measured by the production of the Aβ40 related product from the C100FLAG substrate.[6] Activities were expressed as the percentage relative to Aβ40 that was produced in absence of any compound.

γ-Secretase Assay and IC_{50} Determination. C100Flag was added to HeLa cell membranes containing the indicated concentrations of L-685,458, compound **2** and compound **3** in the presence of CHAPSO (0.25%) in assay buffer (50 mM PIPES, pH 7.0, 5 mM $MgCl_2$, 5 mM $CaCl_2$, 150 mM KCl) at 37 °C. The reactions were stopped by adding RIPA (150 mM NaCl, 1.0% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris HCl, pH 8.0) and boiling for 5 min.[6] After 150 min, aliquots were assayed for

Aβ40 production by electrochemiluminescence technology as described.[6] Activity levels are relative to that observed in the absence of inhibitor (assigned 100%). Inhibition data was plotted using Kaleidagraph and curve-fitted to a sigmoidal function to determine IC_{50} values (Figure 1').

3. References

(1) Chen, P.; Cheng, P. T. W.; Spergel, S. H.; Zahler, R.; Wang, X. B.; Thottathil, J.; Barrish, J. C.; Polniaszek, R. P. *Tetrahedron Lett.* **1997**, *38*, 3175, and references cited therein.

(2) Evans, E. E.; Rittle, K. E.; Homnick, C. F.; Springer, J. P.; Hirshfield, J.; Veber, D. F. *J. Org. Chem.* **1985**, *50*, 4615.

(3) Nadin, A.; Lopez, J. M. S.; Neduvelil, J. G.; Thomas, S. R. *Tetrahedron* **2001**, *57*, 1861.

(4) Vabeno, J.; Brisander, M.; Lejon, T.; Luthman, K. *J. Org. Chem.* **2002**, *67*, 9186, and references cited therein.

(5) Matsuda, K.; Ulrich, G.; Iwamura, H. *J. Chem. Soc., Perkin Trans. 2* **1998**, 1581.

(6) Li, Y. M.; Lai, M. T.; Xu, M.; Huang, Q.; DiMuzio-Mower, J.; Sardana, M. K.; Shi, X. P.; Yin, K. C.; Shafer, J. A.; Gardell, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 6138.

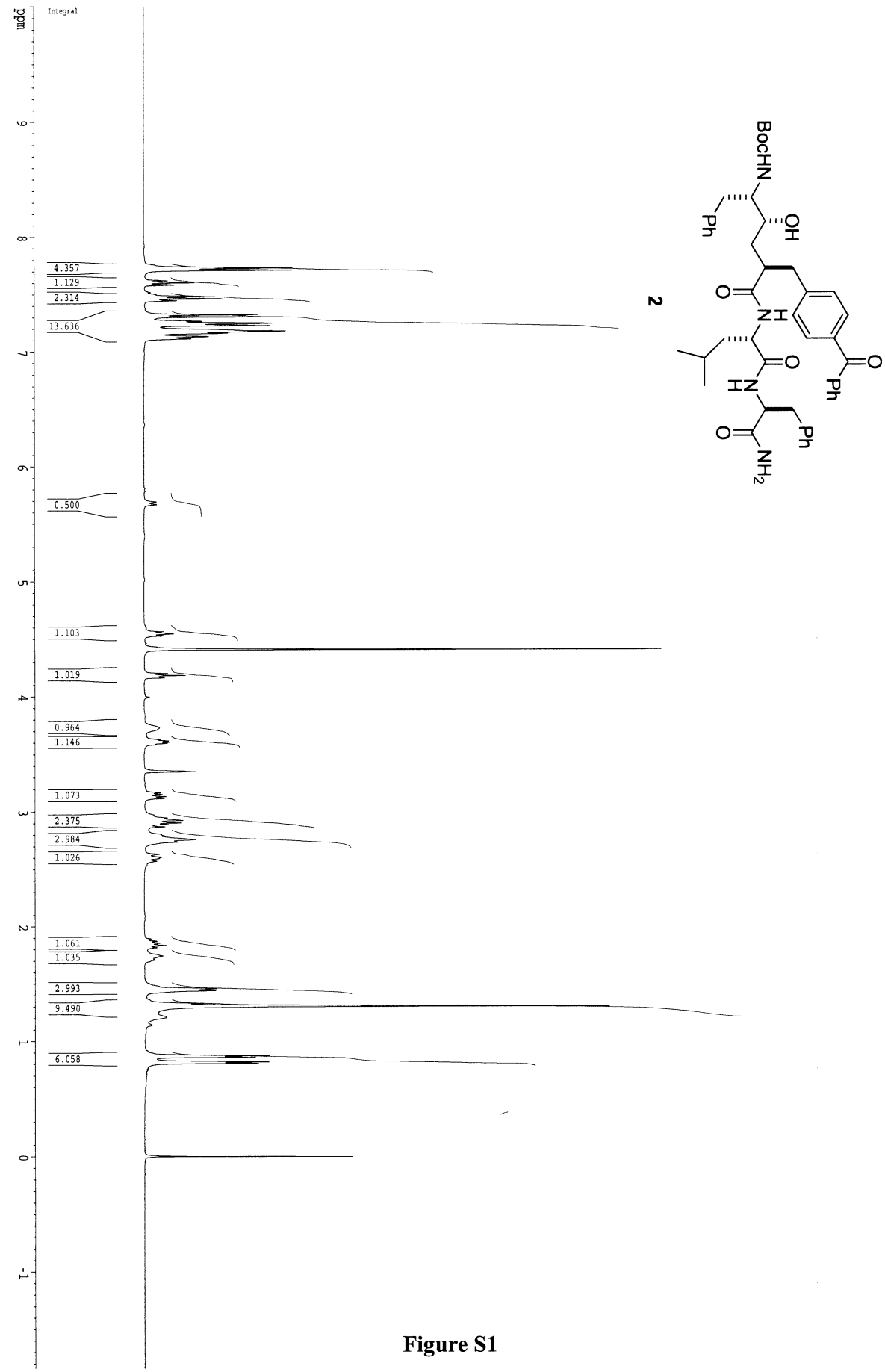
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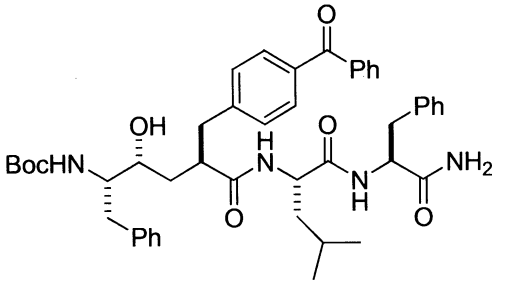
Figure S1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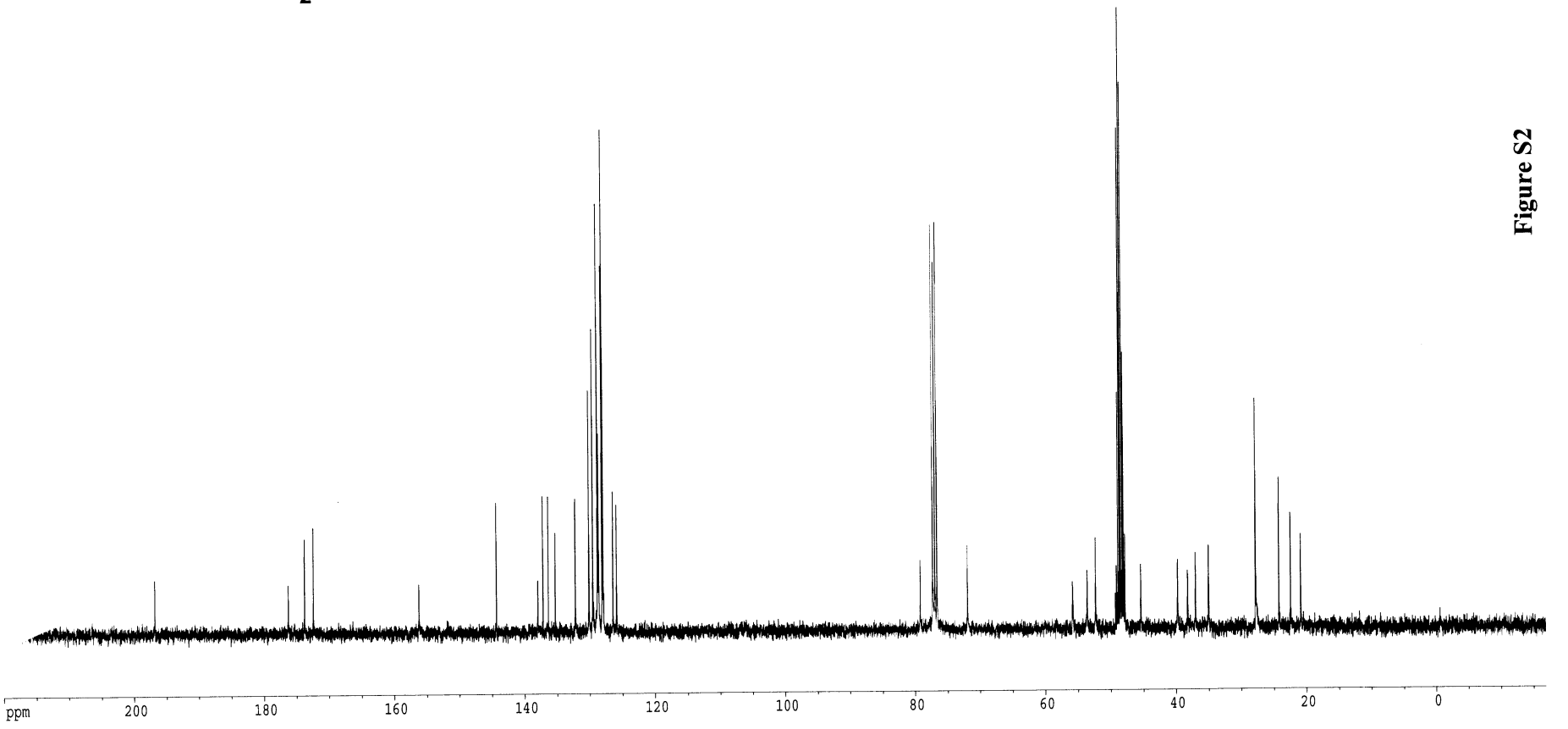
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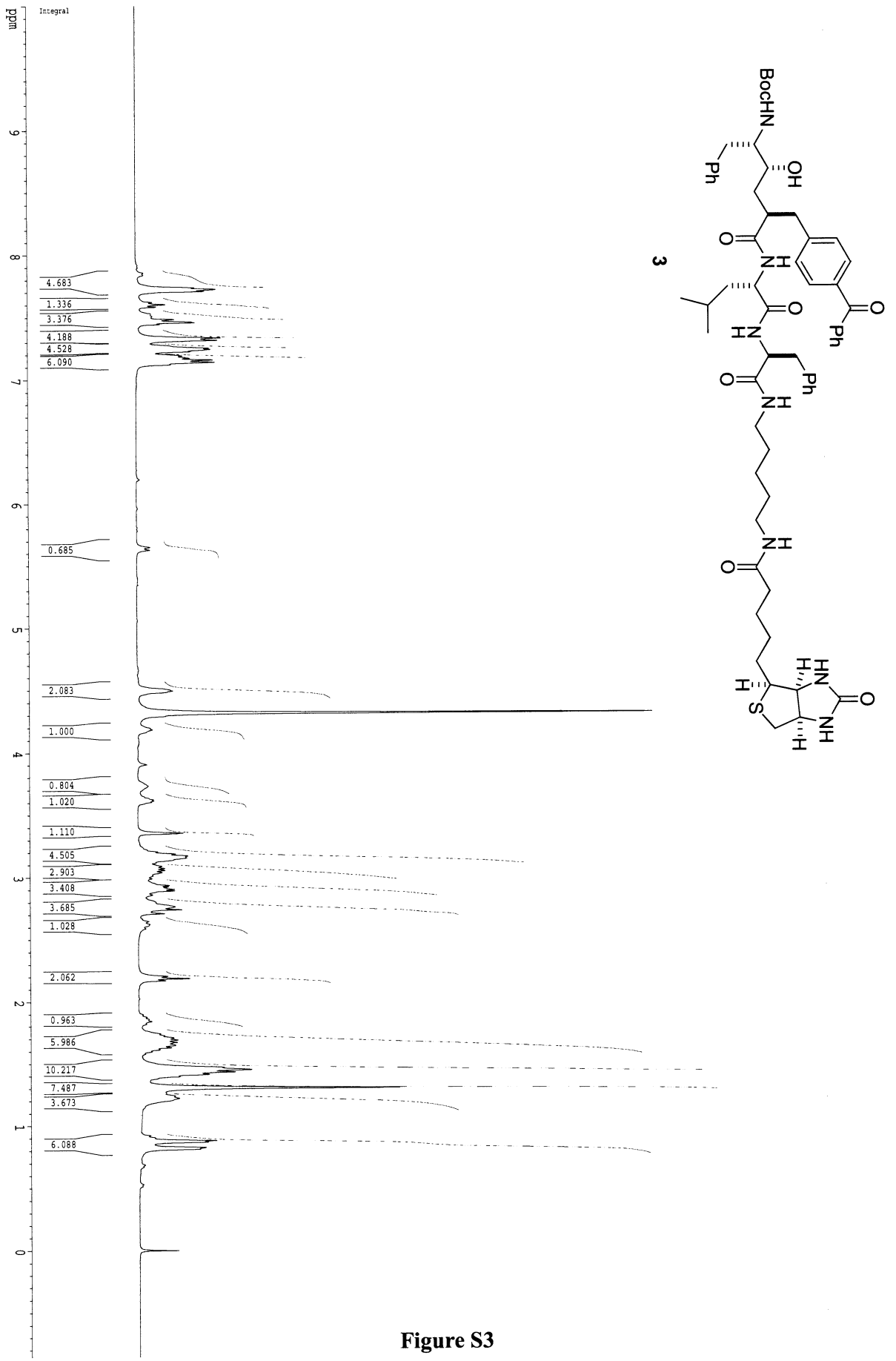
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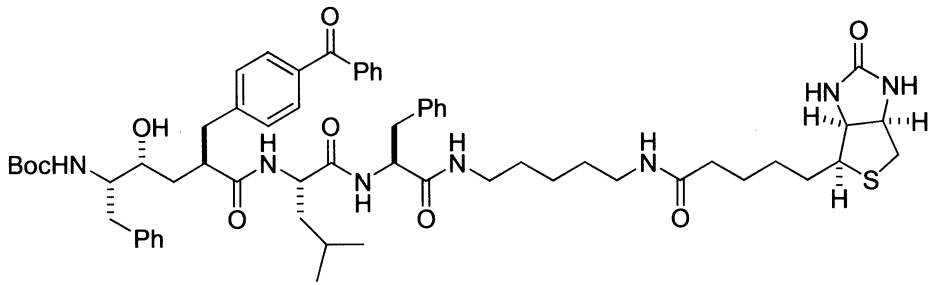
Figure S3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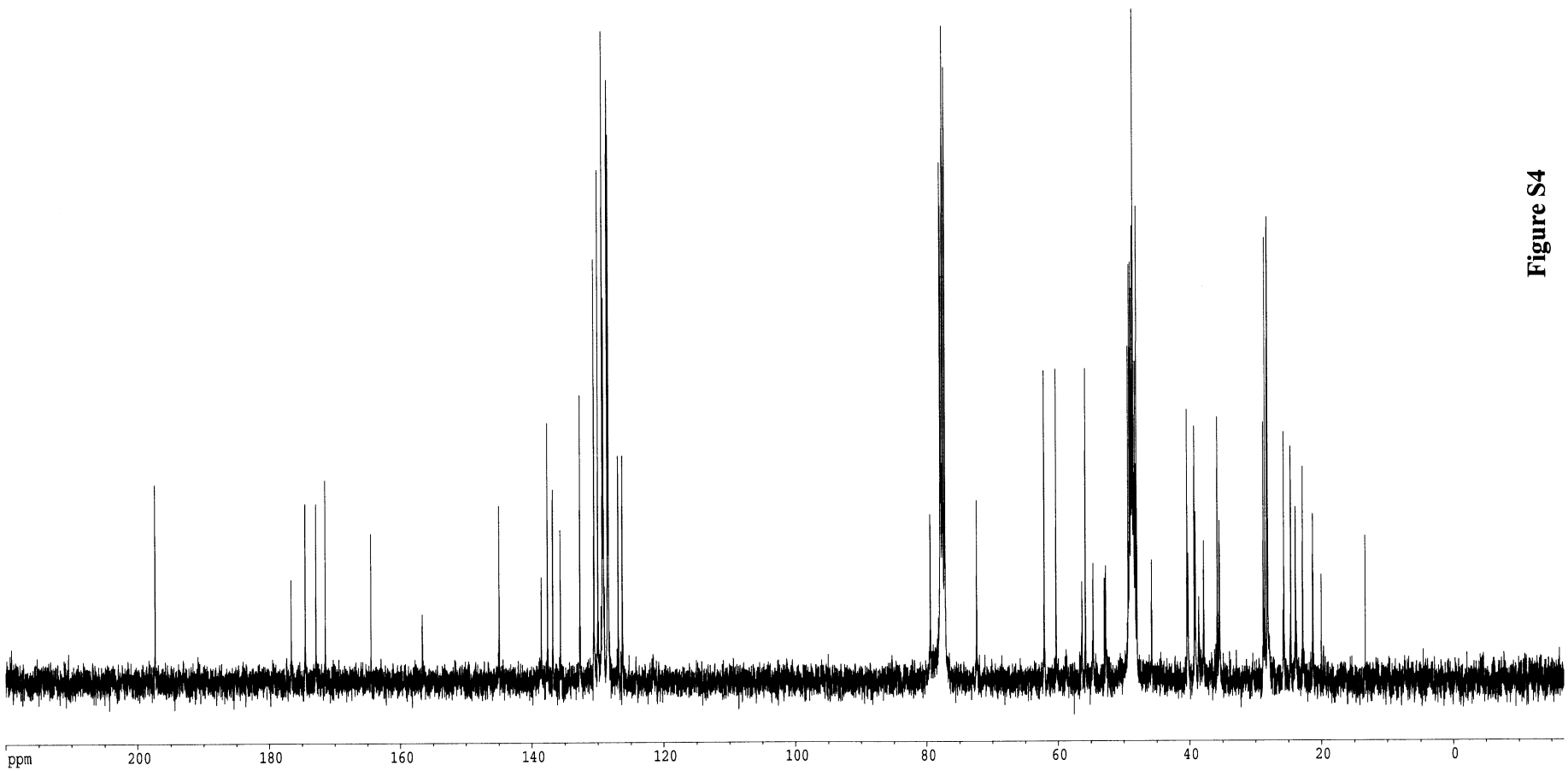
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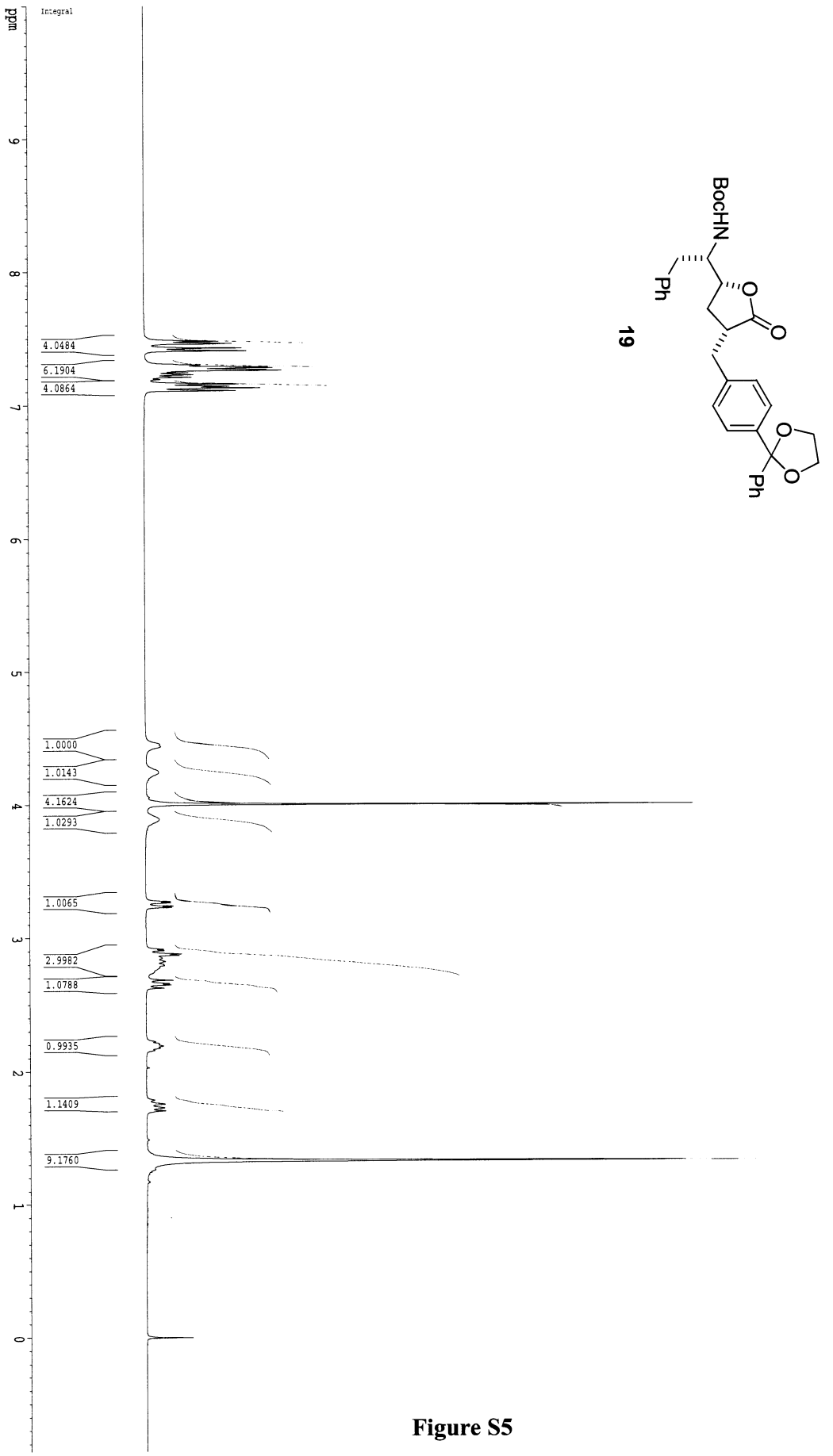
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19

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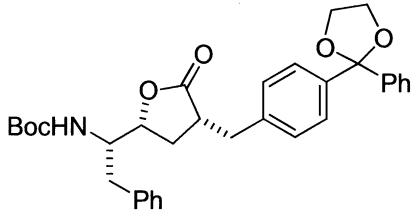

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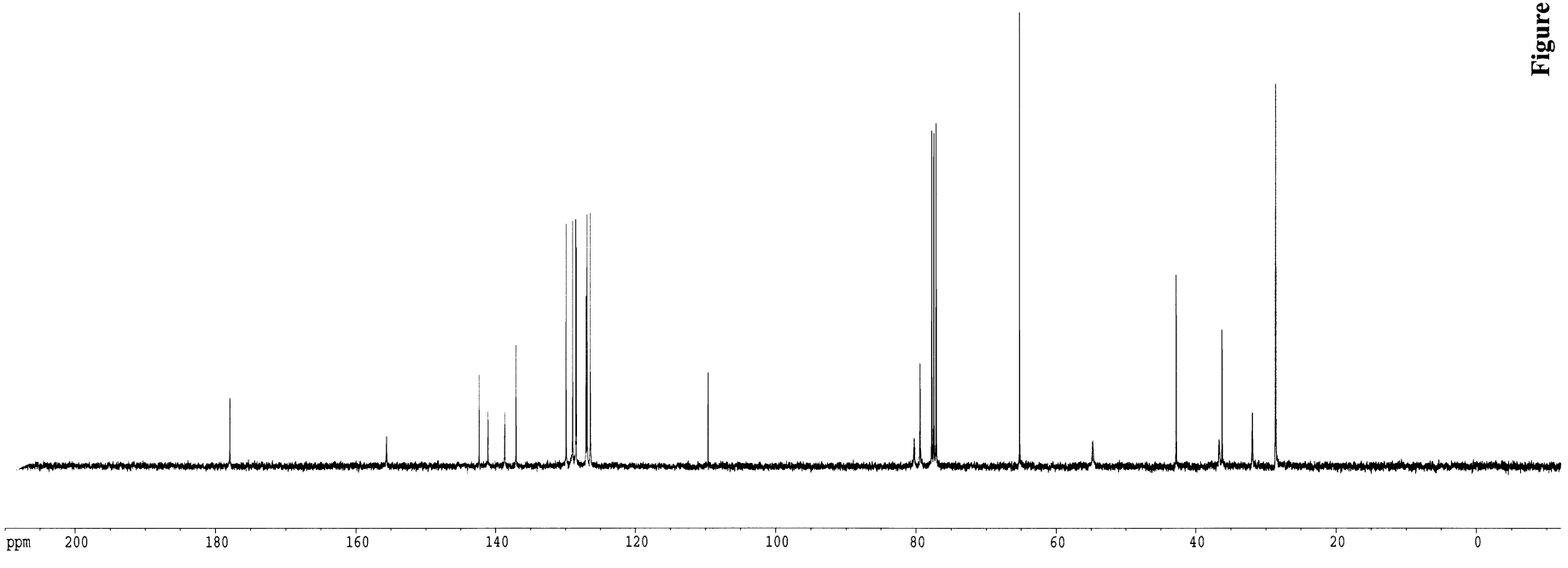

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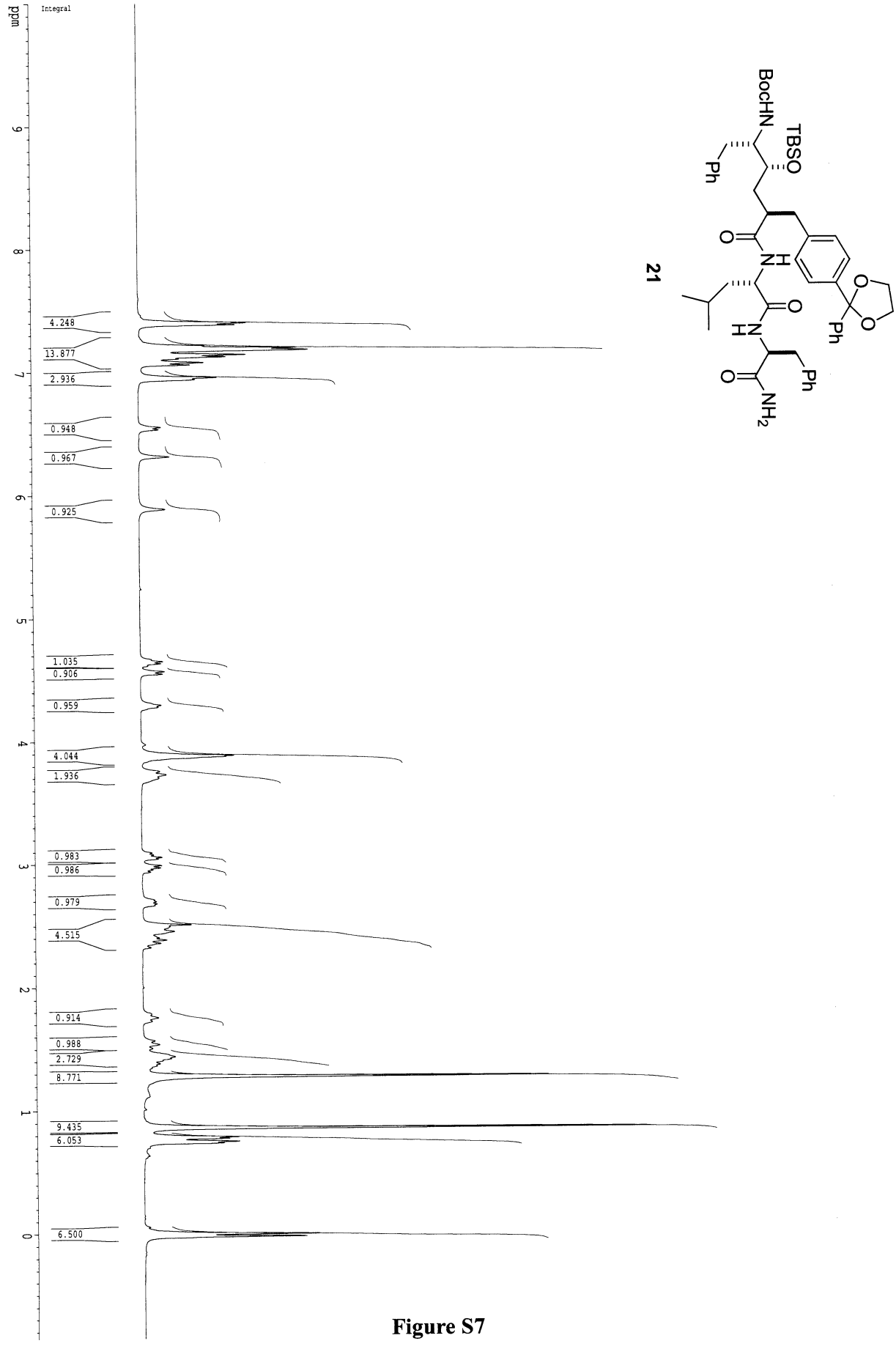
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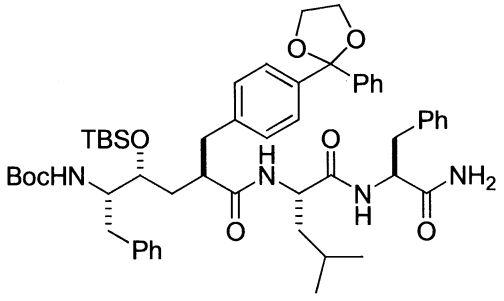
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NH2
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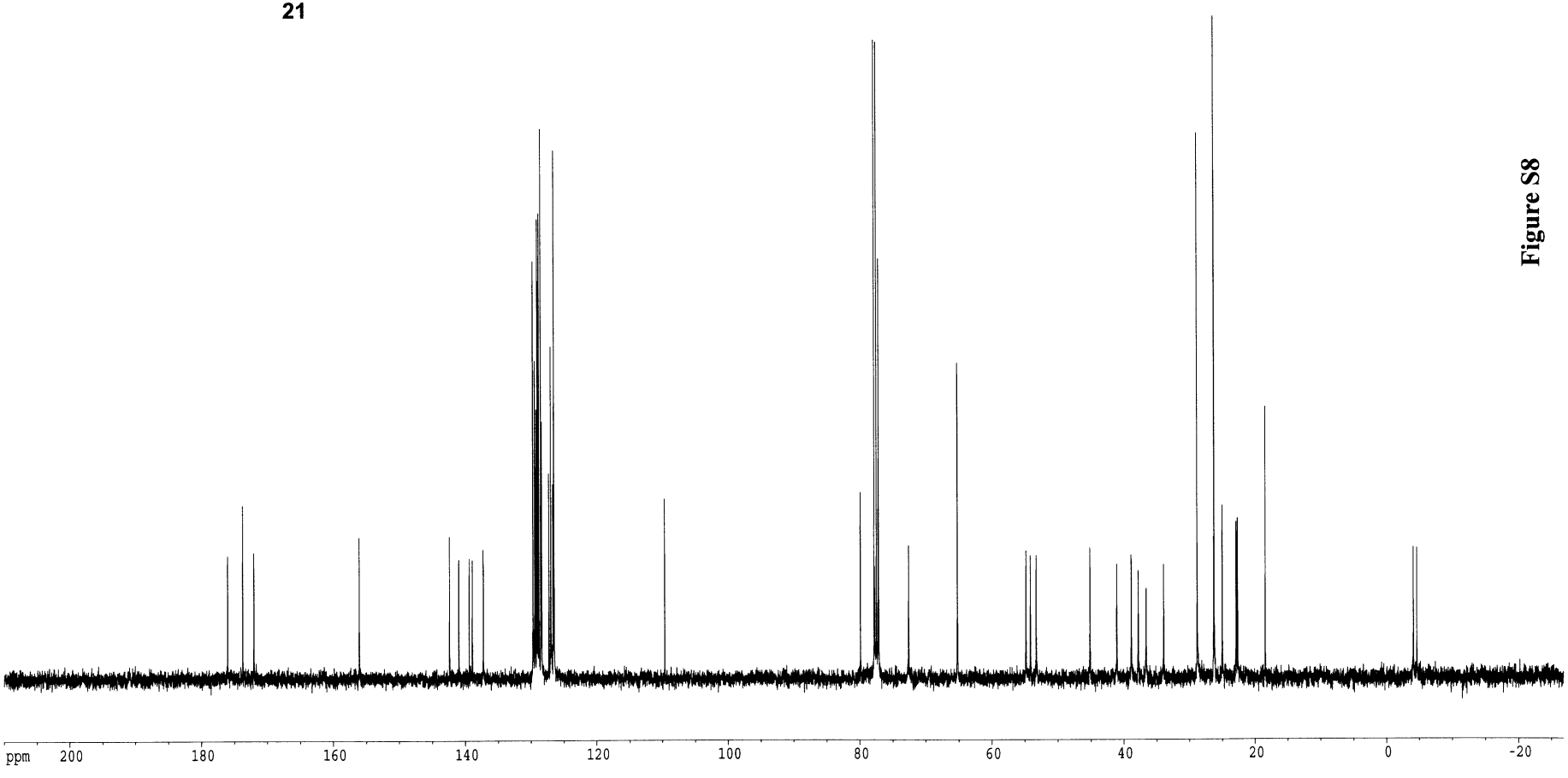
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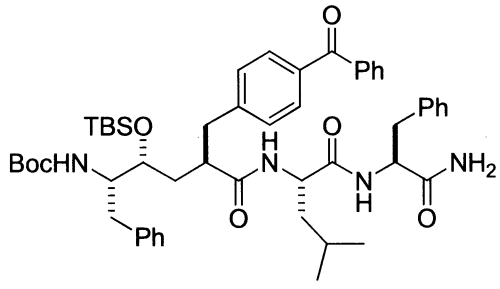
Figure S8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22

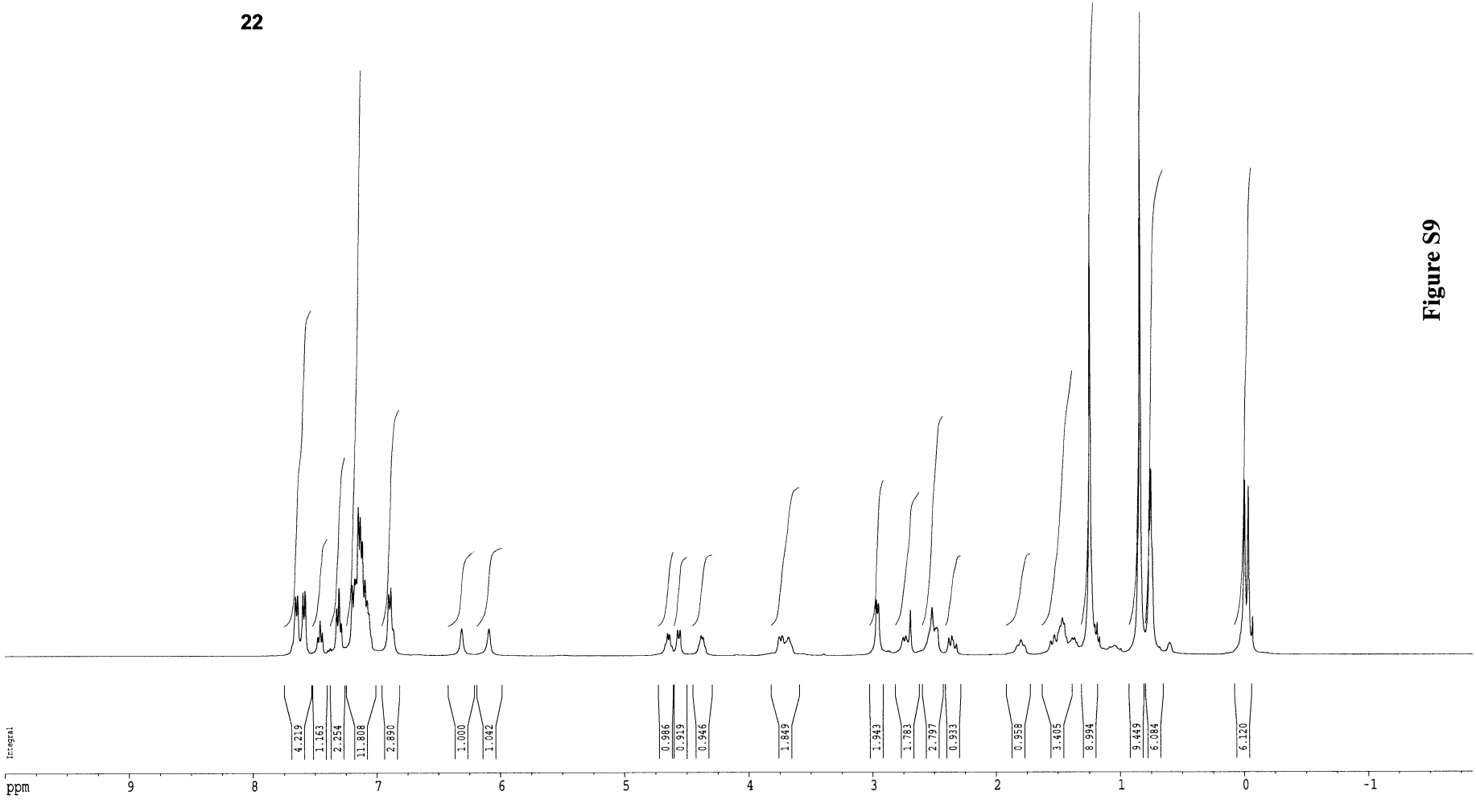
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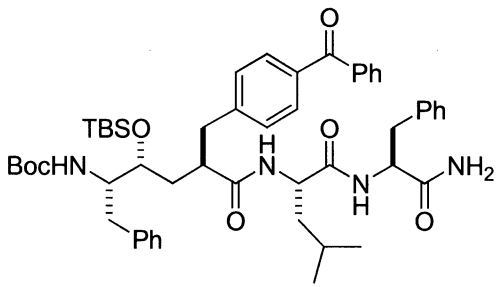
Figure S9



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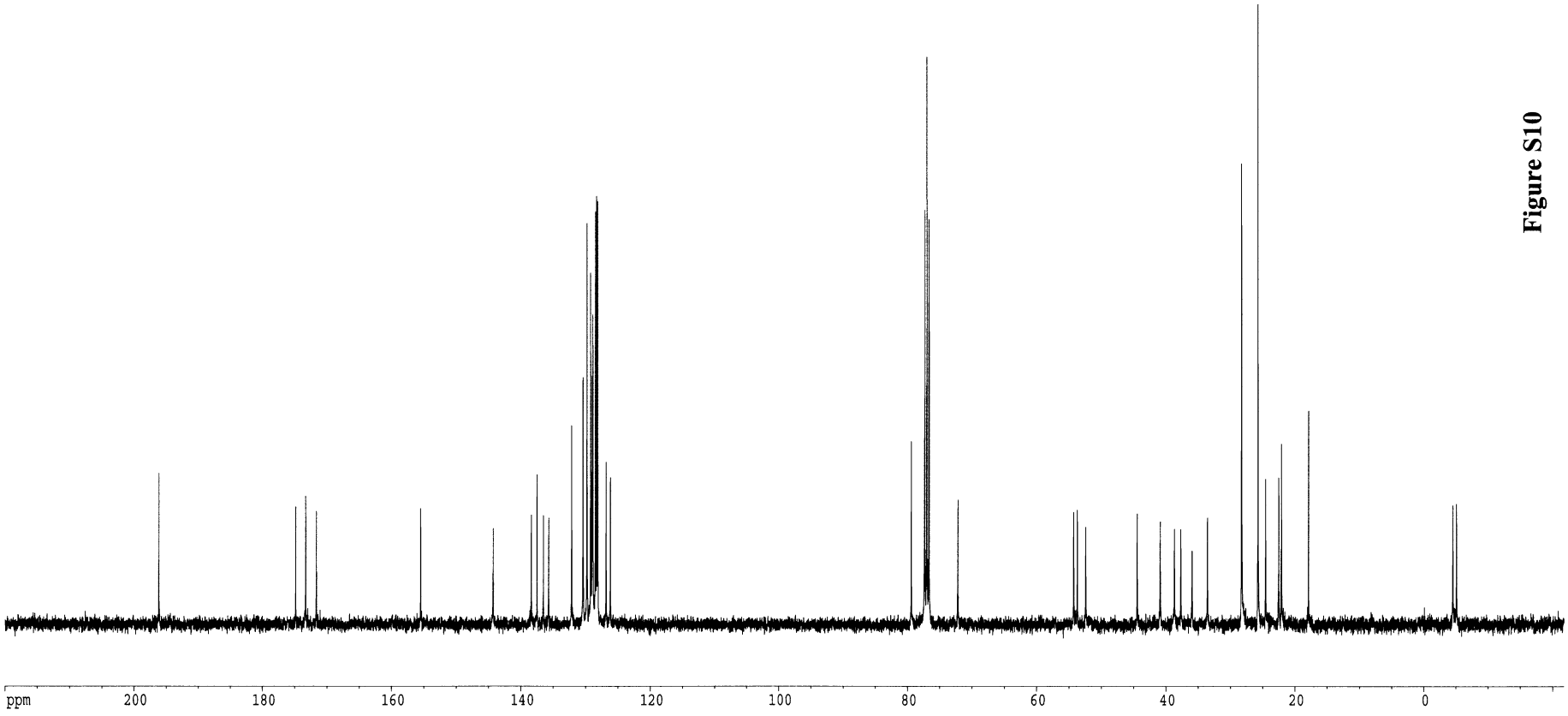

Figure S10

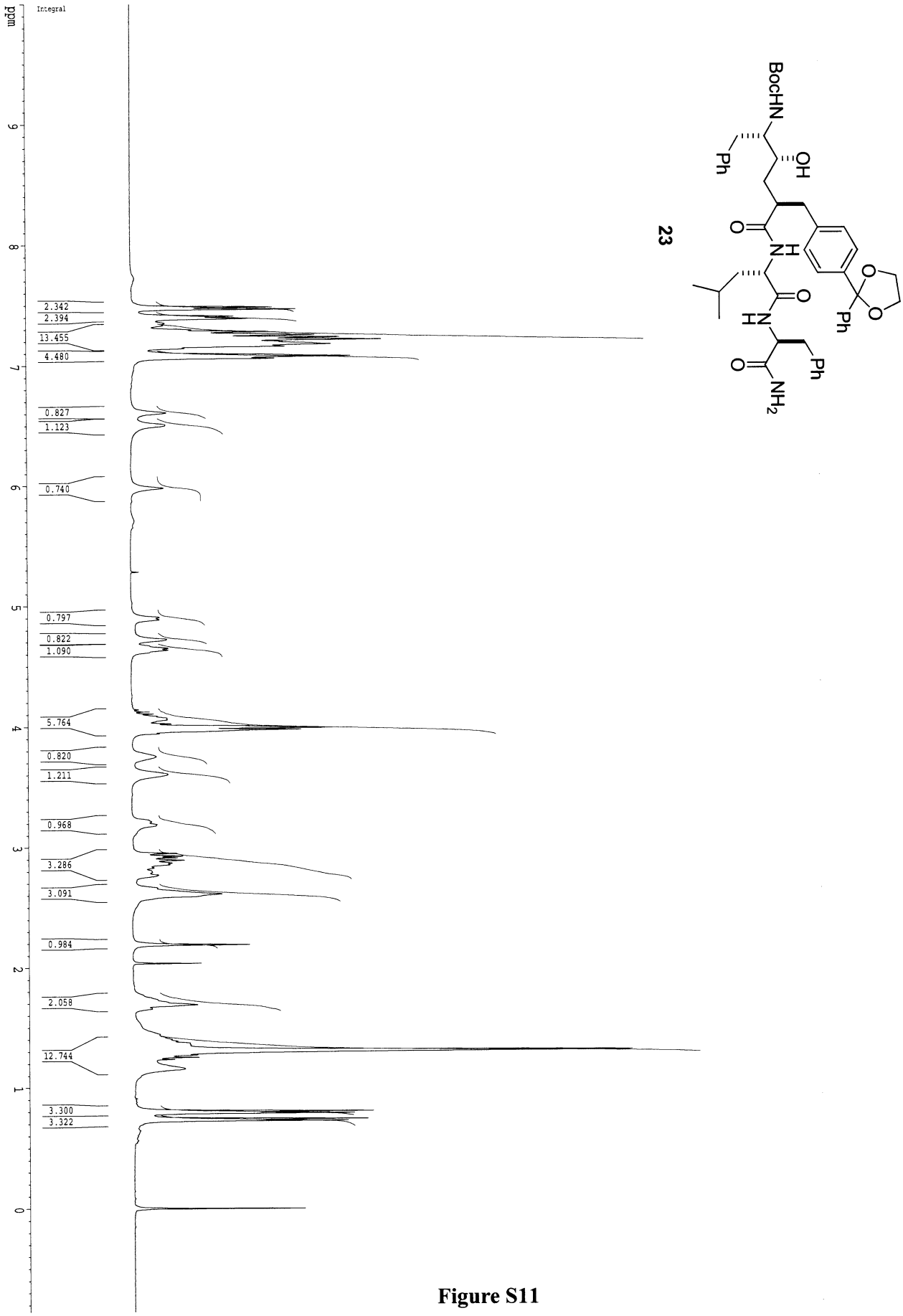

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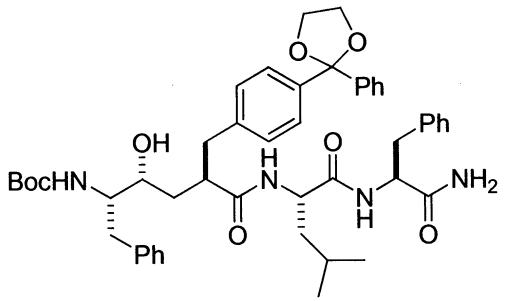
Figure S11



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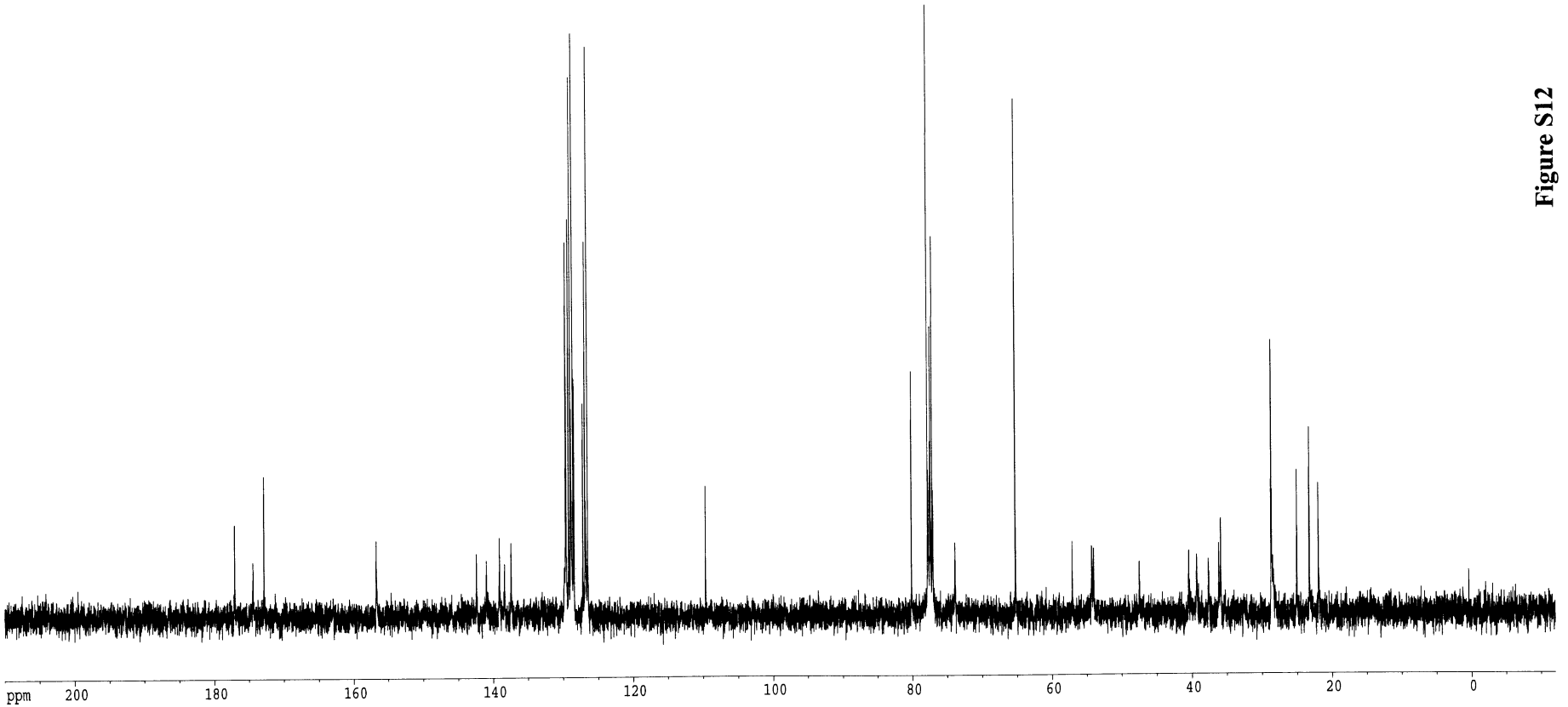

Figure S12

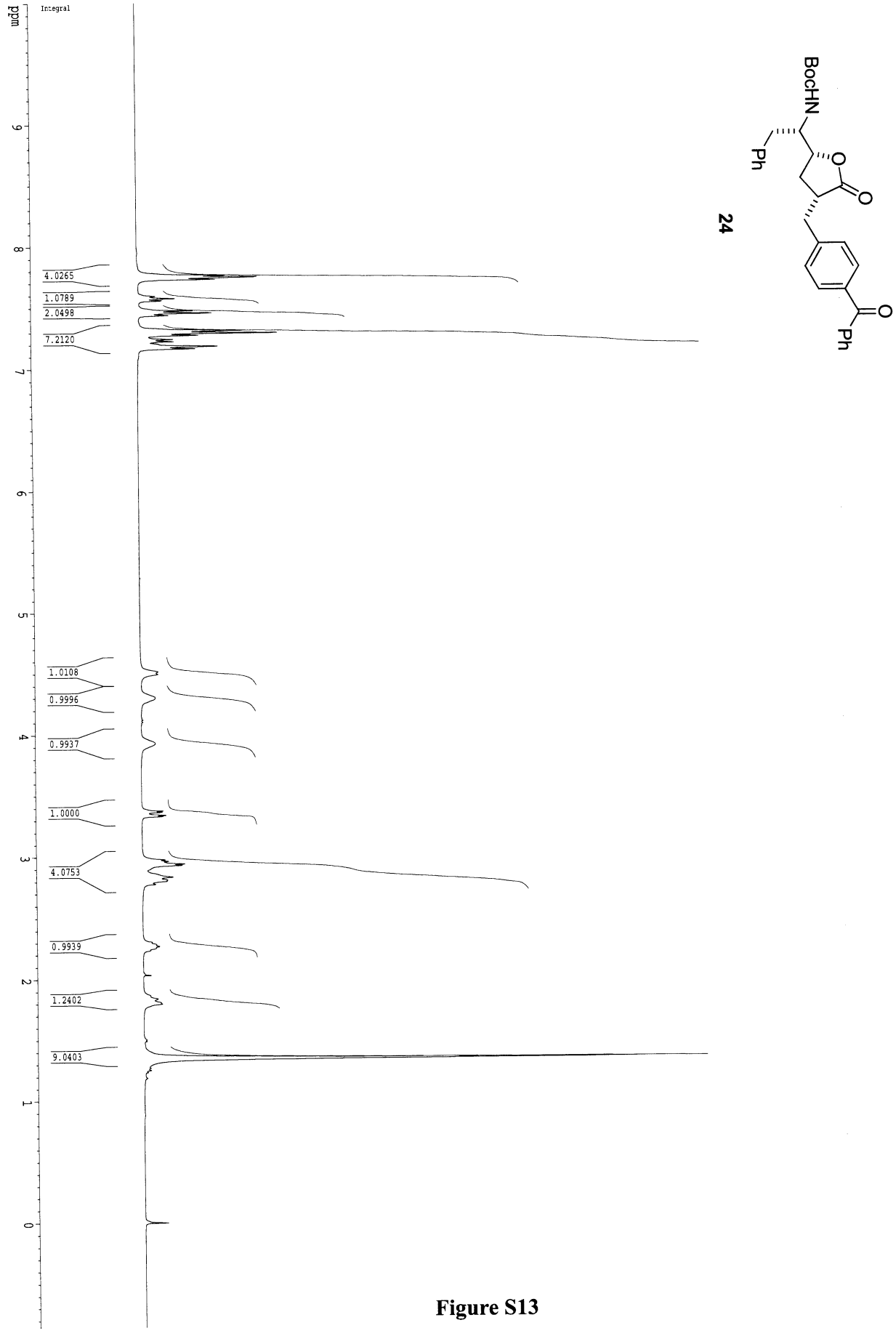
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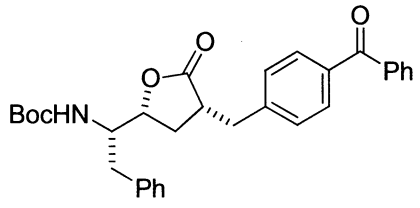
Figure S13



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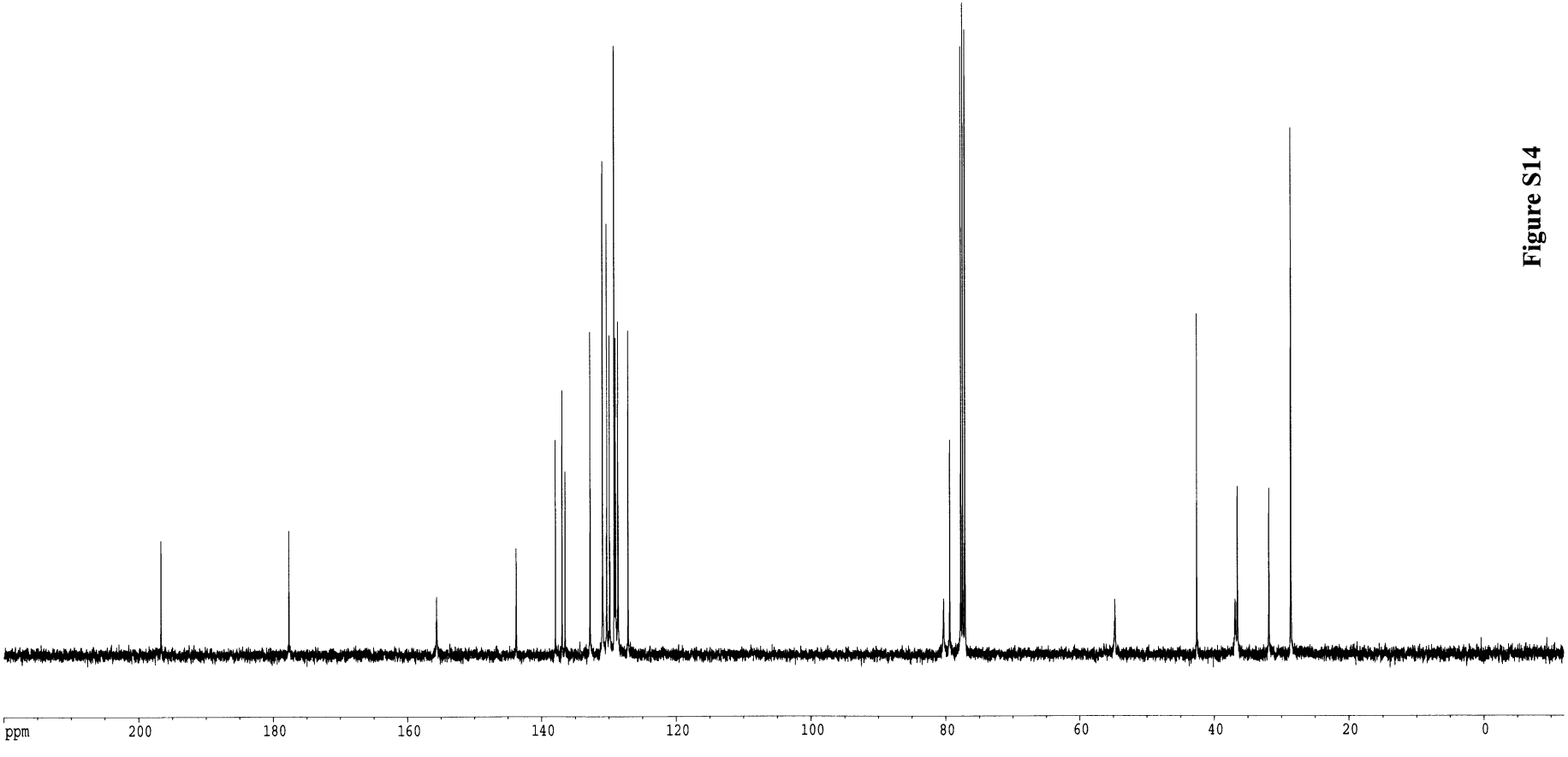

Figure S14