Supporting Information

Selective Inhibitors of the Serine Protease Plasmin: Probing the S3 and S3' Subsites Using a Combinatorial Library

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1. General Methods.

All experiments were conducted using anhydrous conditions under an atmosphere of nitrogen, except where stated. Oven-dried glassware and standard techniques for handling air-sensitive materials were used in all experiments. All reagents were used as received. Aqueous solutions of sodium bicarbonate and sodium chloride (brine) were saturated.

Analytical thin layer chromatography was carried out on Merck Kieselgel 60F254 plates with visualization by ultraviolet, ninhydrin, or phosphomolybdic acid (PMA). Flash column chromatography was carried out on Merck Kieselgel 60 (230-400 mesh) under a positive pressure of nitrogen.

Resins and amino acids. Wang resins that were pre-loaded with Fmoc amino acids were purchased from Calbiochem-Novabiochem Corp. Eighteen of the 20 common amino acids were incorporated into the library. Hydroxyproline (Hyp) and ornithine (Orn) were selected to as replacements for Cys and Met to avoid problems associated with sulfur oxidation. Amino acids and their side chain protecting groups were used as follows: Ala, Arg(Pmc), Asn(Trt), Asp(*t*-Bu), Gln(Trt), Glu(*t*-Bu), Gly, His(Trt), Hyp(*t*-Bu), Ile, Leu, Lys(Boc), Orn(Boc), Phe, Pro, Ser(*t*-Bu), Thr(*t*-Bu), Trp, Tyr(*t*-Bu), and Val.

Synthesis of the Initial Library. Dry Wang resins that were pre-loaded with the 20 Fmoc amino acids (0.05 mmol each) were combined in a flask and allowed to swell in 100 mL of DMF for 6 h. The Fmoc protecting groups were removed with 30 mL of a

1:1 solution of DMF and piperidine for 30 min. After washing with 5 × 25 mL of DMF, a positive Kaiser's test indicated the presence of free amines. Coupling reactions were performed using HBTU (1.5 mmol) and DIEA (3 mmol). A negative Kaiser's test indicated the absence of the free amines after each couplinc cycle. The resin was capped after each coupling reaction with Ac_2O (5 mmol) and DIEA (5 mmol) for 30 min. For the splitting step, the beads were dried and split into 20 even batches. The beads were placed into 20 Econo-Columns (1 × 10 cm, Biorad) that served as synthesis vessels. After the synthesis of the library was complete, the peptides in each batch were cleaved from the beads using 2.5 mL of a solution of TFA (95%), H_2O (2.5%), and TIS (2.5%).

NMR spectra. ^1H Nuclear magnetic resonance spectra were recorded on Avance-300 (300 MHz) or Avance-400 (400 MHz) spectrometers. ^{13}C NMR spectra were recorded at 75 or 100 MHz, and all chemical shift values are reported in ppm on the δ scale, with an internal reference of δ 77.0 or 49.0 for $CDCl_3$ or MeOD, respectively.

Infra-red spectra. Wavelengths of absorbances are reported in cm^{-1}.

High-resolution mass spectra. Electron impact (EI), chemical ionisation (CI) or fast atom bombardment (FAB) spectra were recorded on a JEOL JMS-600 mass spectrometer.

HPLC analyses. A rainin HPLC system was used with a Rainin C18 analytical column and UV detection. Semipreparative HPLC was performed on the same system using a semipreparative column (21.4 × 250 mm).

2. Characterization Data.

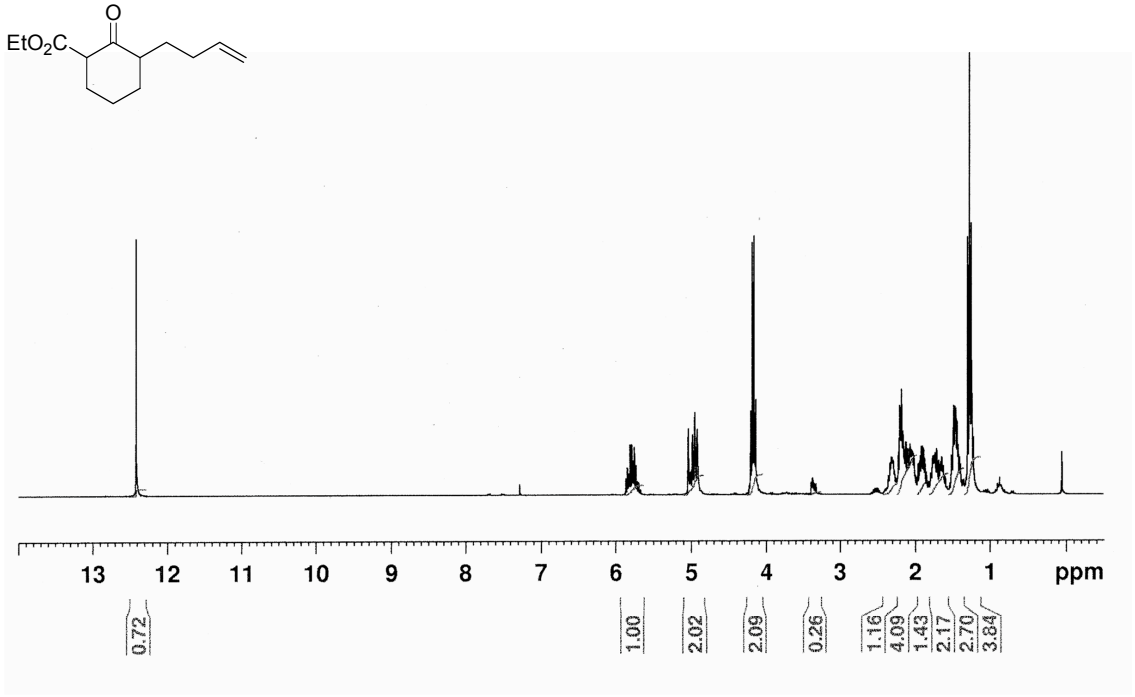


Figure S1. ^{1}H NMR spectrum for compound **5** (mixture of tautomers).

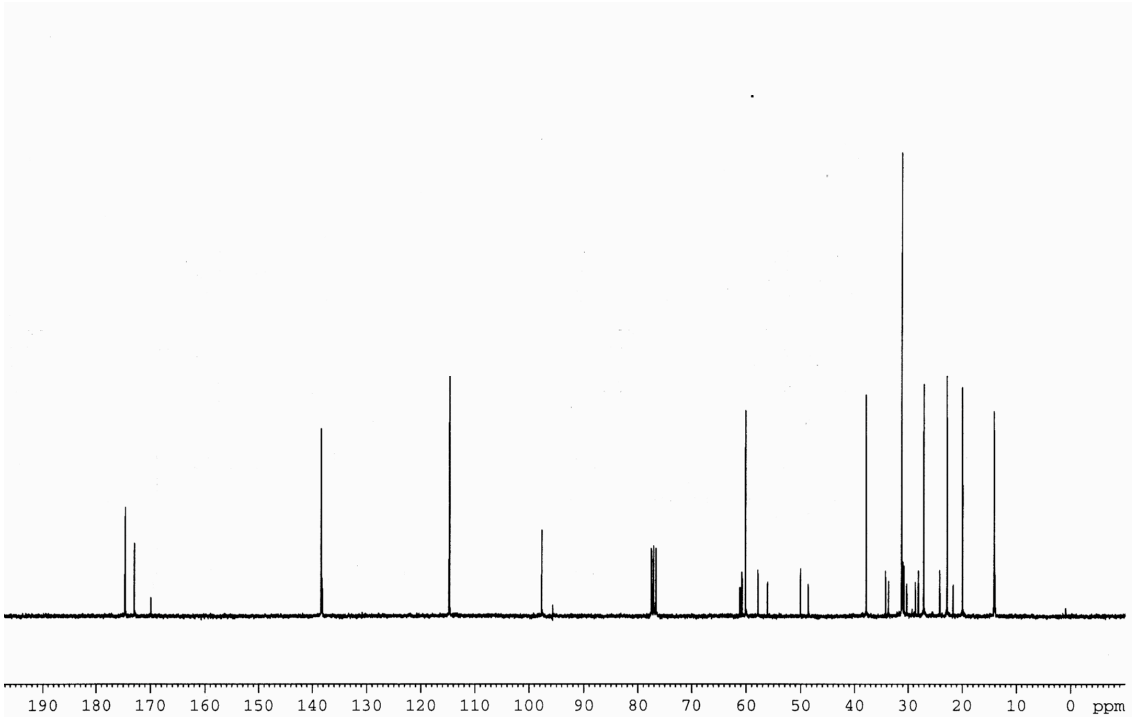


Figure S2. ^{13}C NMR spectrum for compound **5** (mixture of tautomers).

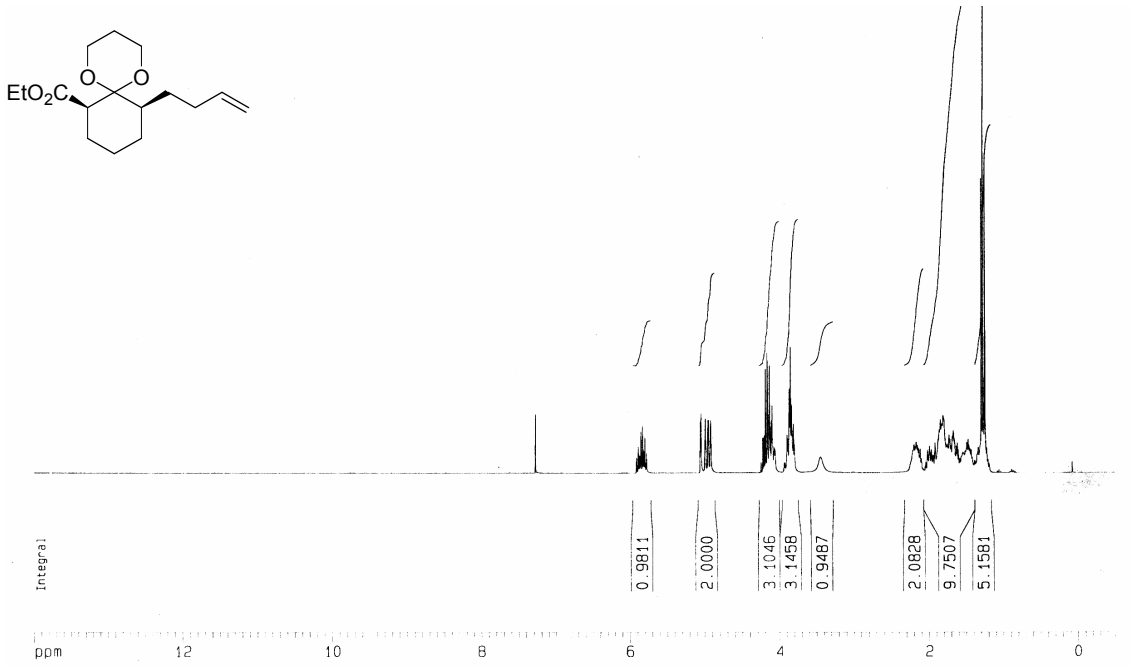


Figure S3. ^{1}H NMR spectrum for compound **6** (racemic).

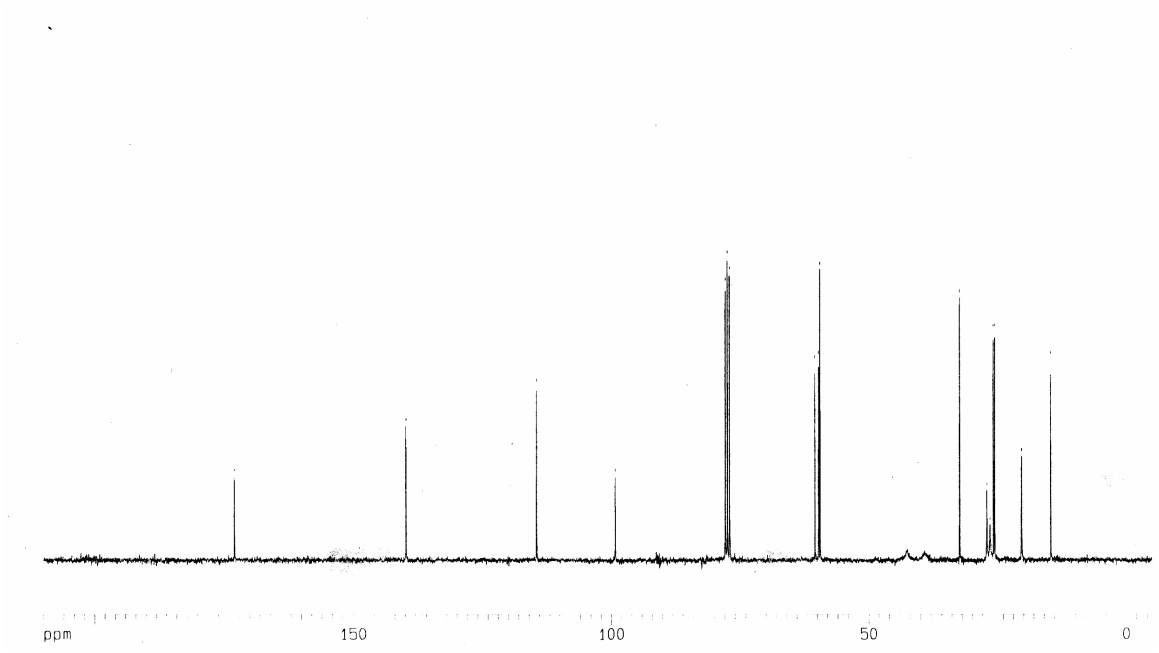


Figure S4. ^{13}C NMR spectrum for compound **6** (racemic).

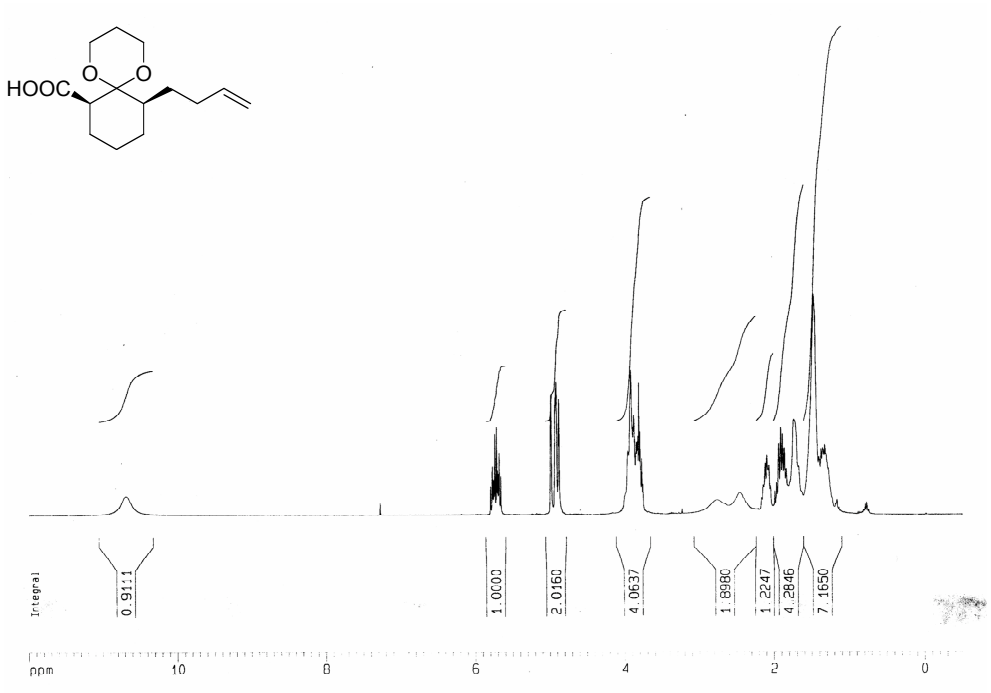


Figure S5. ^{1}H NMR spectrum for compound **7** (racemic).

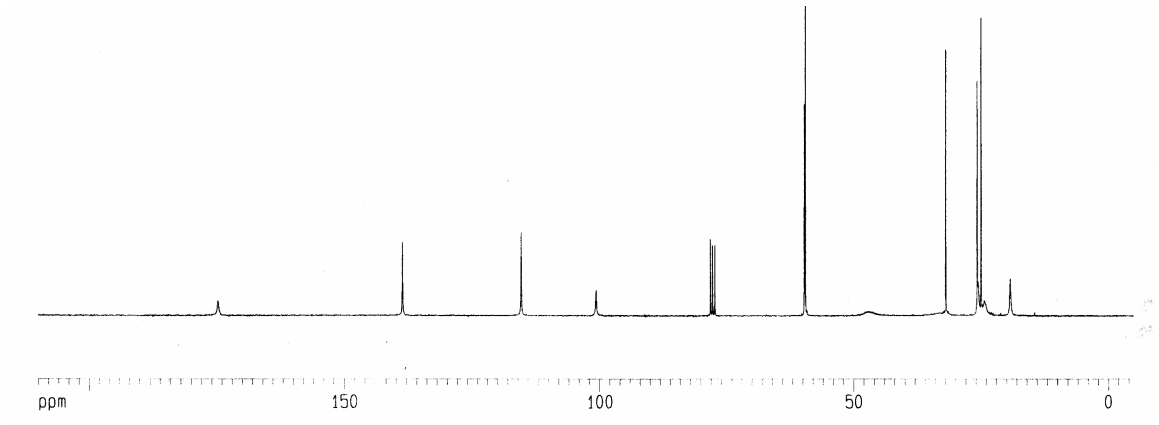
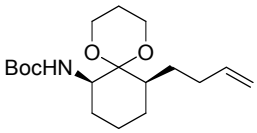


Figure S6. ^{13}C NMR spectrum for compound **7** (racemic).



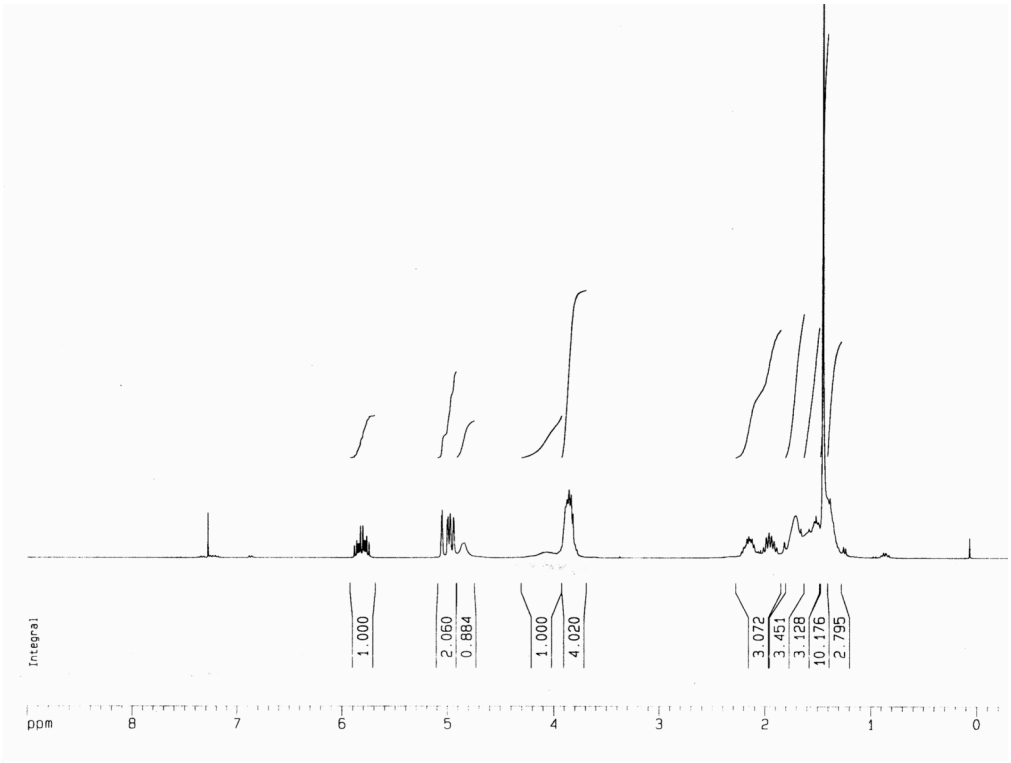


Figure S7. ^{1}H NMR spectrum for compound **8** (racemic).

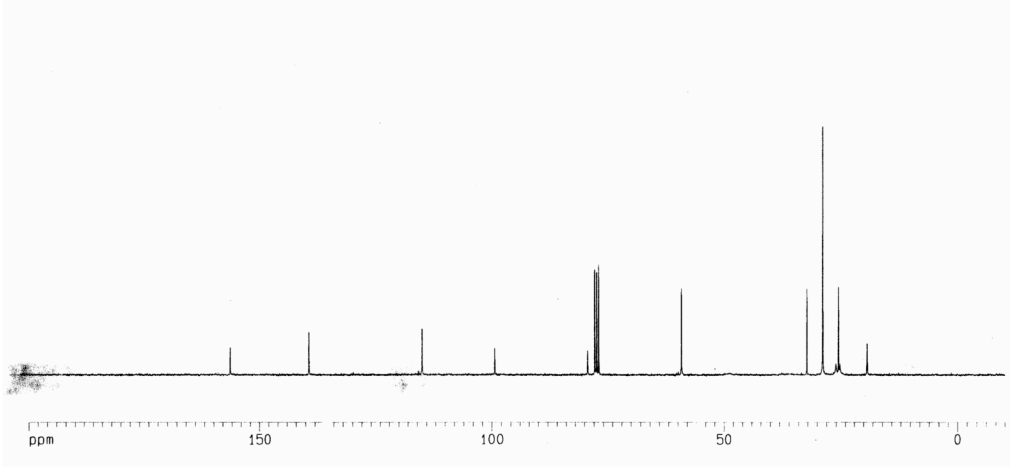
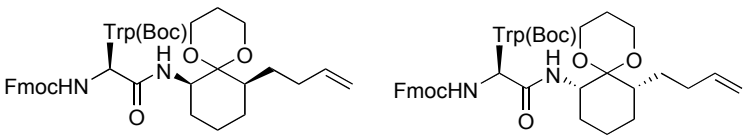


Figure S8. ^{13}C NMR spectrum for compound **8** (racemic).



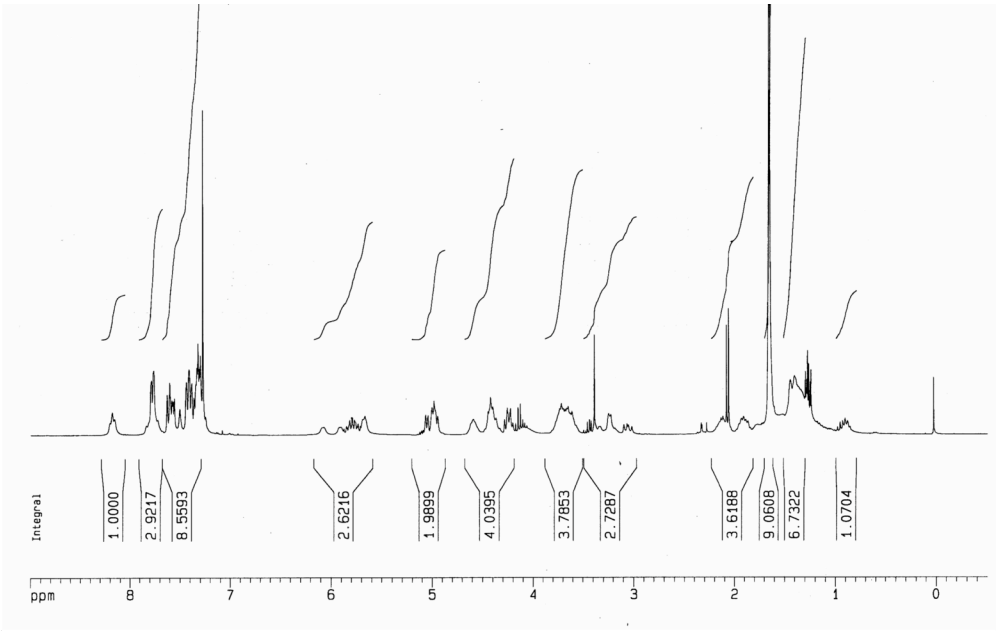


Figure S9. ^{1}H NMR spectrum for compound **13** (mixture of two diastereomers).

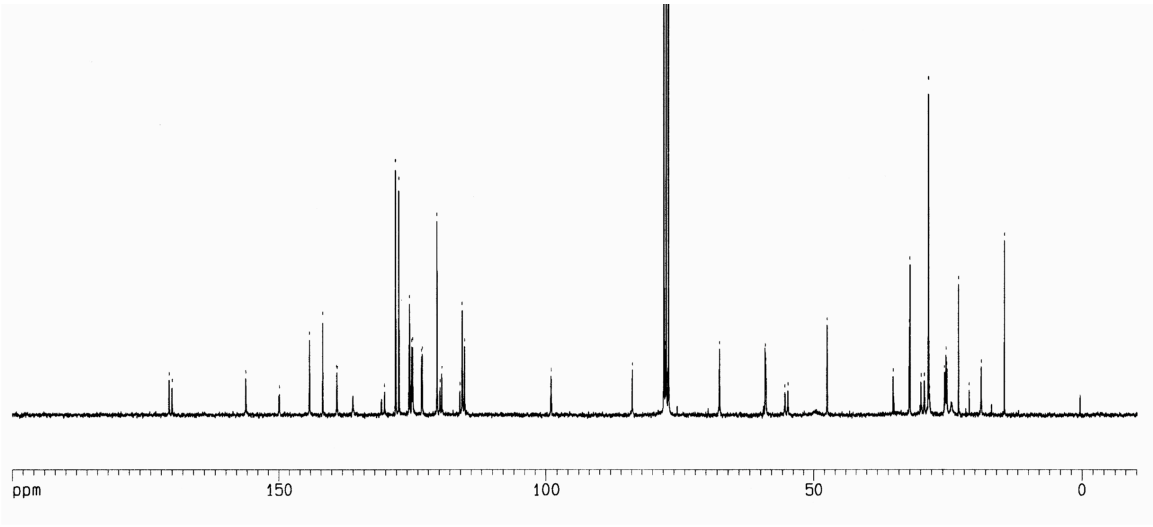
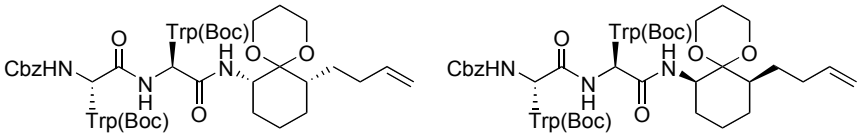


Figure S10. ^{13}C NMR spectrum for compound **13** (mixture of two diastereomers).



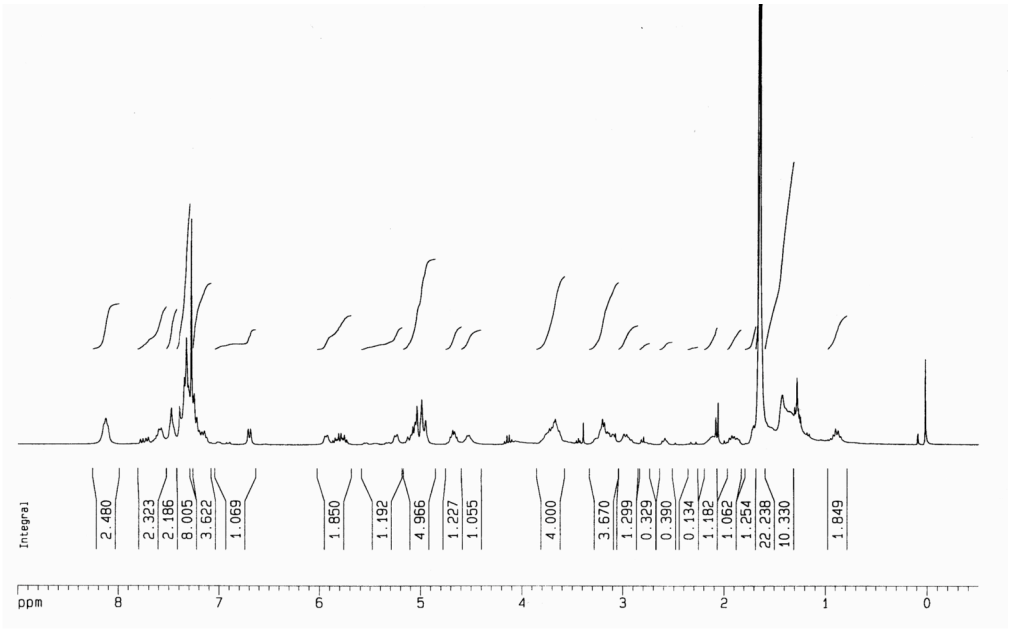


Figure S11. ^{1}H NMR spectrum for compound **14** (mixture of two diastereomers).

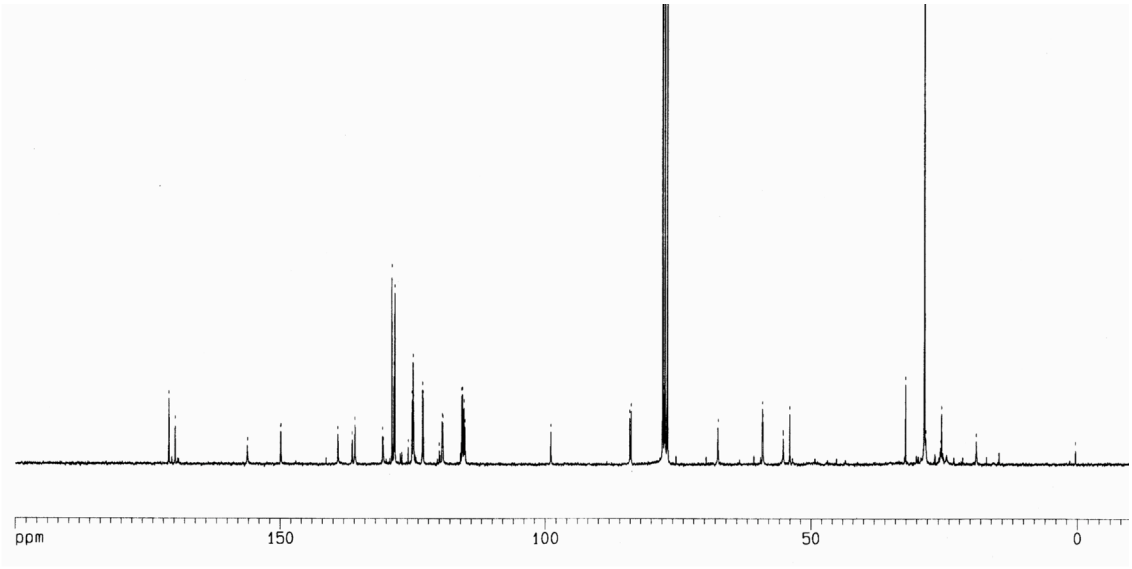


Figure S12. ^{13}C NMR spectrum for compound **14** (mixture of two diastereomers).

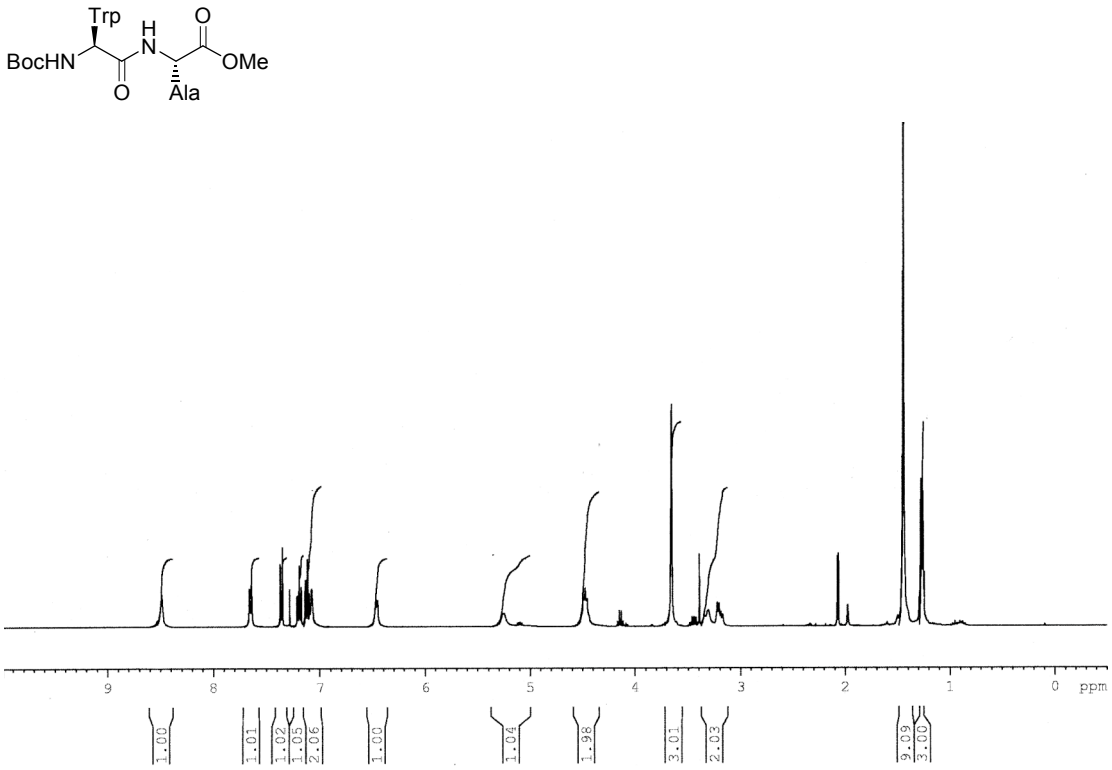


Figure S13. ^{1}H NMR spectrum for compound **15**.

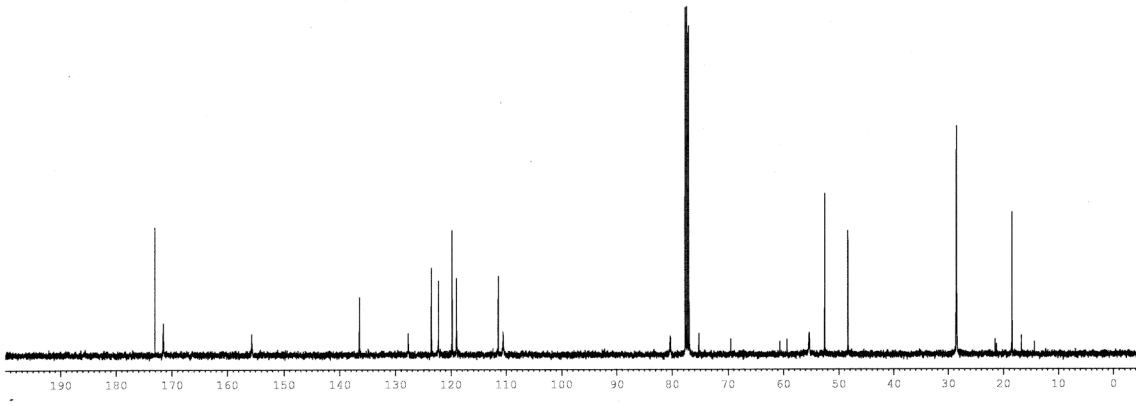


Figure S14. ^{13}C NMR spectrum for compound **15**.

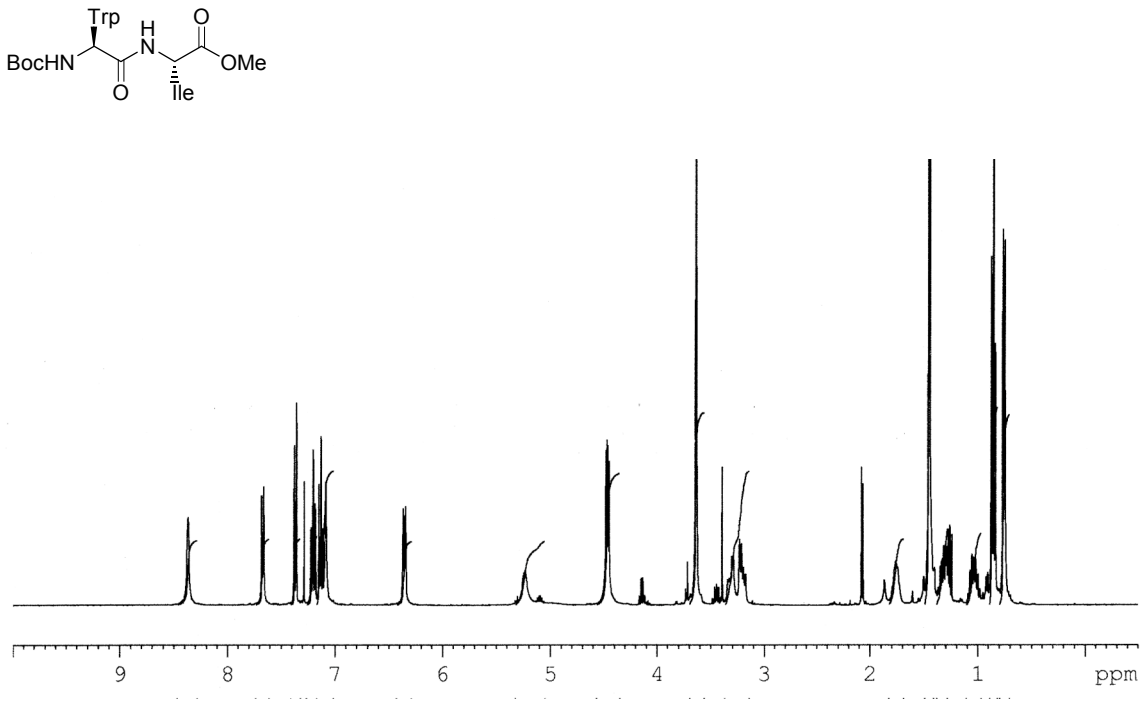


Figure S15. ^{1}H NMR spectrum for compound **16**.

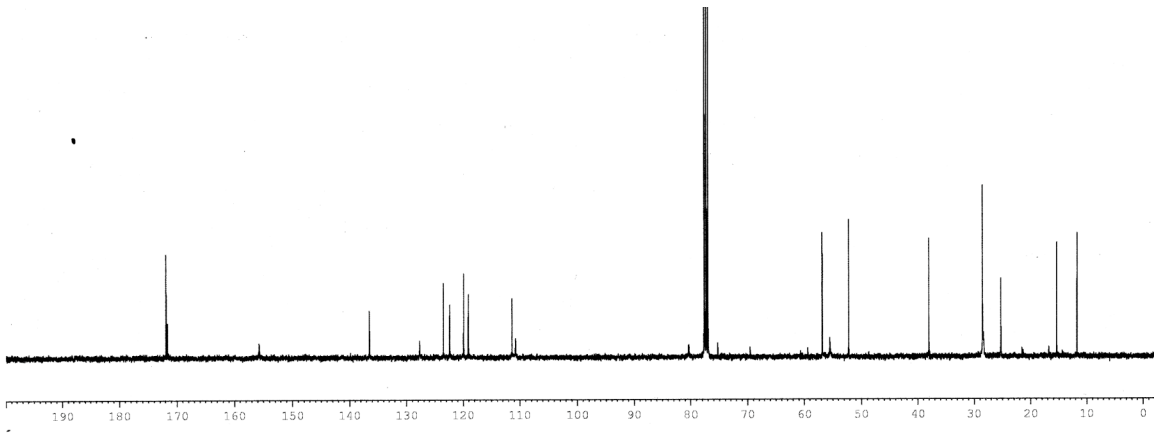


Figure S16. ^{13}C NMR spectrum for compound **16**.

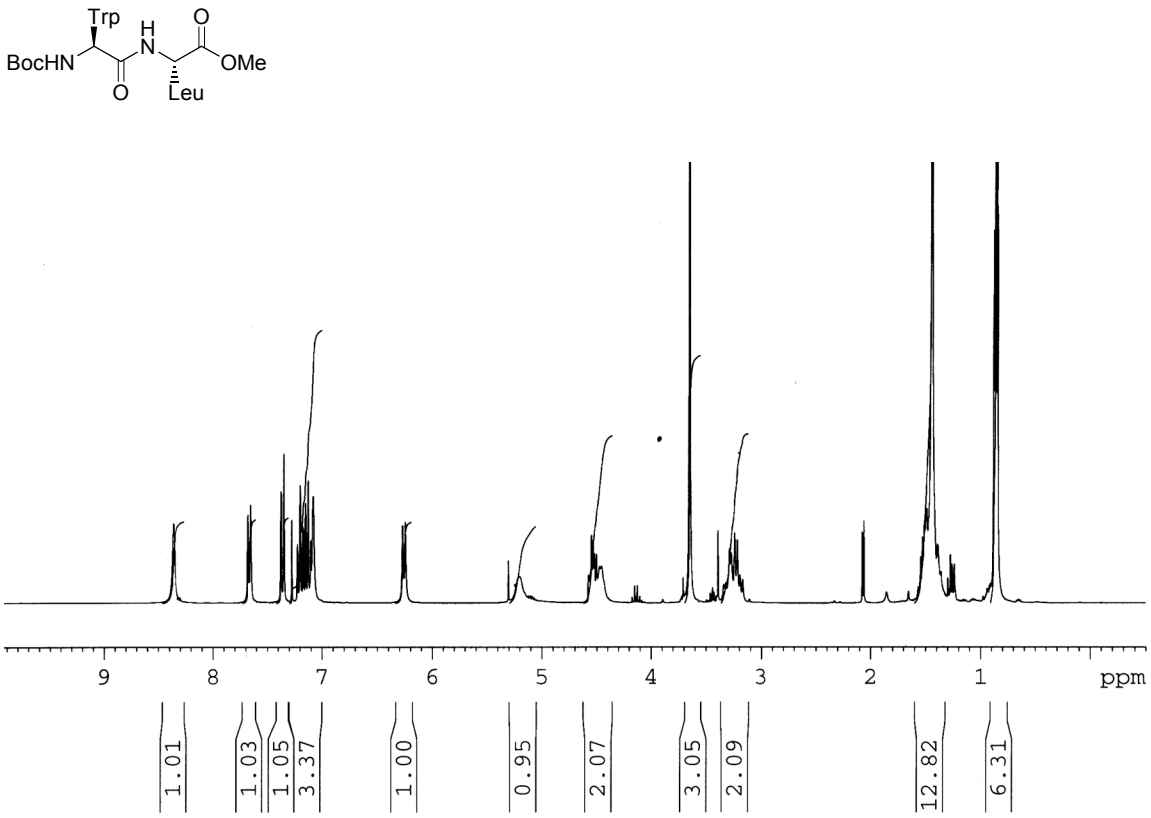


Figure S17. ^{1}H NMR spectrum for compound **17**.

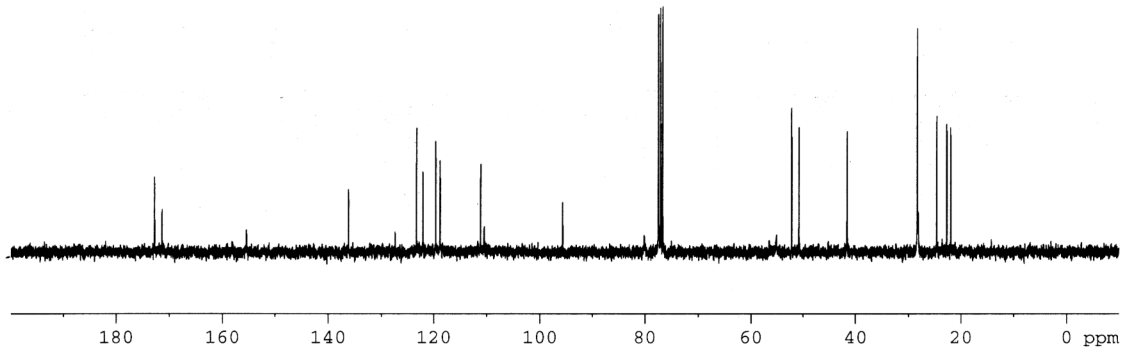


Figure S18. ^{13}C NMR spectrum for compound **17**.

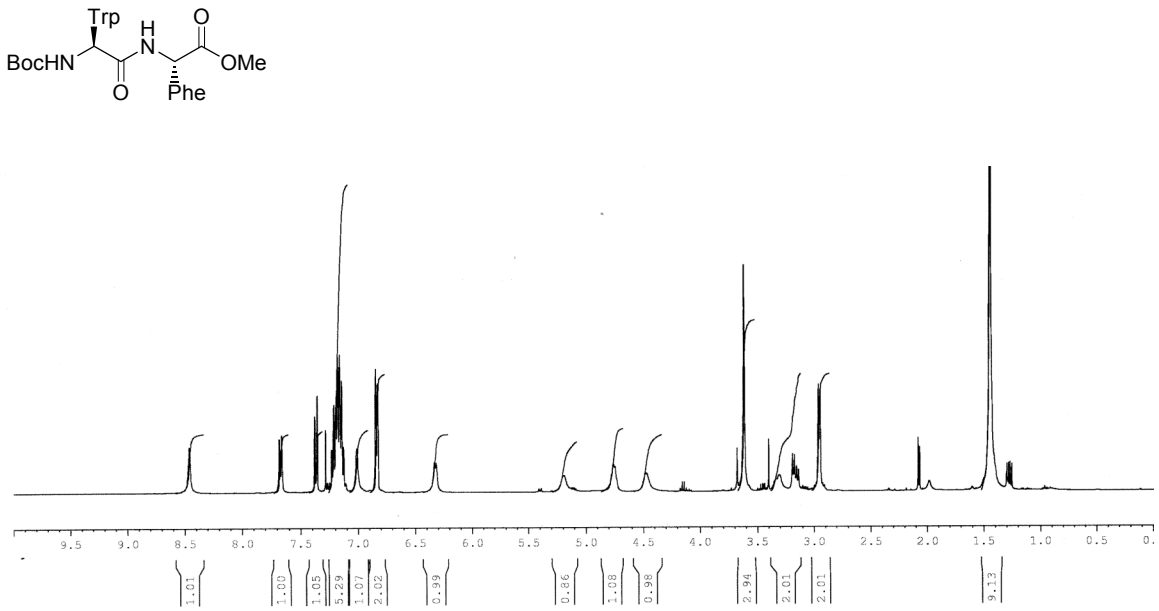


Figure S19. ^{1}H NMR spectrum for compound **18**.

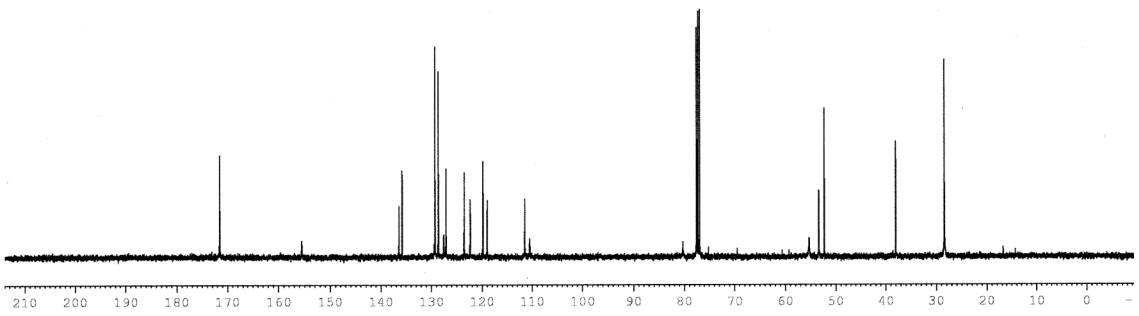


Figure S20. ^{13}C NMR spectrum for compound **18**.

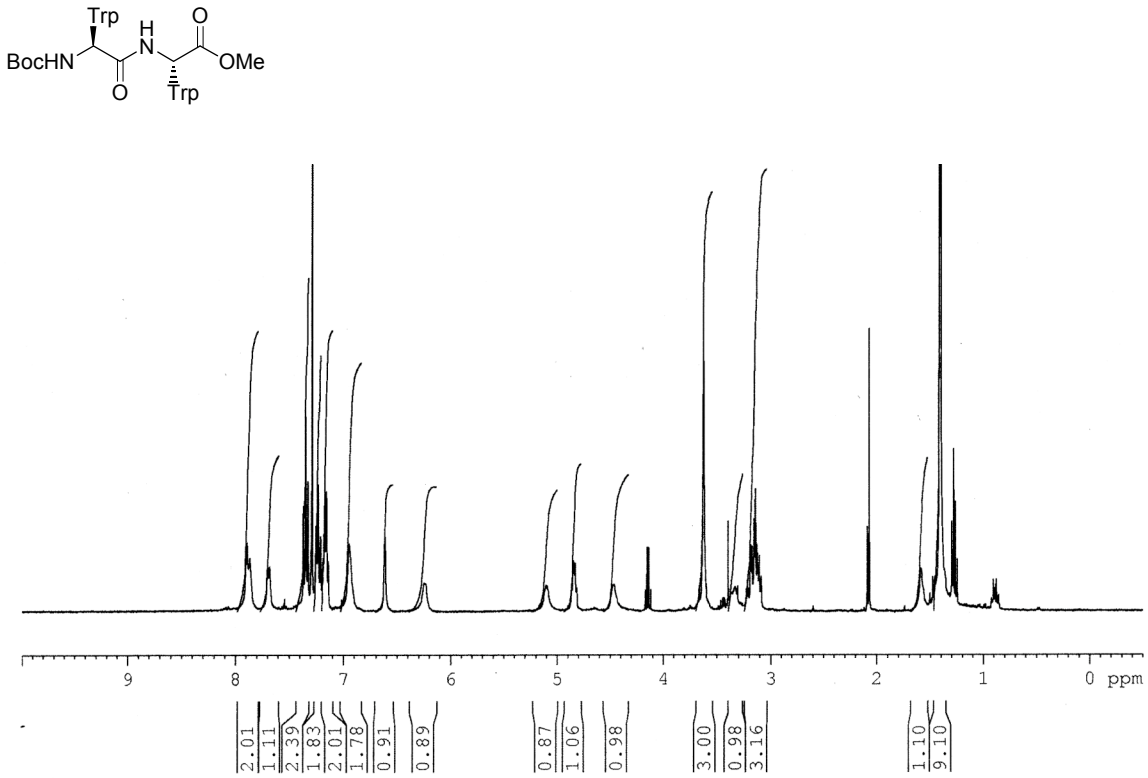


Figure S21. ^{1}H NMR spectrum for compound **19**.

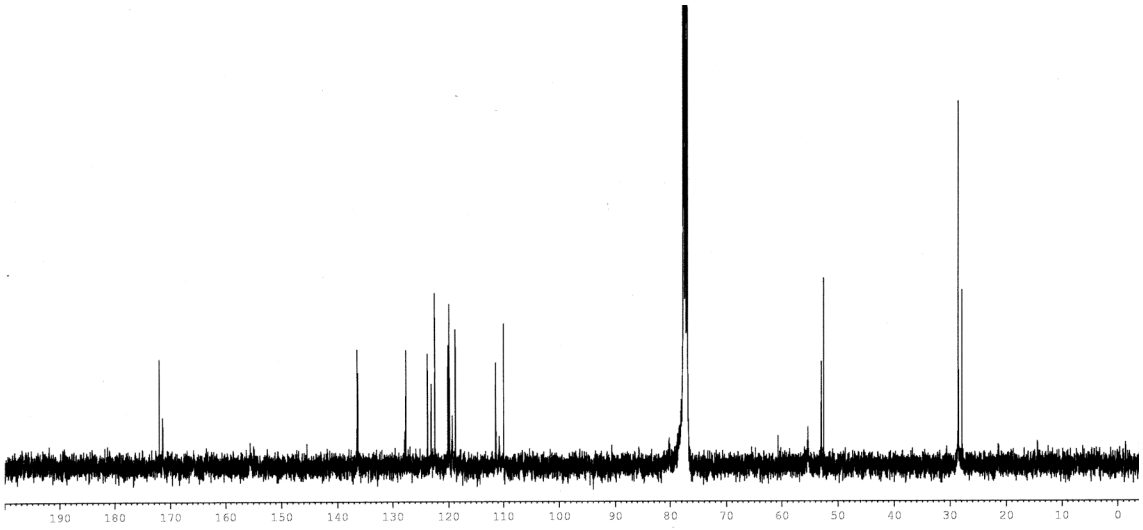


Figure S22. ^{13}C NMR spectrum for compound **19**.

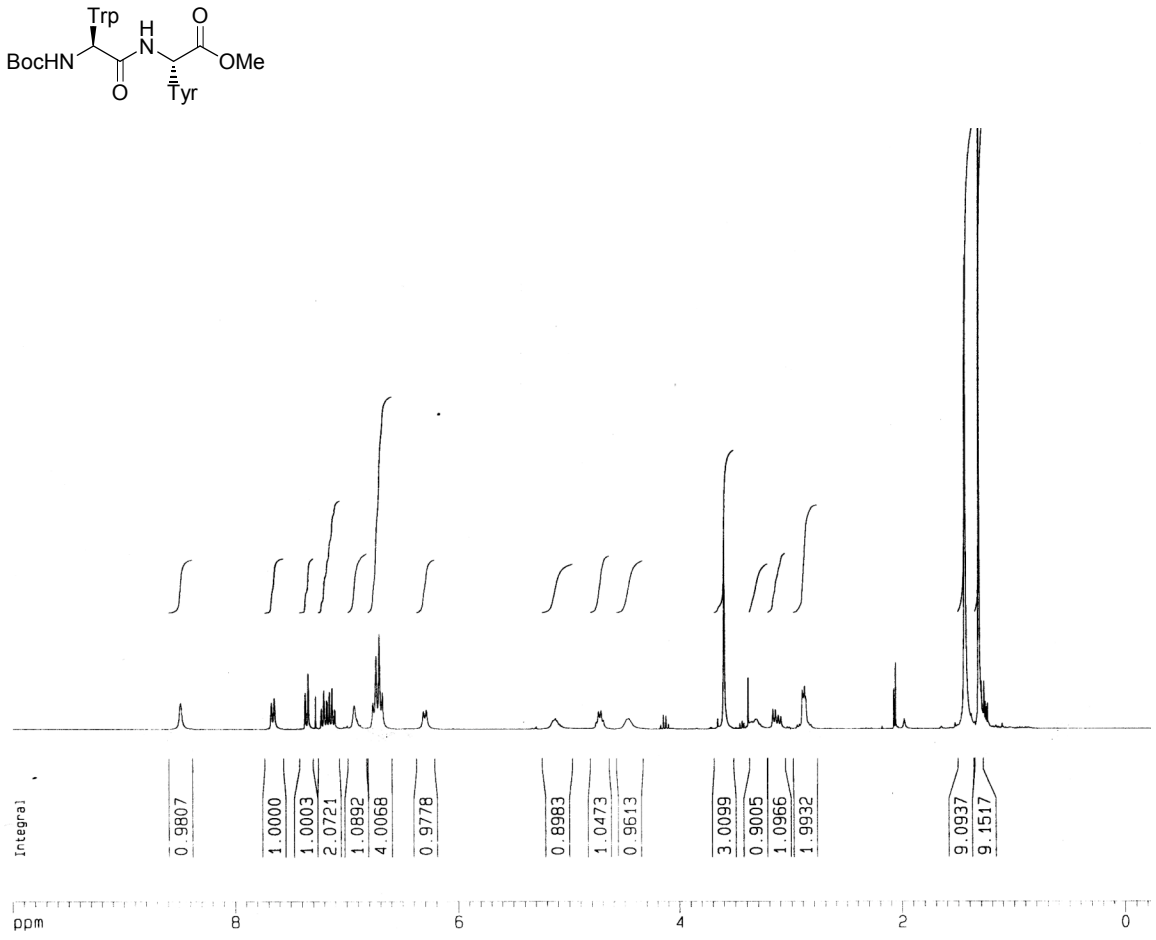


Figure S23. ^{1}H NMR spectrum for compound **20**.

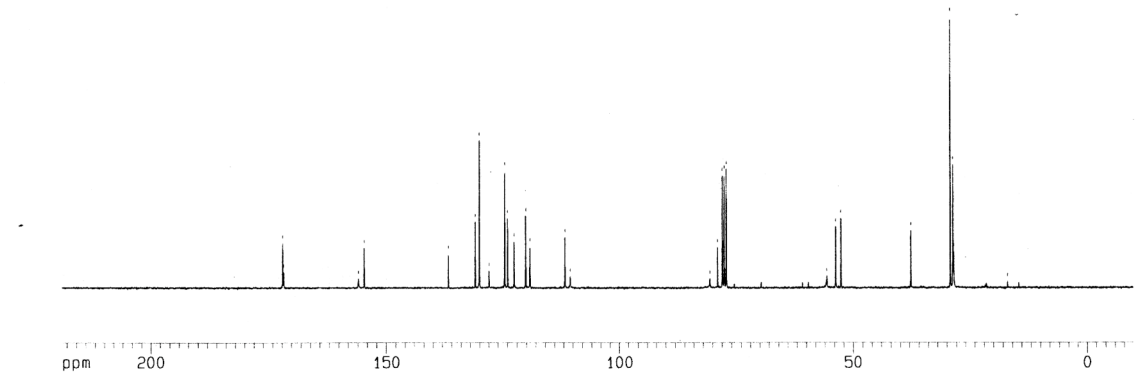


Figure S24. ^{13}C NMR spectrum for compound **20**.

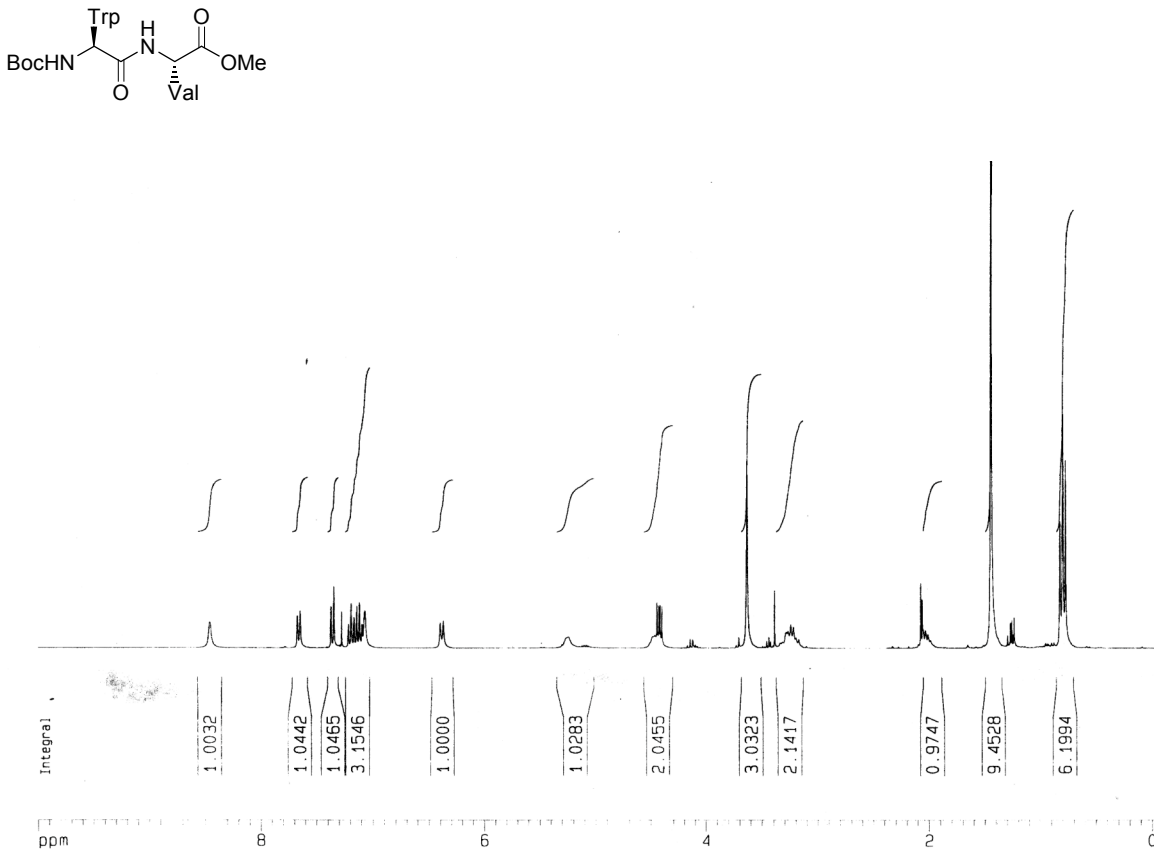


Figure S25. ^{1}H NMR spectrum for compound **21**.

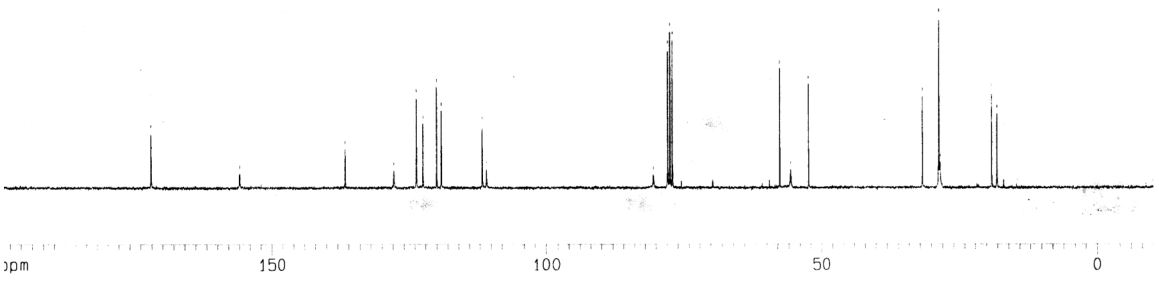


Figure S26. ^{13}C NMR spectrum for compound **21**.

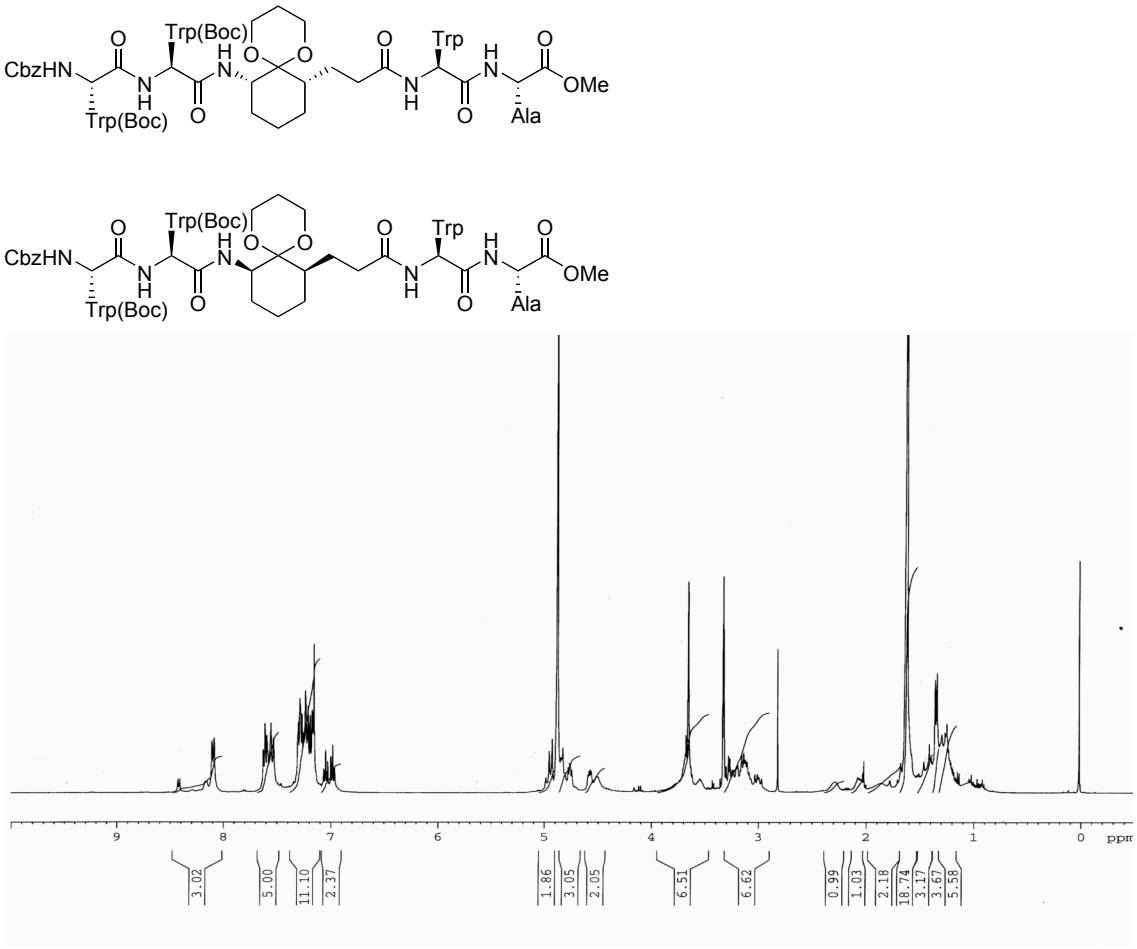


Figure S27. ^{1}H NMR spectrum for compound **22** (mixture of two diastereomers).

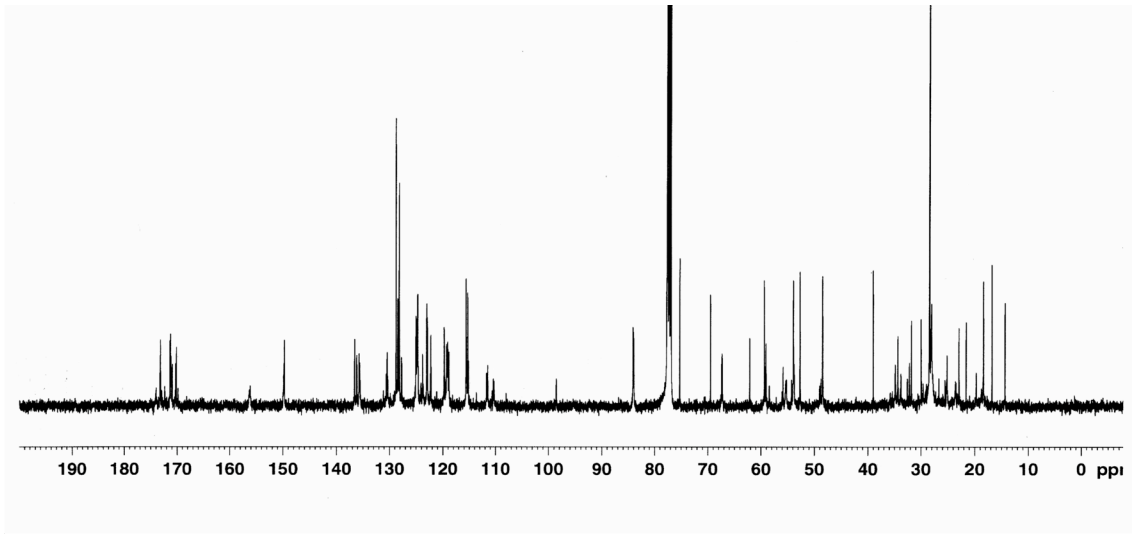
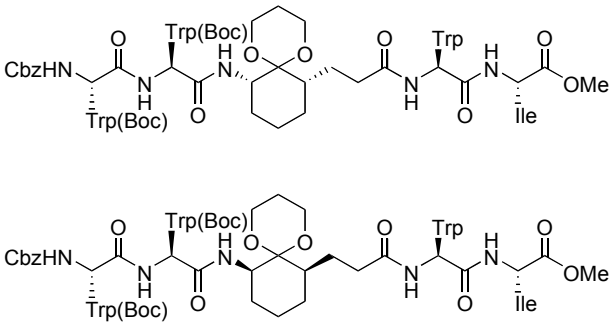


Figure S28. ^{13}C NMR spectrum for compound **22** (mixture of two diastereomers).



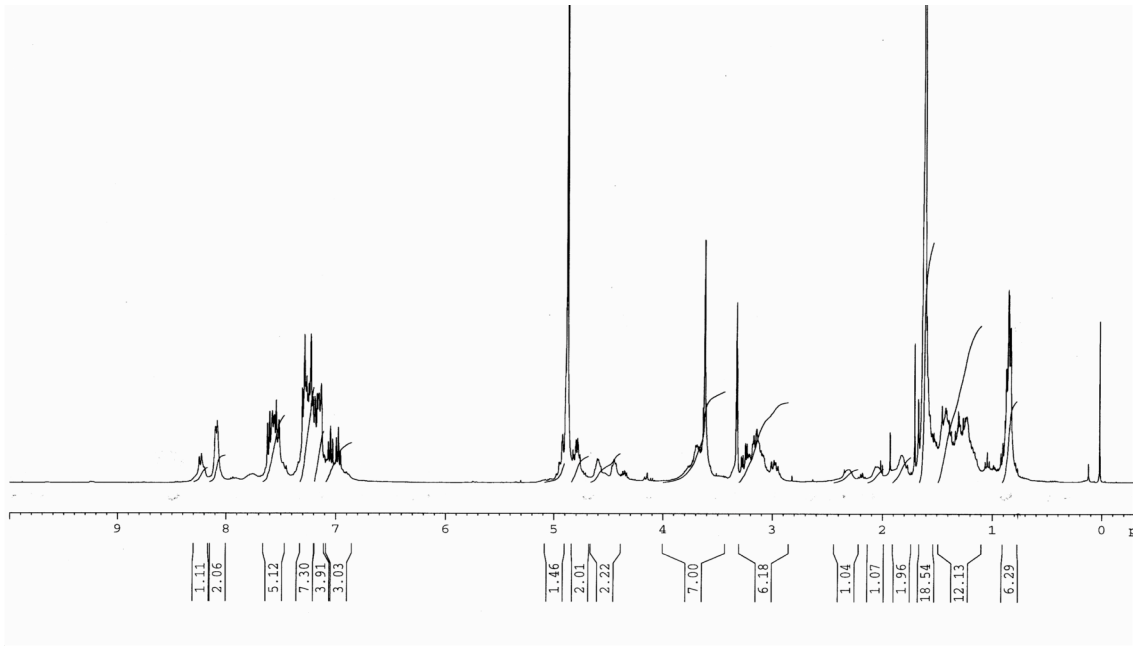


Figure S29. ^{1}H NMR spectrum for compound **23** (mixture of two diastereomers).

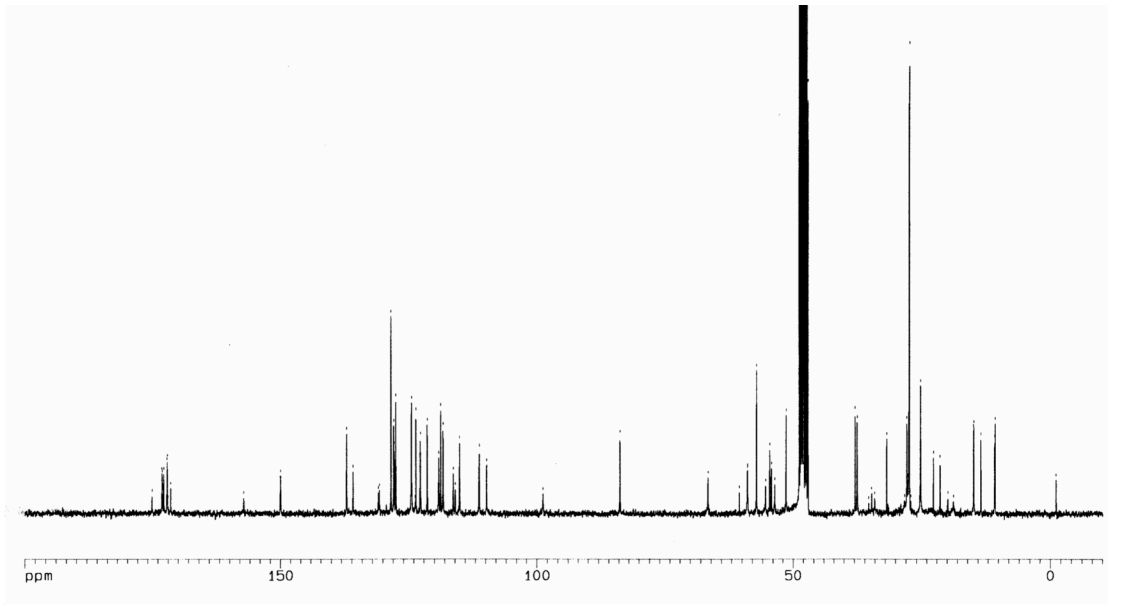


Figure S30. ^{13}C NMR spectrum for compound **23** (mixture of two diastereomers).

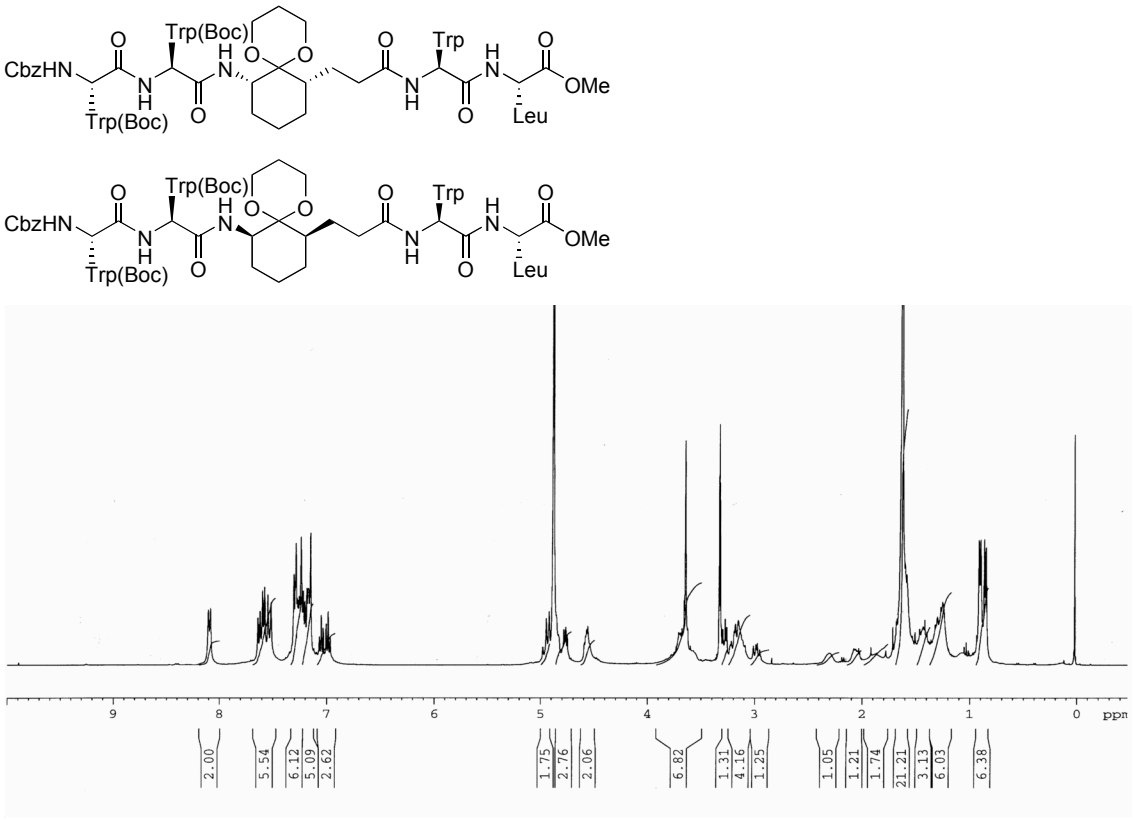


Figure S31. ^{1}H NMR spectrum for compound **24** (mixture of two diastereomers).

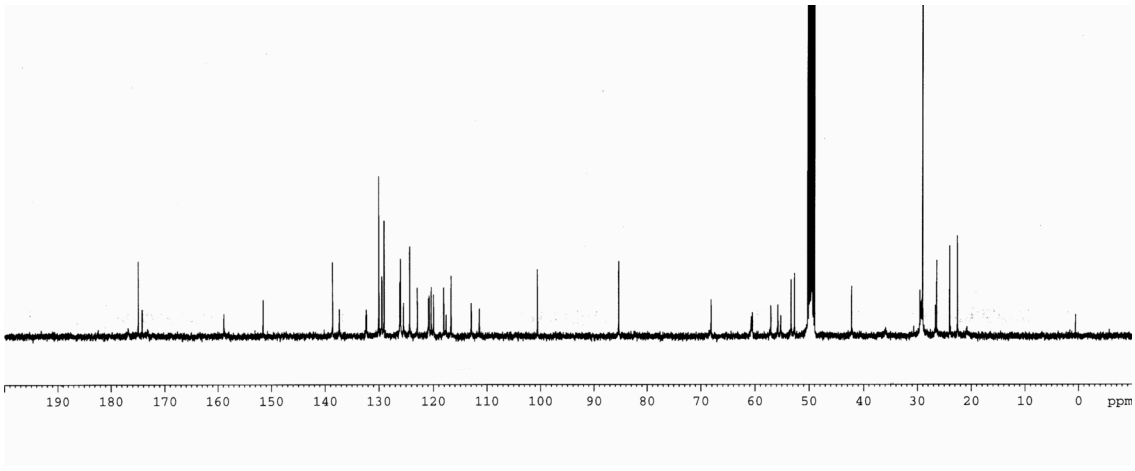


Figure S32. ^{13}C NMR spectrum for compound **24** (mixture of two diastereomers).

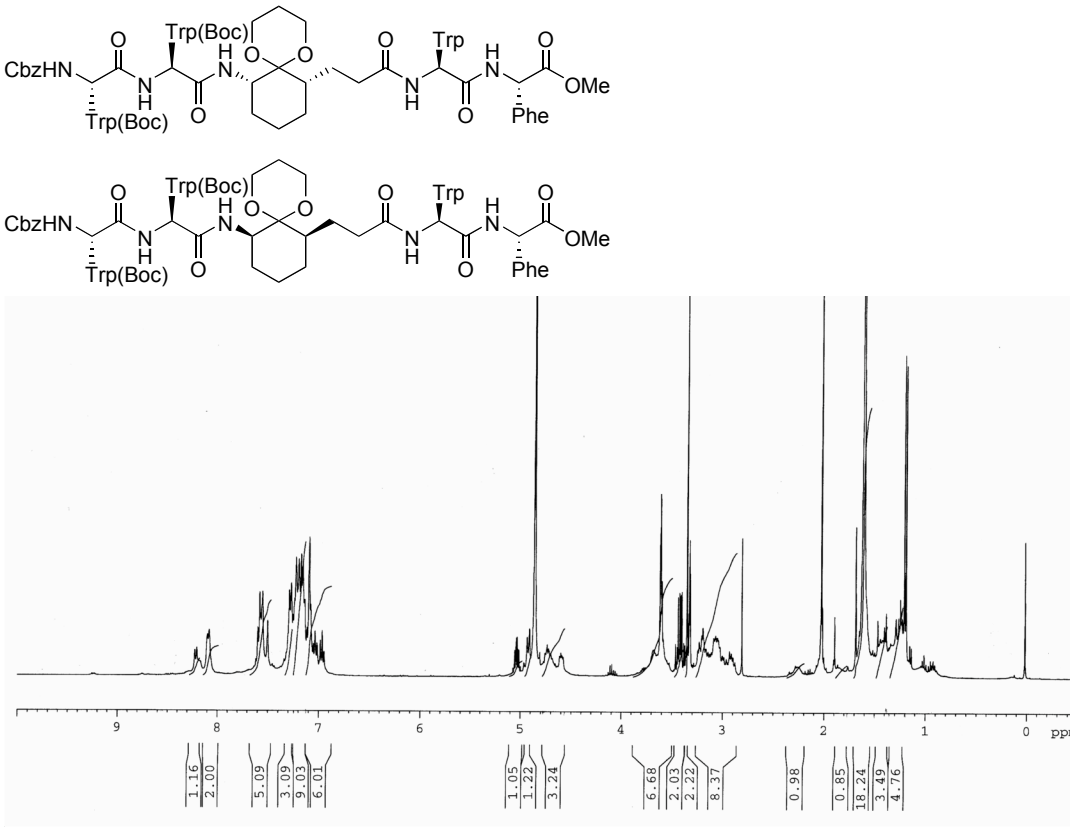


Figure S33. ^{1}H NMR spectrum for compound **25** (mixture of two diastereomers).

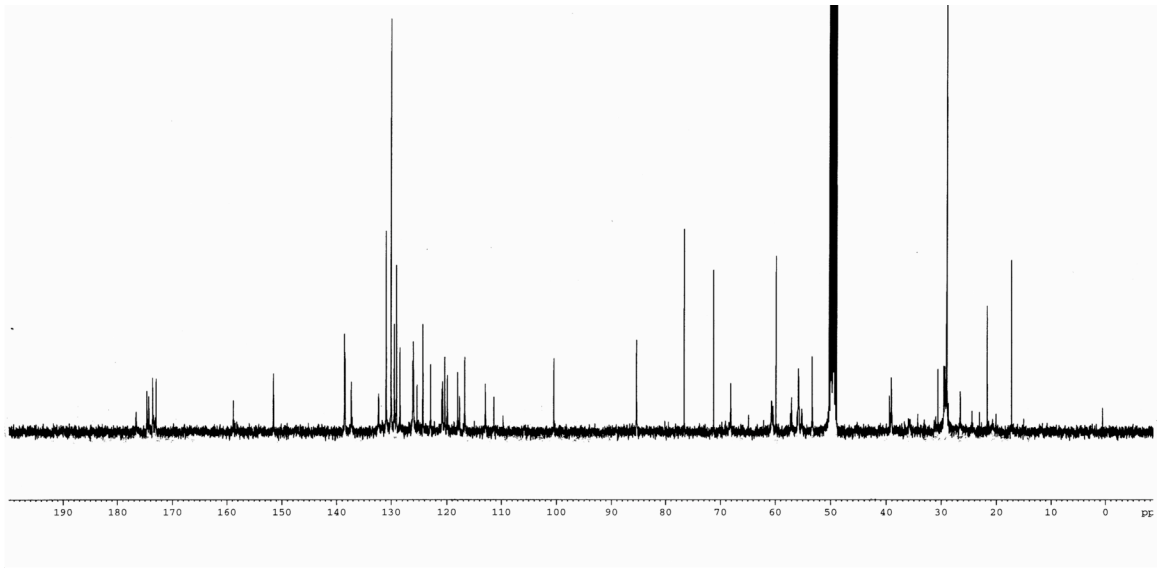


Figure S34. ^{13}C NMR spectrum for compound **25** (mixture of two diastereomers).

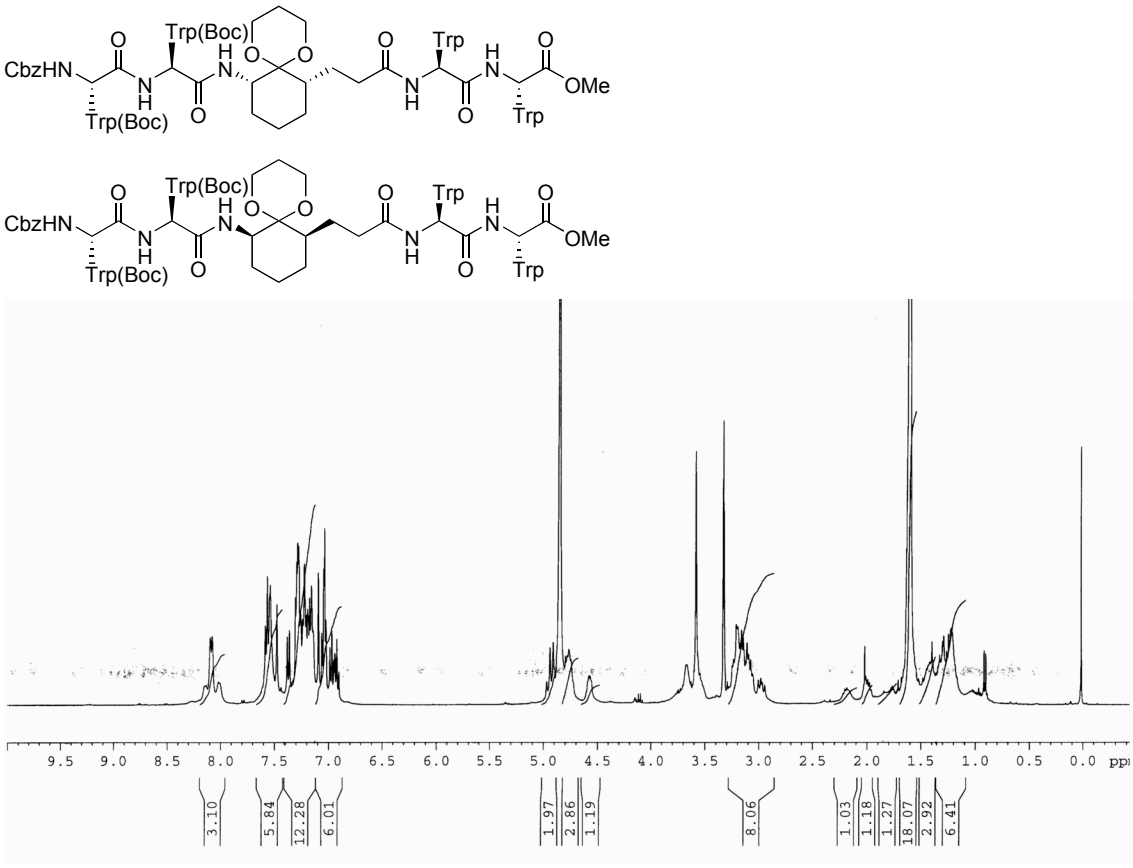


Figure S35. ^{1}H NMR spectrum for compound **26** (mixture of two diastereomers).

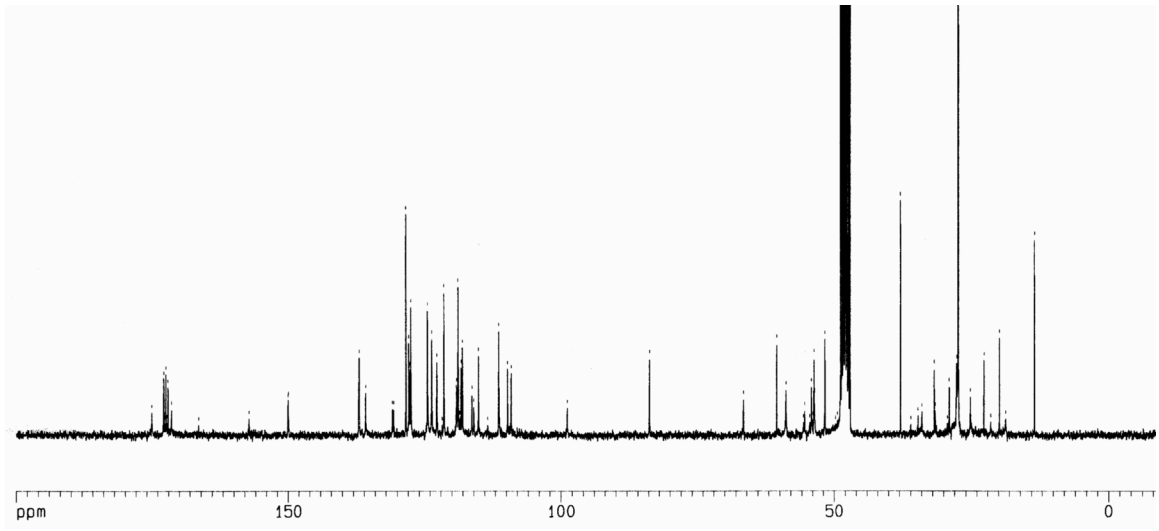


Figure S36. ^{13}C NMR spectrum for compound **26** (mixture of two diastereomers).

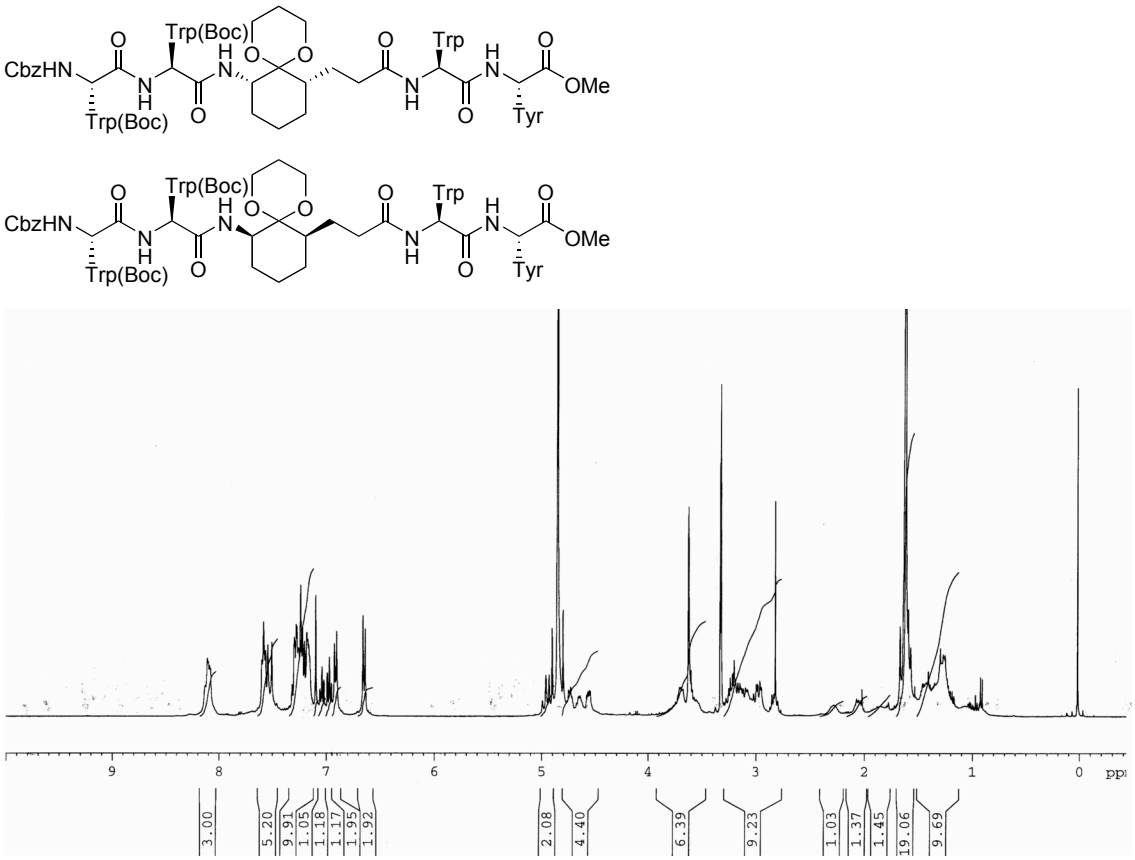


Figure S37. ^{1}H NMR spectrum for compound **27** (mixture of two diastereomers).

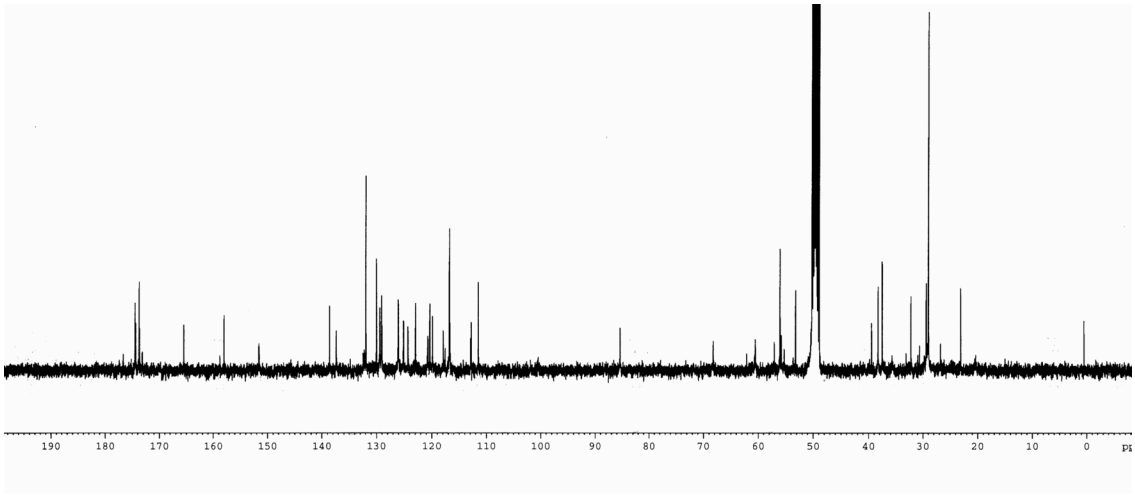


Figure S38. ^{13}C NMR spectrum for compound **27** (mixture of two diastereomers).

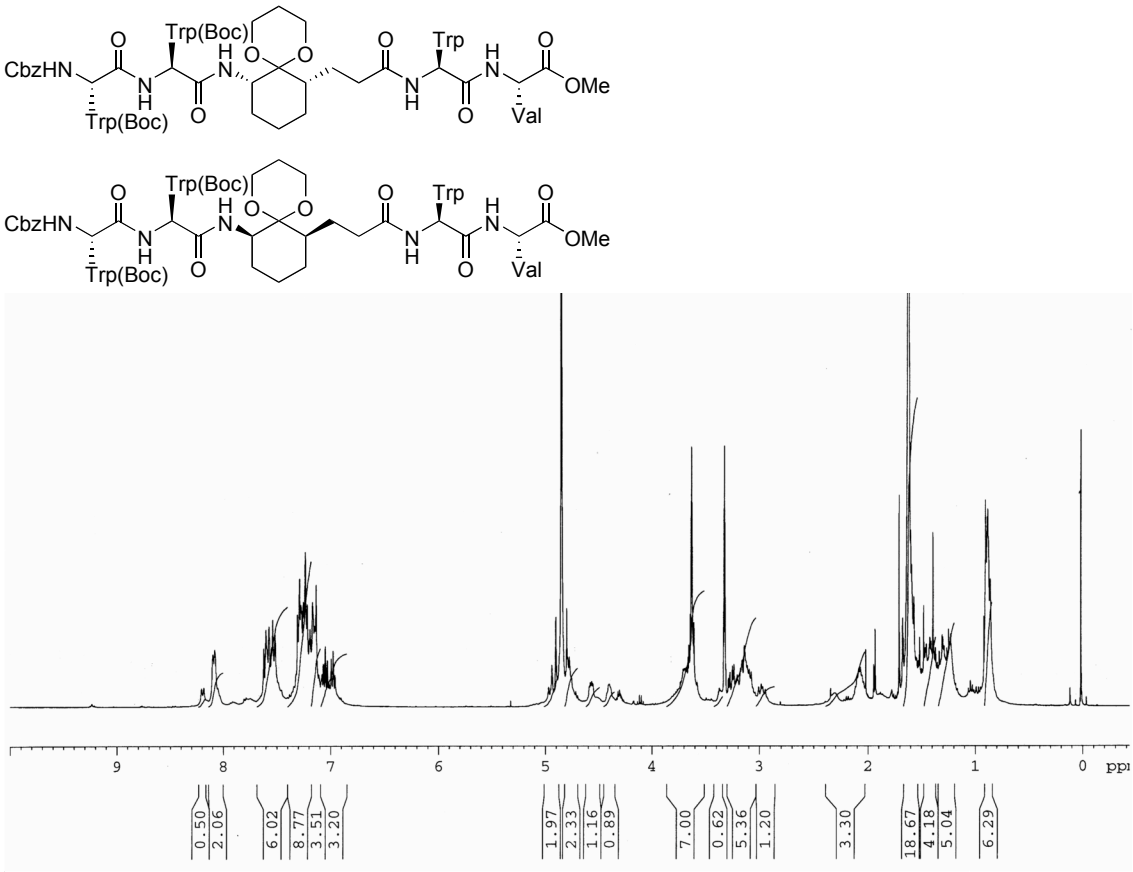


Figure S39. ^{1}H NMR spectrum for compound **28** (mixture of two diastereomers).

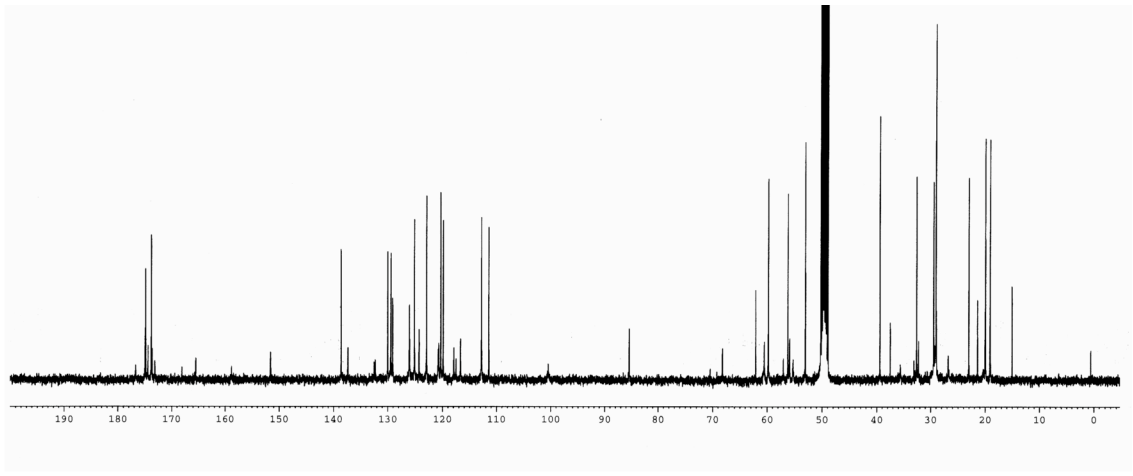
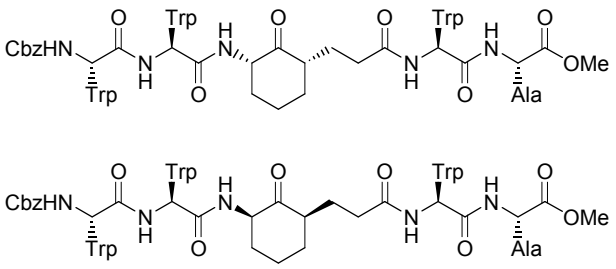


Figure S40. ^{13}C NMR spectrum for compound **28** (mixture of two diastereomers).



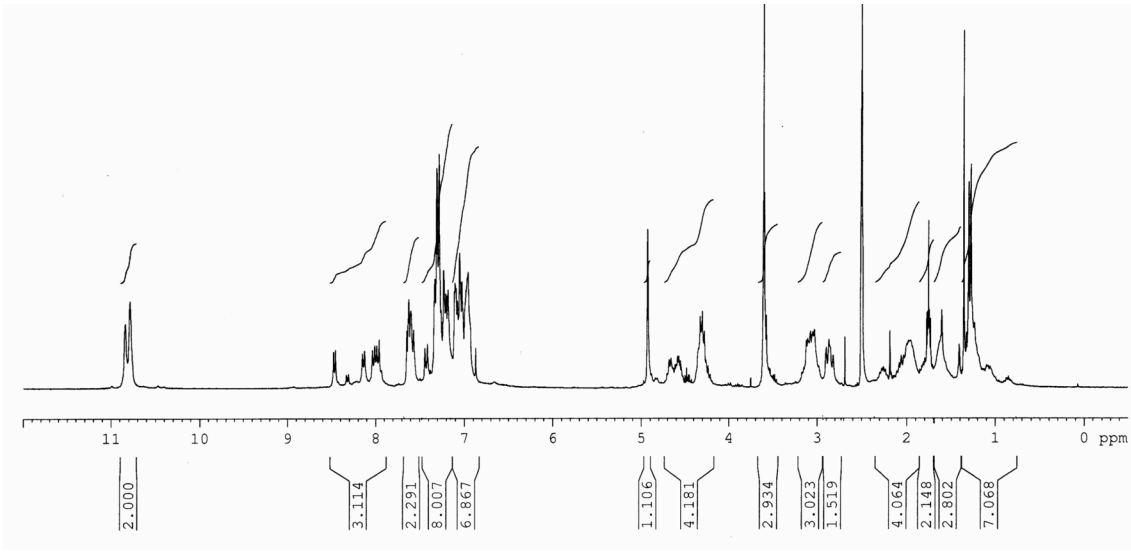


Figure S41. ^{1}H NMR spectrum for inhibitor **29** (mixture of two diastereomers).

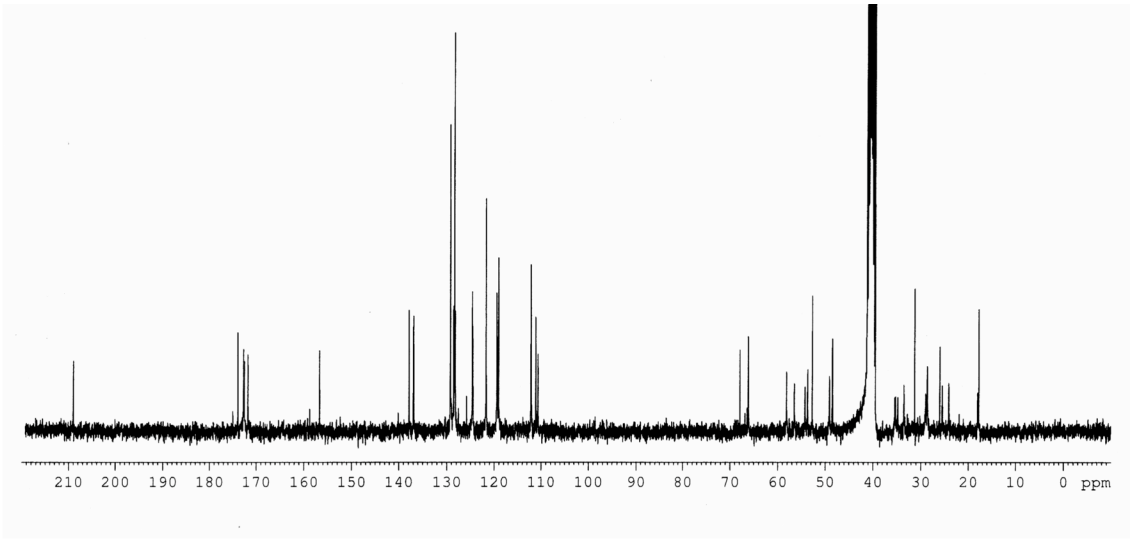
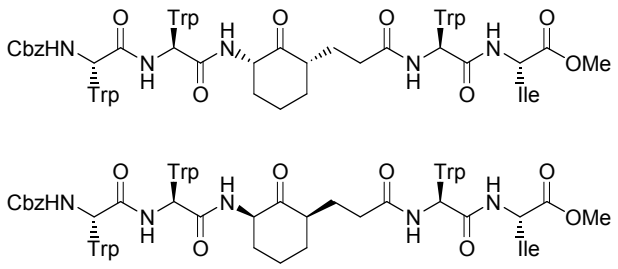


Figure S42. ^{13}C NMR spectrum for inhibitor **29** (mixture of two diastereomers).



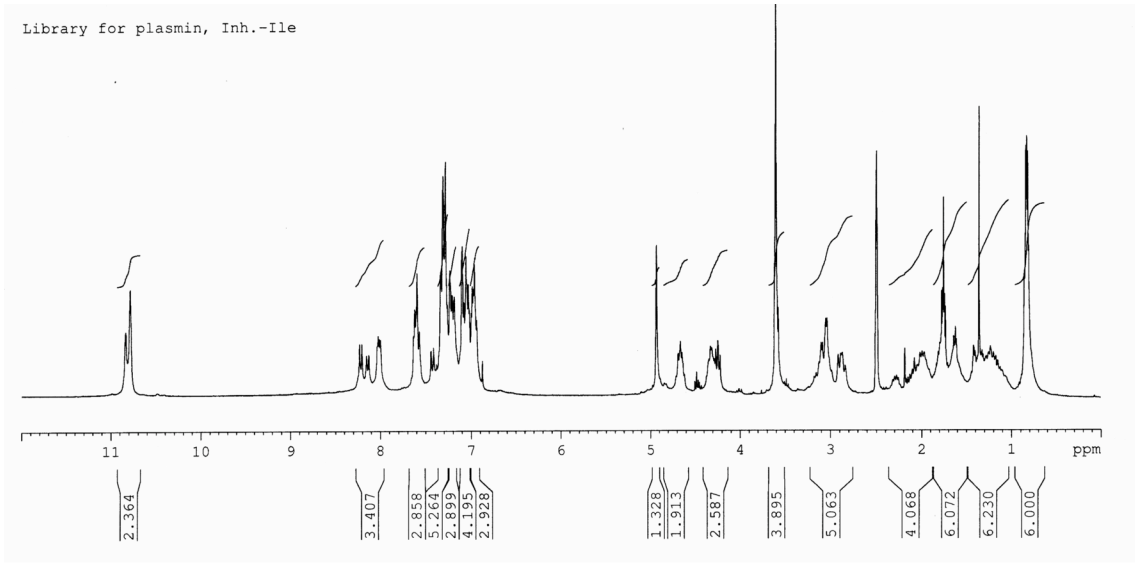


Figure S43. ^{1}H NMR spectrum for inhibitor **30** (mixture of two diastereomers).

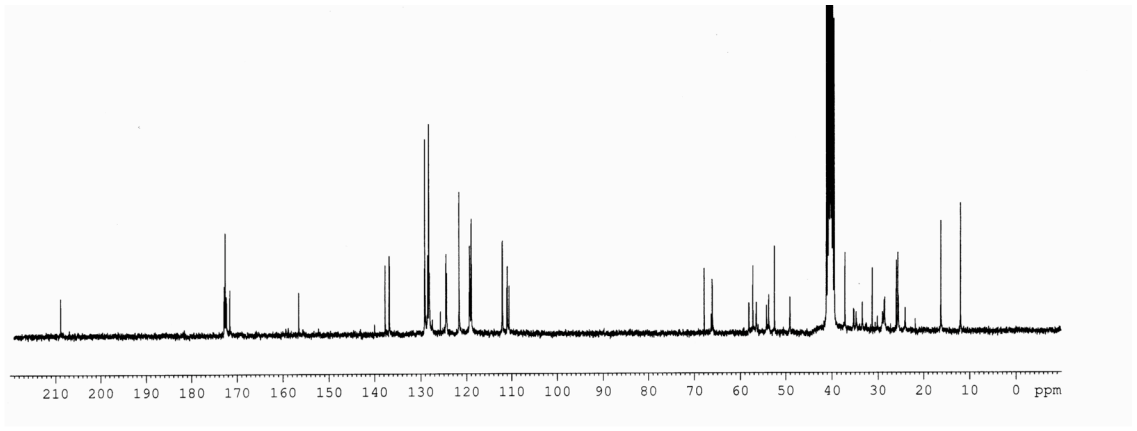


Figure S44. ^{13}C NMR spectrum for inhibitor **30** (mixture of two diastereomers).

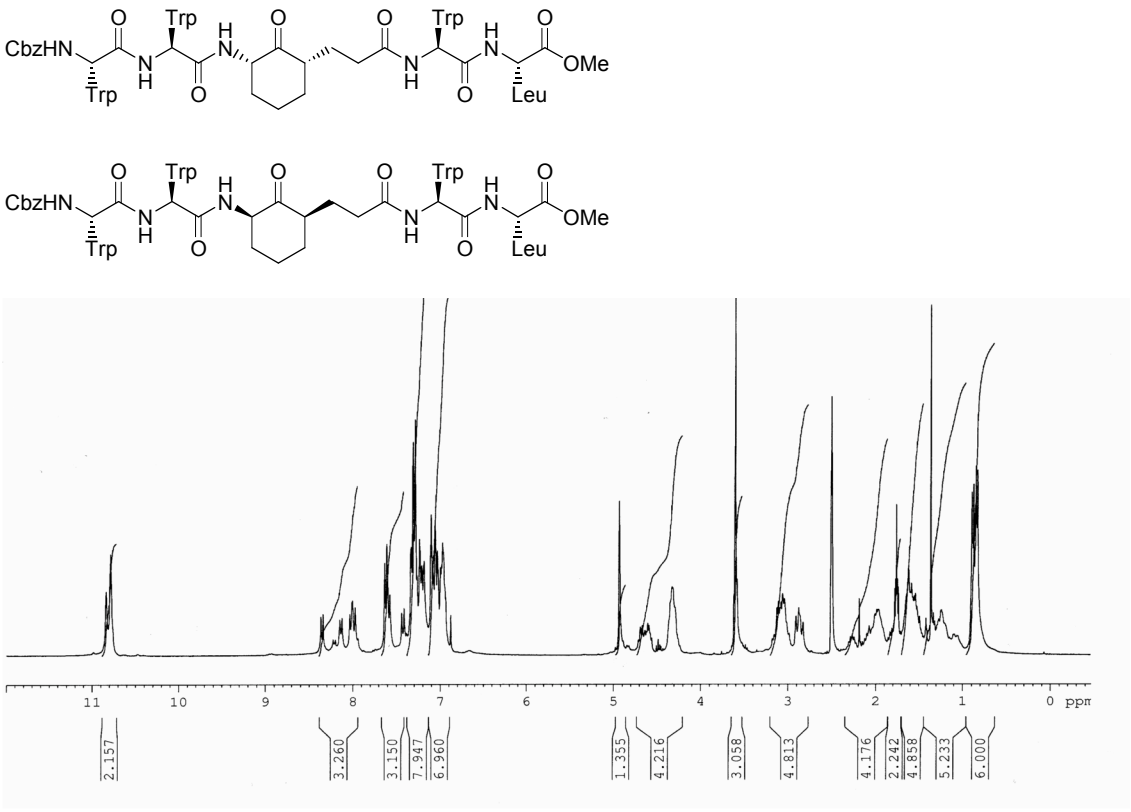


Figure S45. ^{1}H NMR spectrum for inhibitor **31** (mixture of two diastereomers).

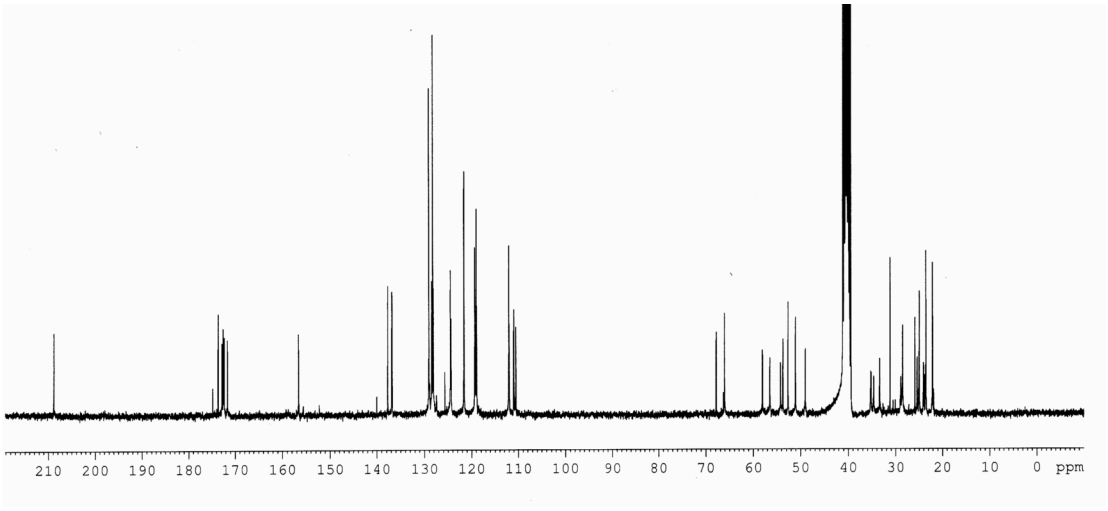


Figure S46. ^{13}C NMR spectrum for inhibitor **31** (mixture of two diastereomers).

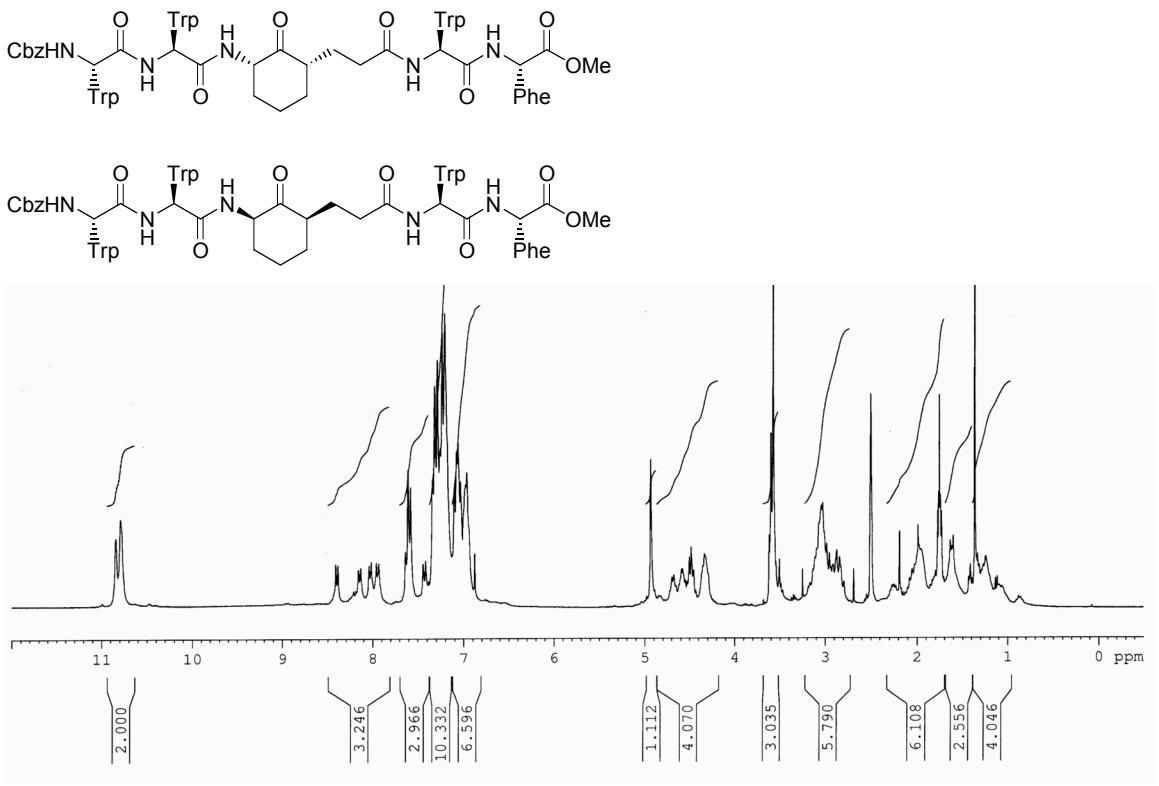


Figure S47. ^{1}H NMR spectrum for inhibitor **32** (mixture of two diastereomers).

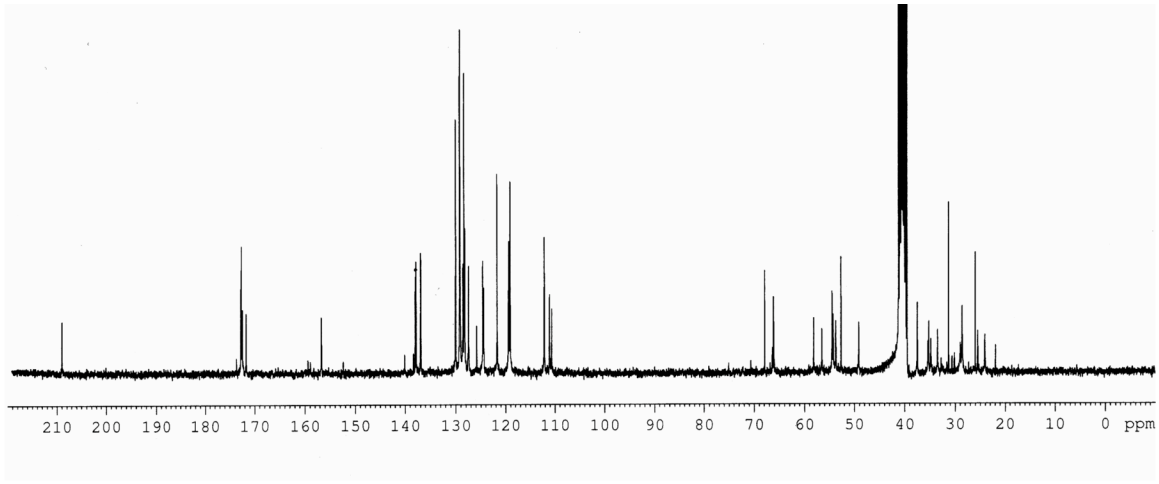
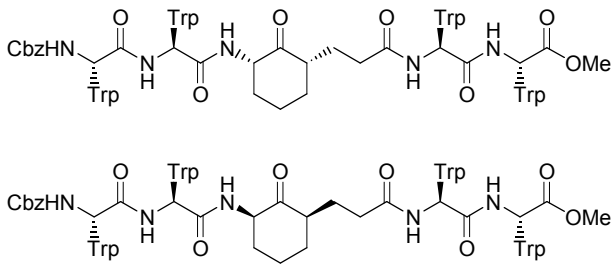


Figure S48. ^{13}C NMR spectrum for inhibitor **32** (mixture of two diastereomers).



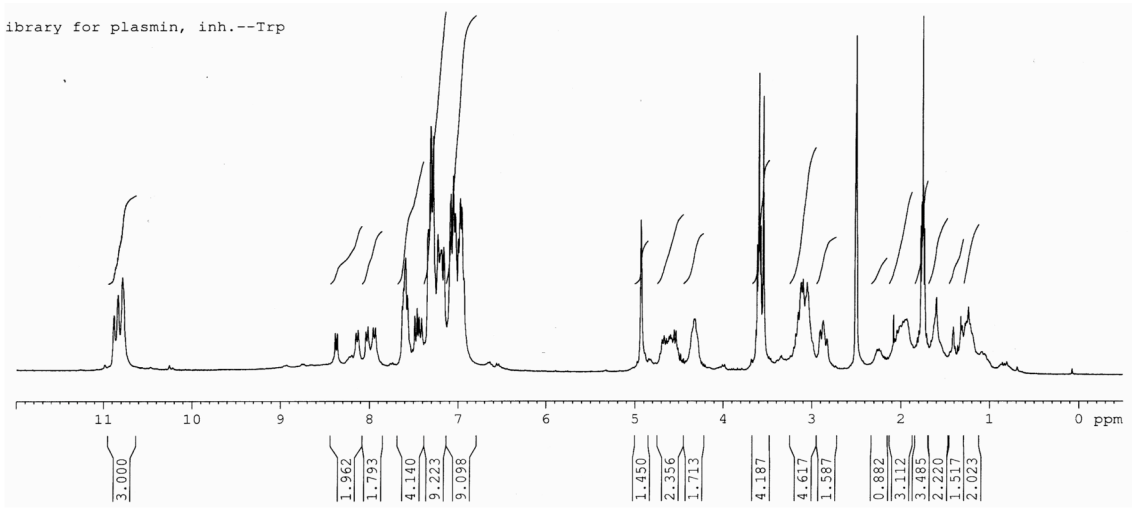


Figure S49. ^{1}H NMR spectrum for inhibitor **33** (mixture of two diastereomers).

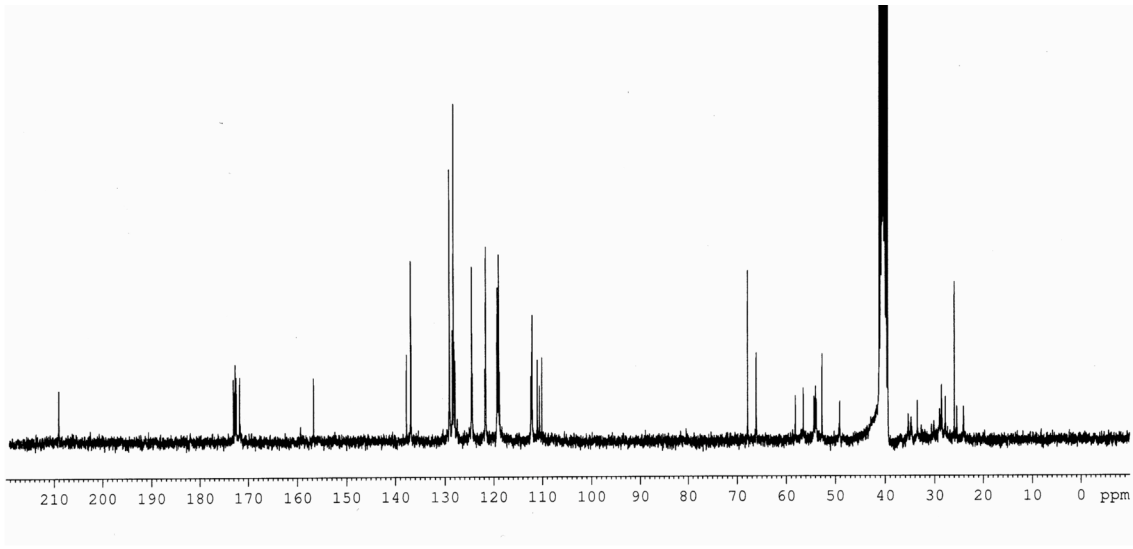


Figure S50. ^{13}C NMR spectrum for inhibitor **33** (mixture of two diastereomers).

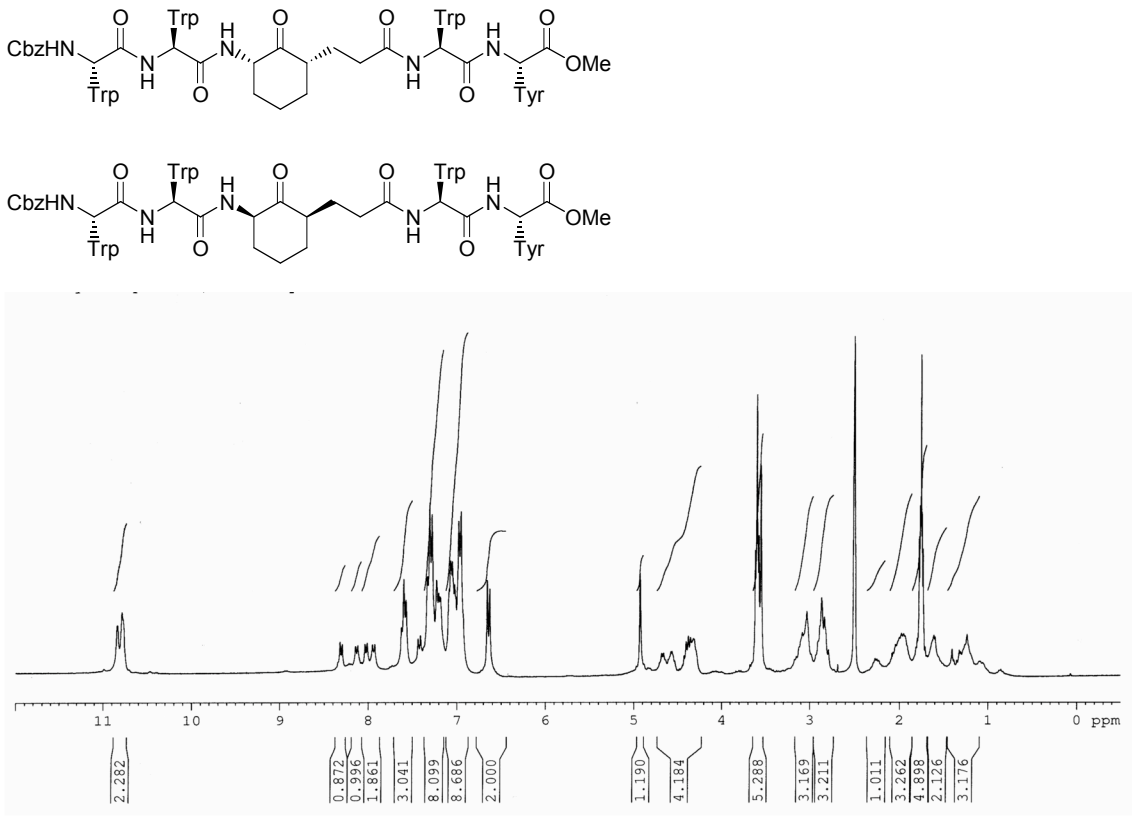


Figure S51. ^{1}H NMR spectrum for inhibitor **34** (mixture of two diastereomers).

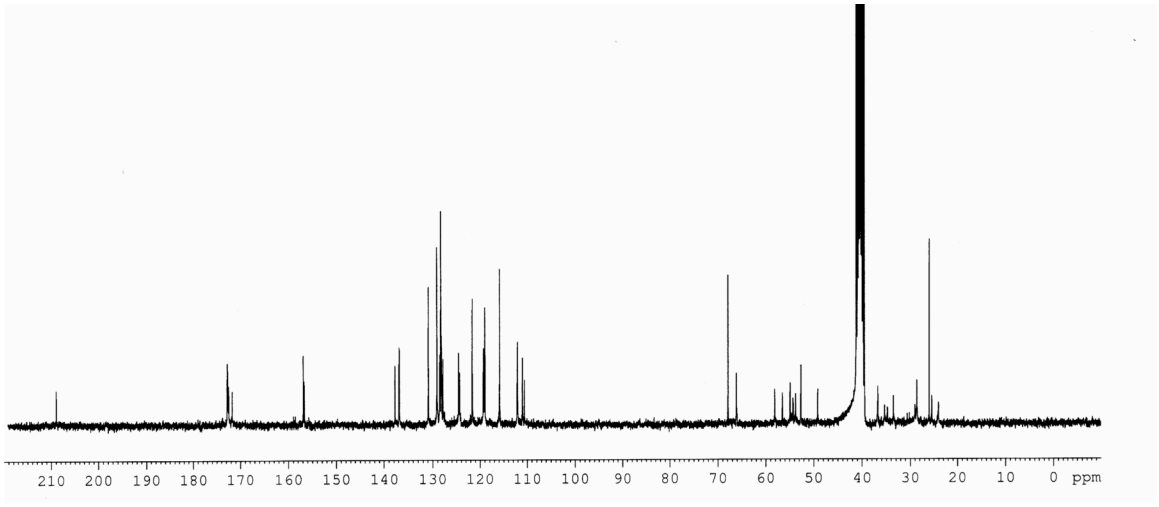


Figure S52. ^{13}C NMR spectrum for inhibitor **34** (mixture of two diastereomers).

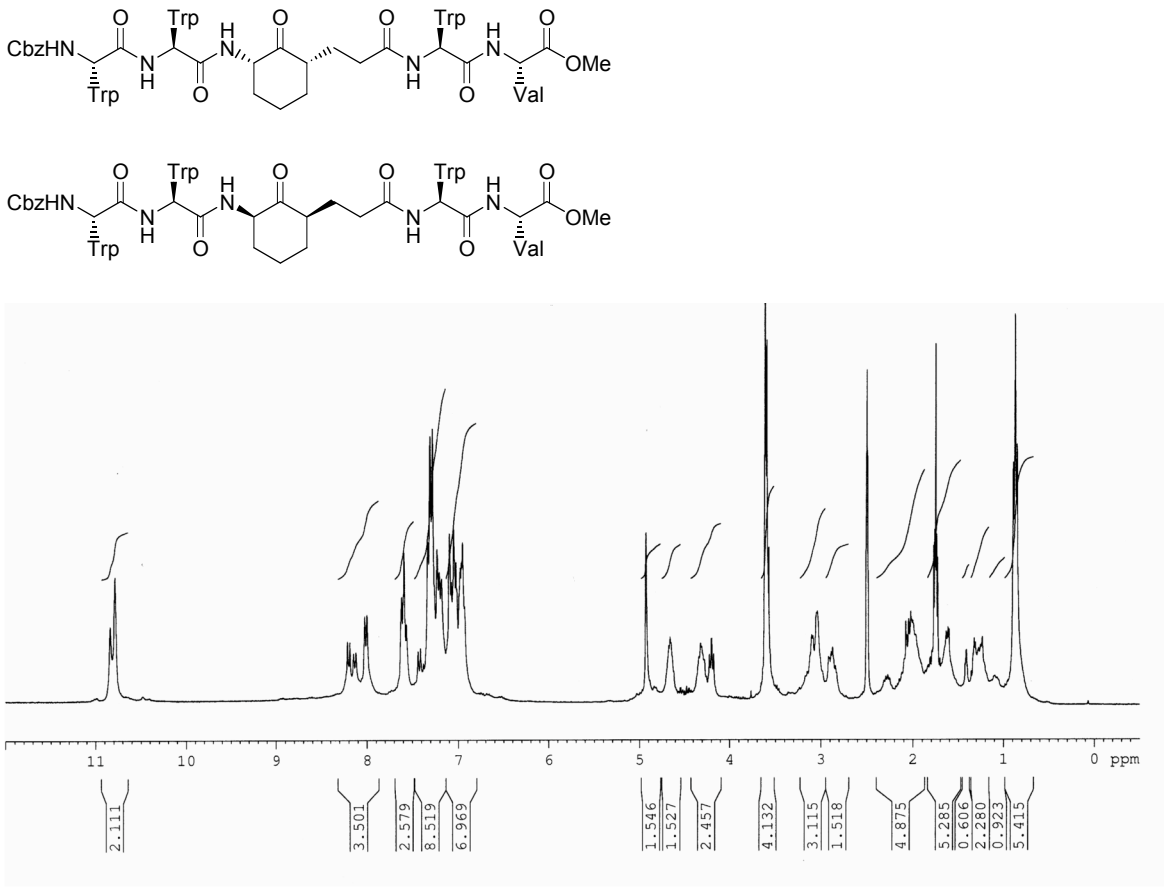


Figure S53. ^{1}H NMR spectrum for inhibitor **35** (mixture of two diastereomers).

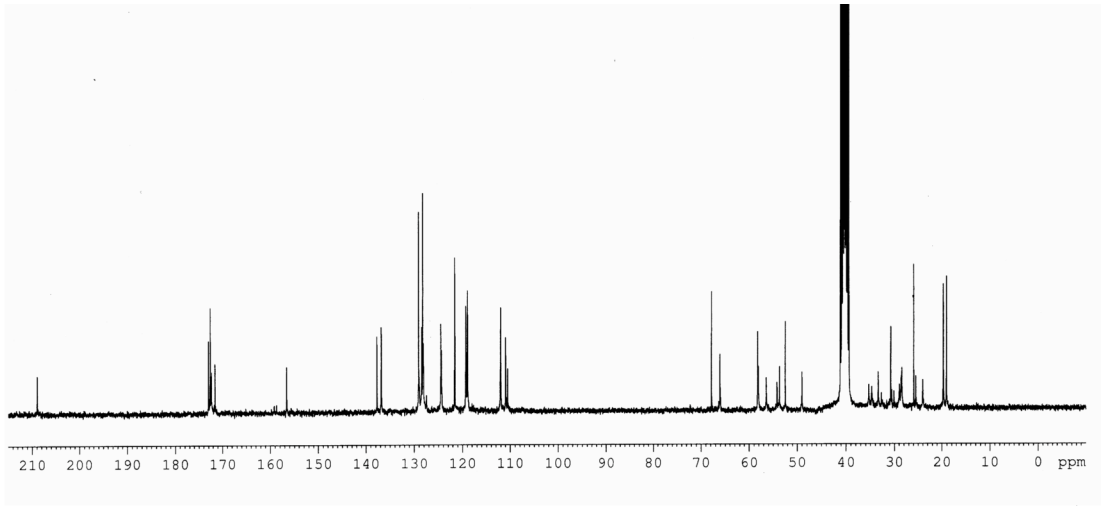


Figure S54. ^{13}C NMR spectrum for inhibitor **35** (mixture of two diastereomers).

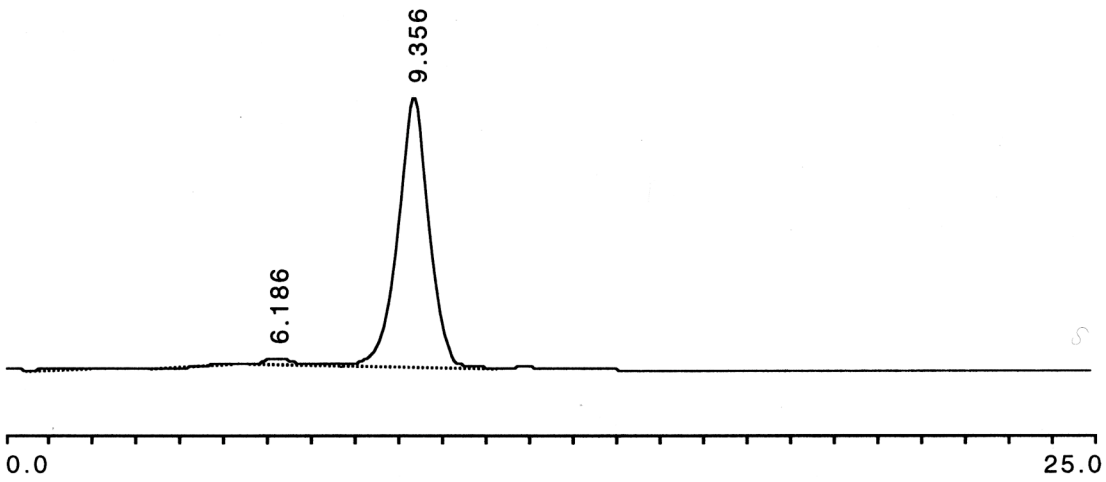


Figure S55. HPLC trace of inhibitor **29** using a reverse phase C-18 column eluted with 50% CH_3CN in water with a total of 0.1% TFA. Detection was performed at 254 nm.

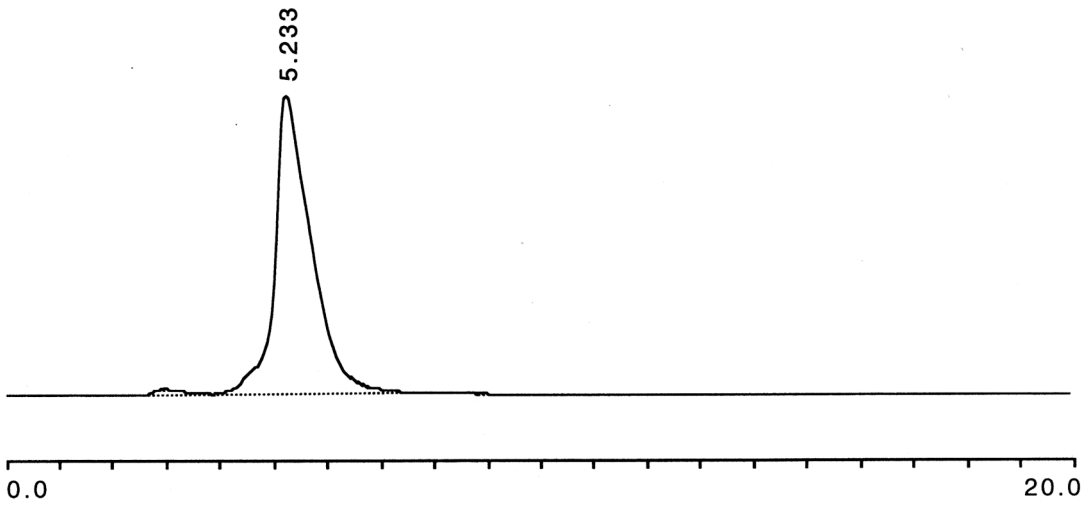


Figure S56. HPLC trace of inhibitor **29** using a reverse phase C-18 column eluted with 80% MeOH in water with a total of 0.1% TFA. Detection was performed at 254 nm.

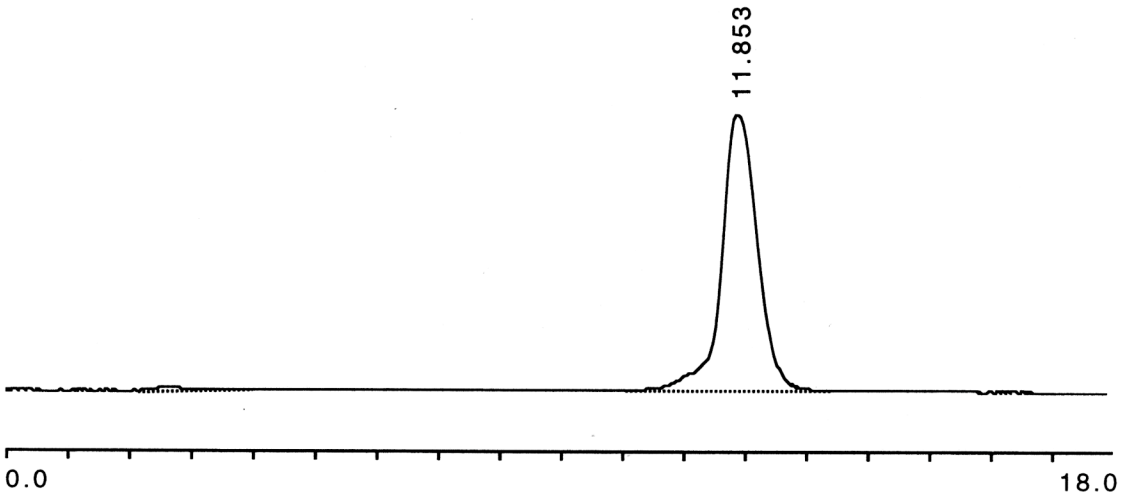


Figure S57. HPLC trace of inhibitor **30** using a reverse phase C-18 column eluted with 50% CH_3CN in water with a total of 0.1% TFA. Detection was performed at 254 nm.

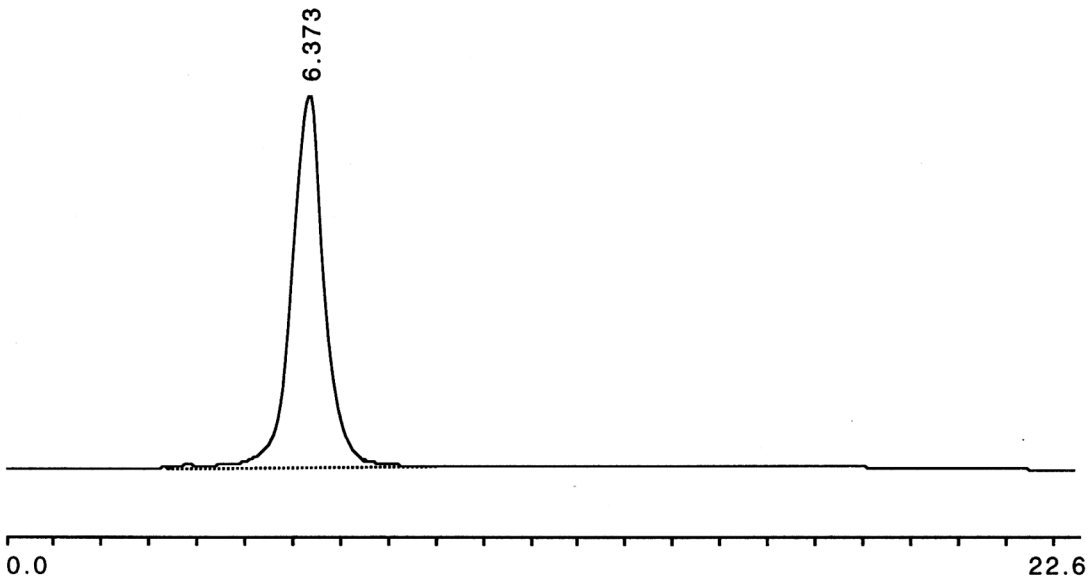


Figure S58. HPLC trace of inhibitor **30** using a reverse phase C-18 column eluted with 80% MeOH in water with a total of 0.1% TFA. Detection was performed at 254 nm.

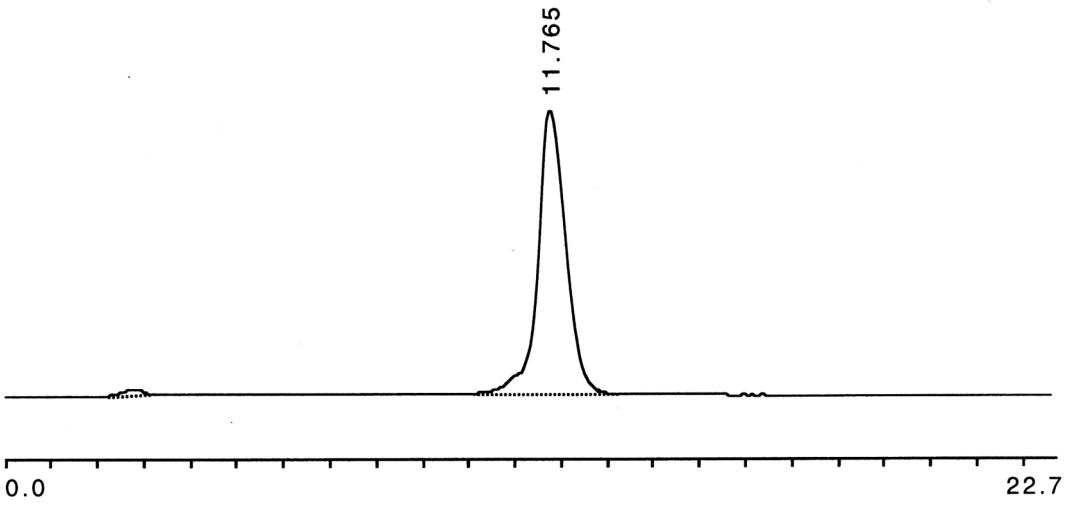


Figure S59. HPLC trace of inhibitor **31** using a reverse phase C-18 column eluted with 50% CH_3CN in water with a total of 0.1% TFA. Detection was performed at 254 nm.

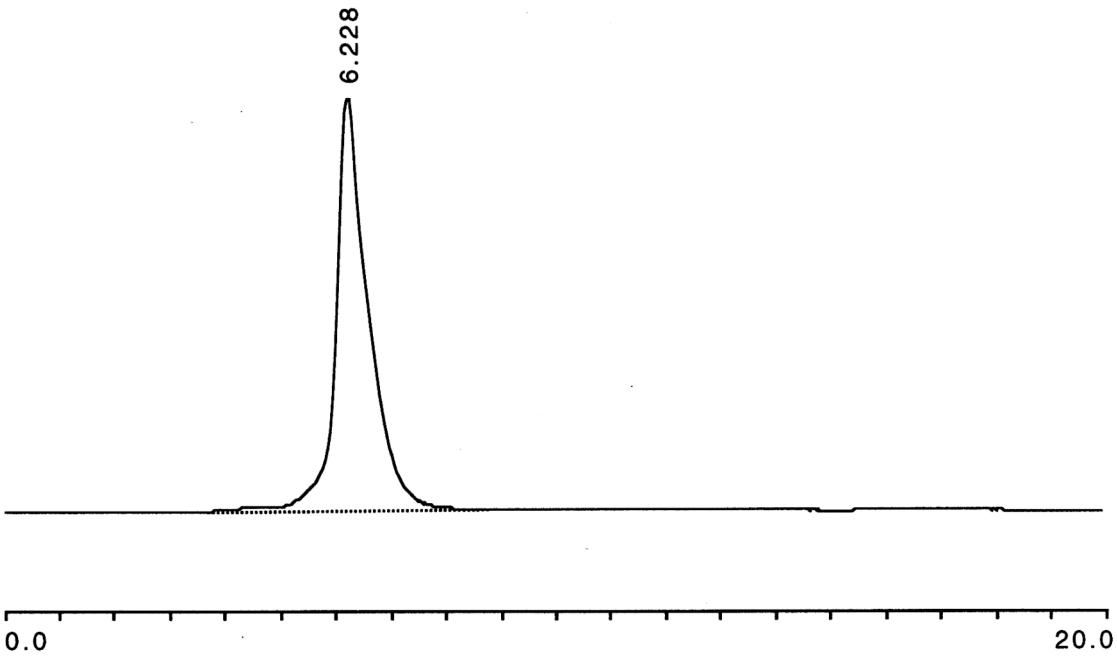


Figure S60. HPLC trace of inhibitor **31** using a reverse phase C-18 column eluted with 80% MeOH in water with a total of 0.1% TFA. Detection was performed at 254 nm.

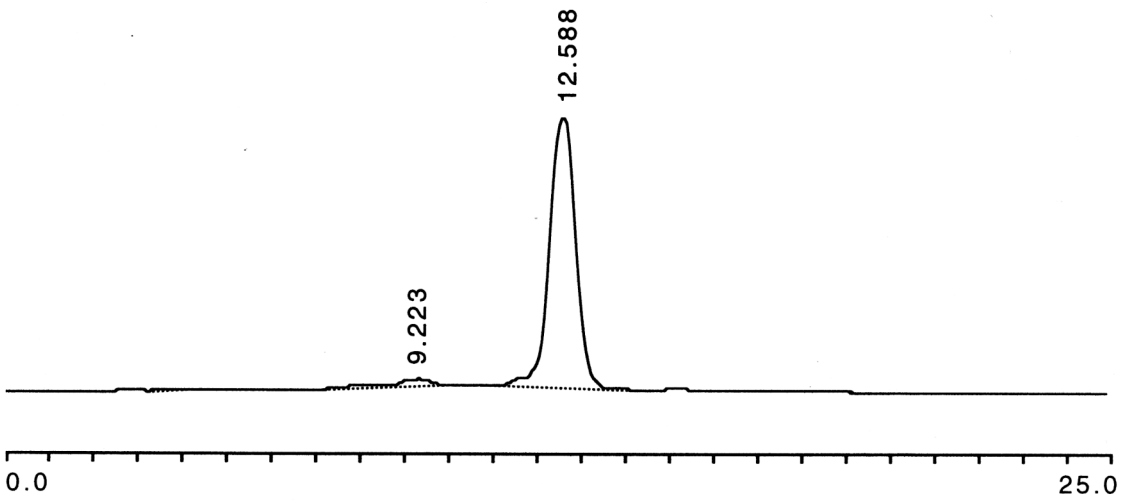


Figure S61. HPLC trace of inhibitor **32** using a reverse phase C-18 column eluted with 50% CH_3CN in water with a total of 0.1% TFA. Detection was performed at 254 nm.

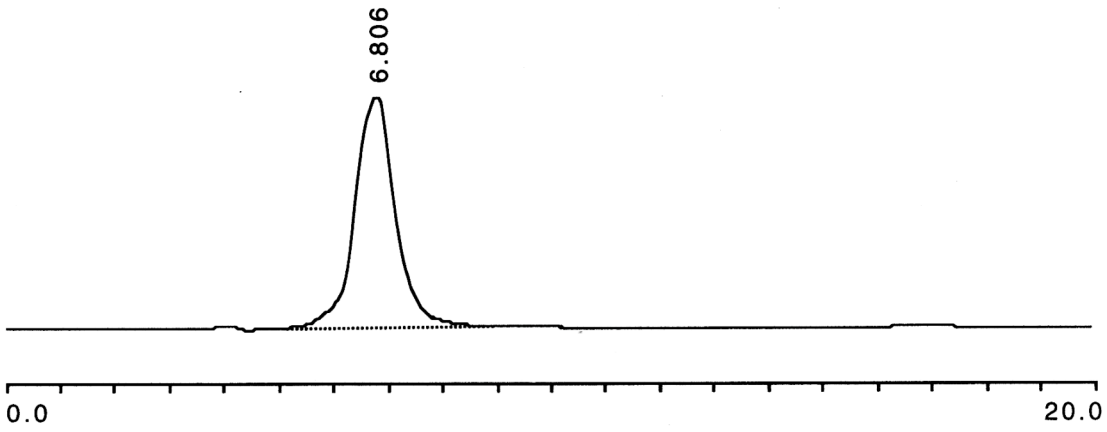


Figure S62. HPLC trace of inhibitor **32** using a reverse phase C-18 column eluted with 80% MeOH in water with a total of 0.1% TFA. Detection was performed at 254 nm.

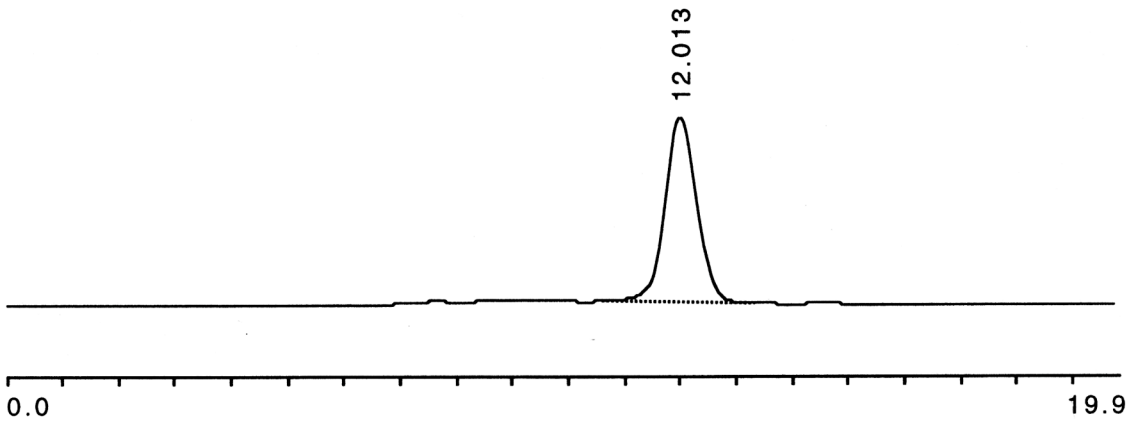


Figure S63. HPLC trace of inhibitor **33** using a reverse phase C-18 column eluted with 50% CH_3CN in water with a total of 0.1% TFA. Detection was performed at 254 nm.

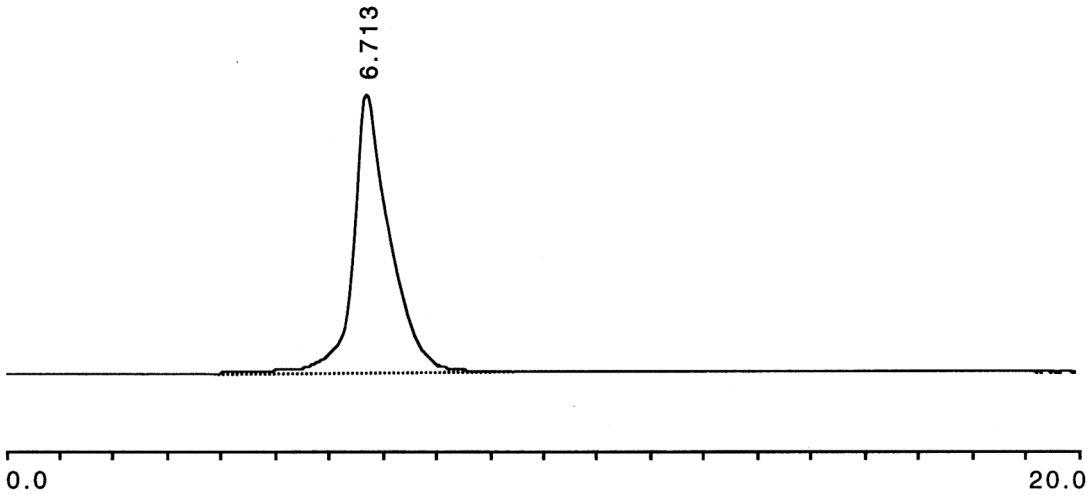


Figure S64. HPLC trace of inhibitor **33** using a reverse phase C-18 column eluted with 80% MeOH in water with a total of 0.1% TFA. Detection was performed at 254 nm.

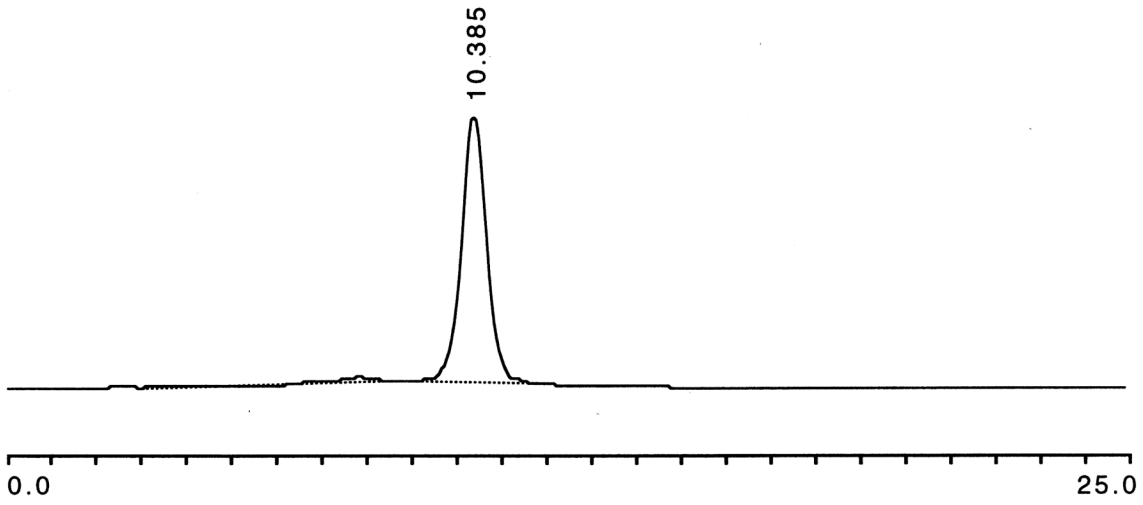


Figure S65. HPLC trace of inhibitor **34** using a reverse phase C-18 column eluted with 50% CH_3CN in water with a total of 0.1% TFA. Detection was performed at 254 nm.

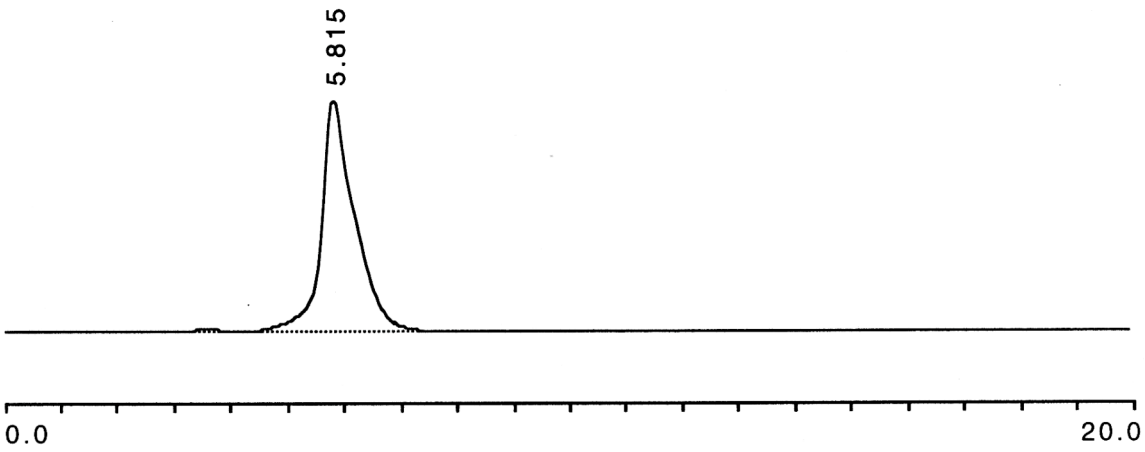


Figure S66. HPLC trace of inhibitor **34** using a reverse phase C-18 column eluted with 80% MeOH in water with a total of 0.1% TFA. Detection was performed at 254 nm.

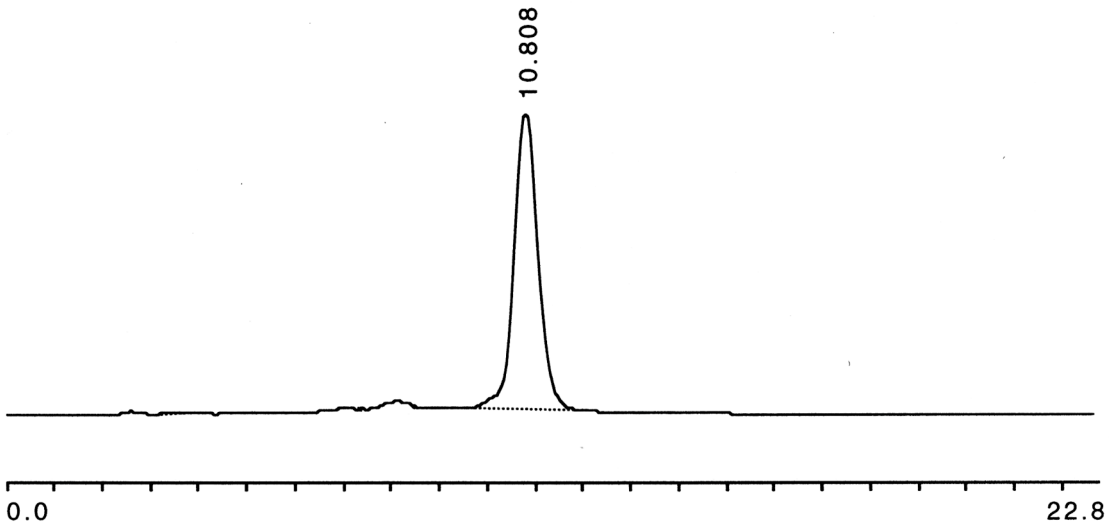


Figure S67. HPLC trace of inhibitor **35** using a reverse phase C-18 column eluted with 50% CH_3CN in water with a total of 0.1% TFA. Detection was performed at 254 nm.

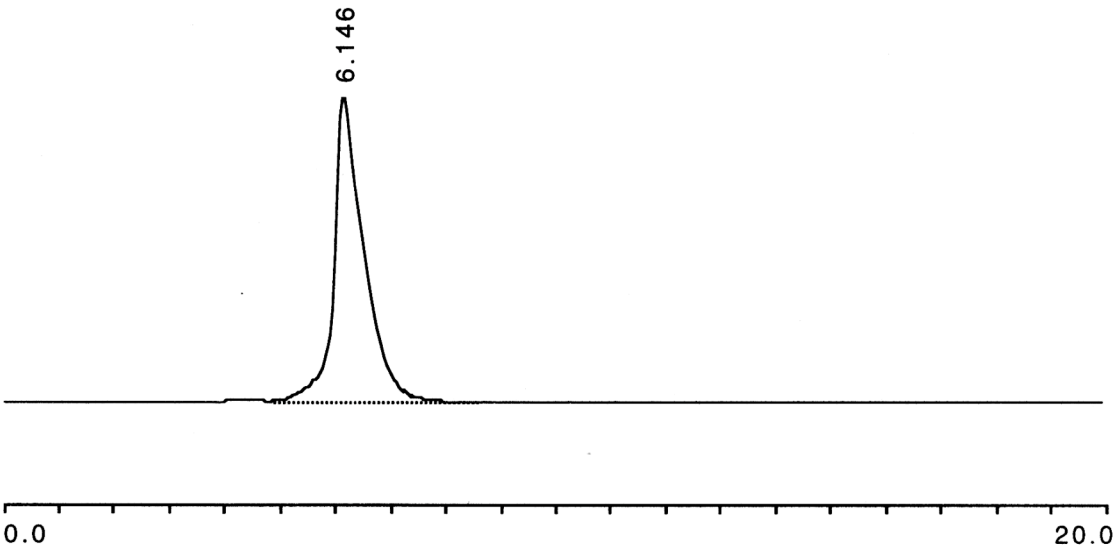


Figure S68. HPLC trace of inhibitor **35** using a reverse phase C-18 column eluted with 80% MeOH in water with a total of 0.1% TFA. Detection was performed at 254 nm.