α,α-Dichloroisoxazolidinones for the Synthesis and Chemoselective Peptide Ligation of α-Peptide α-Ketoacids

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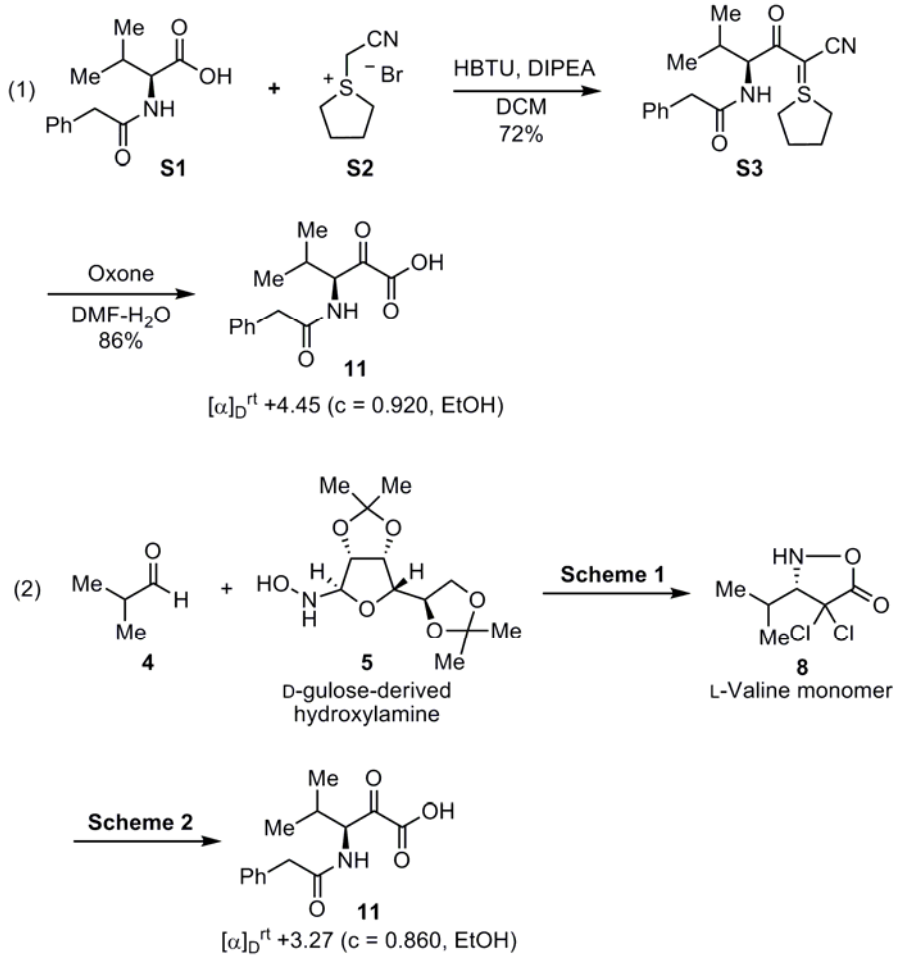
Supporting Information

General Methods. All reactions utilizing air- or moisture-sensitive reagents were performed in dried glassware under an atmosphere of dry nitrogen. Thin layer chromatography (TLC) was performed on EMD precoated plates (silica gel 60 F254, Art 5715) and were visualized by fluorescence quenching under UV light and by staining with phosphomolybdic acid and/or *p*-anisaldehyde, respectively. For flash column chromatography, EMD Silica Gel 60 (230–400 Mesh) and Silica Gel 60 N (Kanto Chemical Co., Inc.) were employed. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) were measured on a Bruker Avance AVII-500 spectrometer with a CryoProbe. Chemical shifts are expressed in parts per million (ppm) with respect to the residual solvent peak. Coupling constants are reported as Hertz (Hz), signal shapes and splitting patterns are indicated as follows: br, broad; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Infrared (IR) spectra were recorded on a SHIMADZU IR Prestige-21 FTIR-8400S and are reported as wavenumber (cm^{-1}). Low- and high-resolution mass spectra were recorded on a Brucker Daltonics microTOF-2focus in the positive and negative detection mode. Optical rotations were measured on JASCO DIP-370 polarimeter operating at the sodium D line with a 100 mm path length cell, and were reported as follows: $[\alpha]_D^T$ (concentration (g:100 mL), solvent).

HPLC Conditions. For analytical HPLC, a Cosmosil 5C_{18}-ARII column (4.6 x 250 mm, Nacalai Tesque, Inc., Kyoto, Japan) was employed with a linear gradient of CH_3CN containing 0.1% (v/v) TFA at a flow rate of 1 cm^3 min^{-1} on a LaChrom Elite HTA system (Hitachi High-Technologies corporation, Ltd., Tokyo, Japan) and JASCO PU-2086 plus (JASCO corporation, Ltd., Tokyo, Japan), and eluting products were detected by UV at 220 nm. Preparative HPLC was performed using a Cosmosil 5C_{18}-AR II column (20 x 250 mm, Nacalai Tesque, Inc.) on a JASCO PU-2087 plus and PU-2089 plus (JASCO corporation, Ltd., Tokyo, Japan) in a suitable gradient mode of CH_3CN solution containing 0.1% (v/v) TFA at a flow rate of 7-10 cm^3 min^{-1}.

Determination of the absolute stereochemistry of phenylacetyl-Val-ketoacid 11. The stereochemistry was assigned by the chemical correlation with the synthetic sample, prepared from phenylacetyl-L-Val-OH **S1**. Coupling of **S1** with the bromide salt **S2**[1] followed by oxone oxidation provided the enatiopure ketoacid **11** (eq 1).

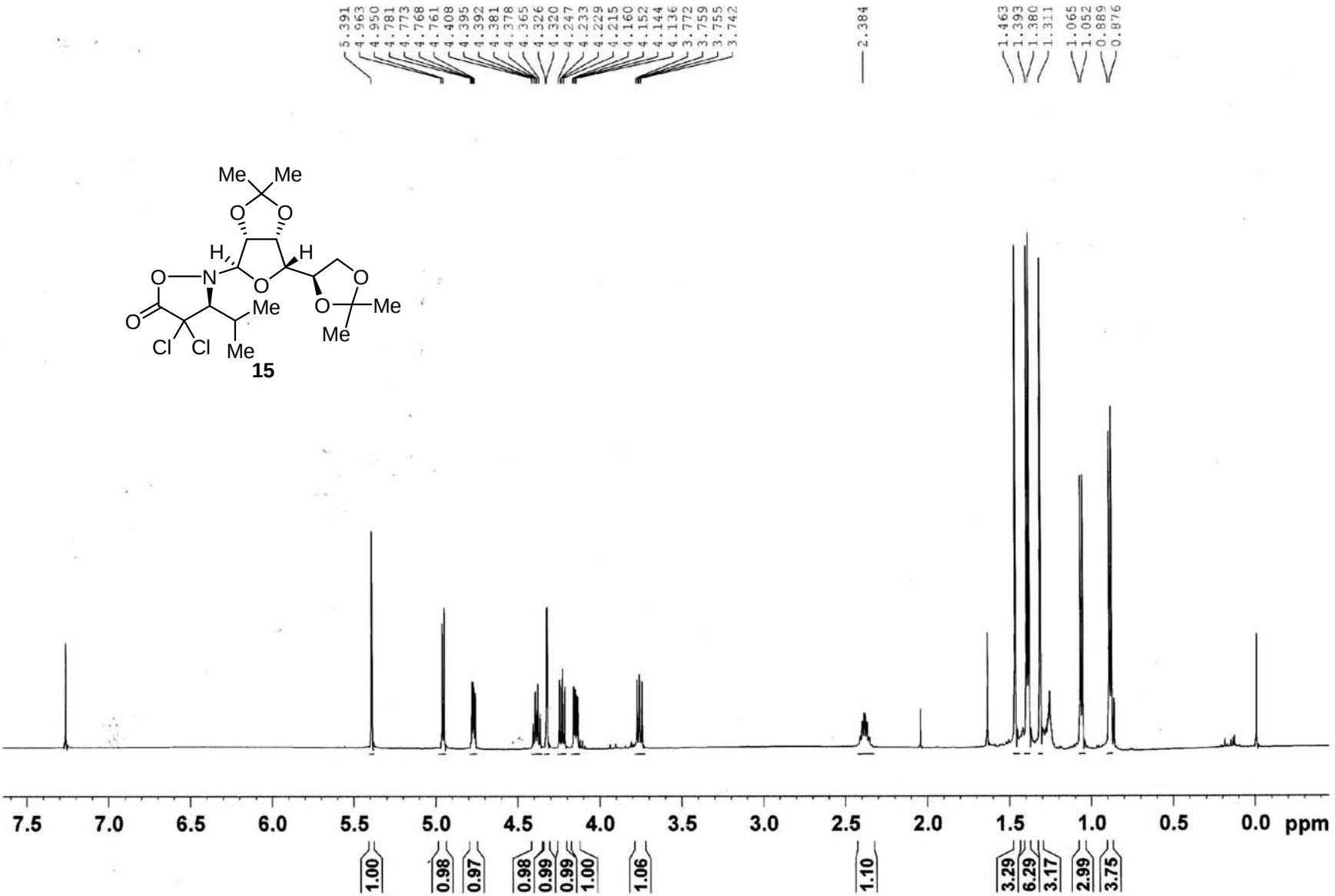


Phenylacetyl-L-Val-SY S3. Phenylacetyl-L-Val-OH **S1** (235.3 mg, 1.00 mmol) was dissolved in CH_2Cl_2 (10 mL). HBTU (417.8 mg, 1.10 mmol), DIPEA (522 μL, 3.00 mmol), and the bromide salt **S2** (270.5 mg, 1.30 mmol) were added and the reaction was stirred at RT for 2 h. The reaction mixture was poured into sat. aq. NH_4Cl and extracted with CH_2Cl_2. The extract was washed with brine and dried over Na_2SO_4. Concentration under reduced pressure followed by flash chromatography over silica gel (EtOAc → 1:1 EtOAc:Acetone → Acetone) gave **S3** (247.7 mg, 72% yield) as a semisolid: $[\alpha]_D^{rt}$ +24.7 (c 1.22, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36-7.24 (m, 5H), 6.12 (d, *J* = 9.0 Hz, 1H), 4.78 (dd, *J* = 8.5, 5.0 Hz, 1H), 3.55-3.63 (m, 2H), 3.36-3.42 (m, 2H), 3.26-3.33 (m, 2H), 2.51-2.62 (m, 2H), 2.03-2.13 (m, 3H), 0.918 (d, *J* = 6.5 Hz, 3H), 0.713 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 189.2, 170.5, 135.1, 129.4, 128.9, 127.2, 119.3, 59.0, 55.2, 45.7, 45.1, 44.0, 32.0, 28.5, 28.4, 19.7, 17.2; HRMS (ESI), *m*/*z* calcd for $C_{19}H_{25}N_2O_2S$ $[M+H]^+$ 345.1631, found 345.1630.

[1] L. Ju, A. R. Lippert, and J. W. Bode, *J. Am. Chem. Soc.*, 2008, **130**, 4253.

(*S*)-4-Methyl-2-oxo-3-(2-phenylacetamido)pentanoic acid (phenylacetyl-L-Val-ketoacid) 11. Phenylacetyl-L-Val-SY **S3** (51.9 mg, 0.145 mmol) was dissolved in DMF:H_2O (1:1, 2.90 mL). Oxone (178.5 mg, 0.290 mmol) was added to the reaction mixture in one portion. After stirring for 30 min at rt, purification by preparative HPLC (Gradient: 0 min, 25% MeCN in H_2O; 90 min, 55% MeCN in H_2O) followed by lyophilization afforded **11** (32.8 mg, 86% yield) as a colorless oil. For spectroscopical data on phenylacetyl-L-Val-ketoacid **11**, see experimental section.

TNR148 D-Gulosyl-L-Val cyclization



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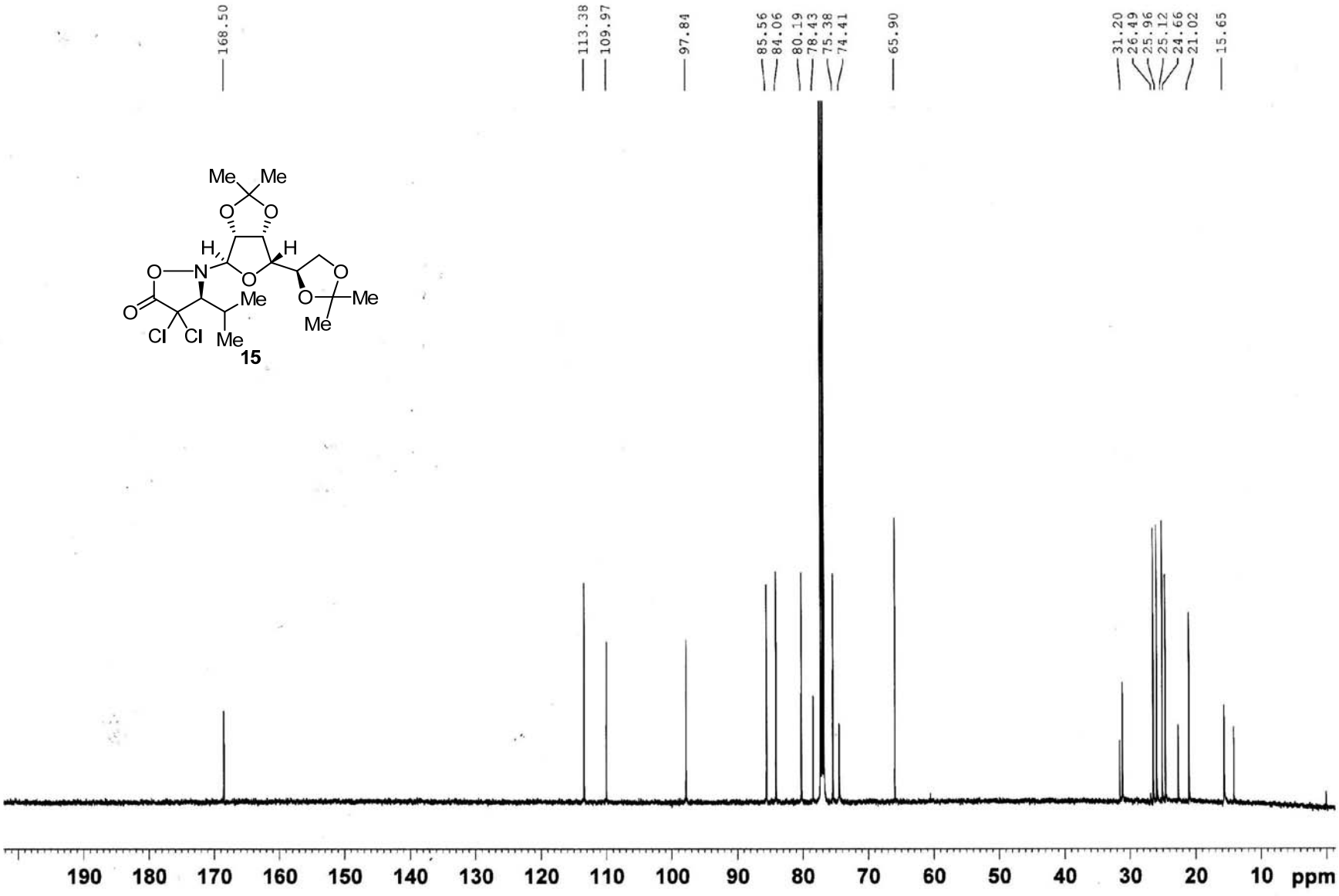
TNR148 D-Gulosyl-L-Val cyclization 13C



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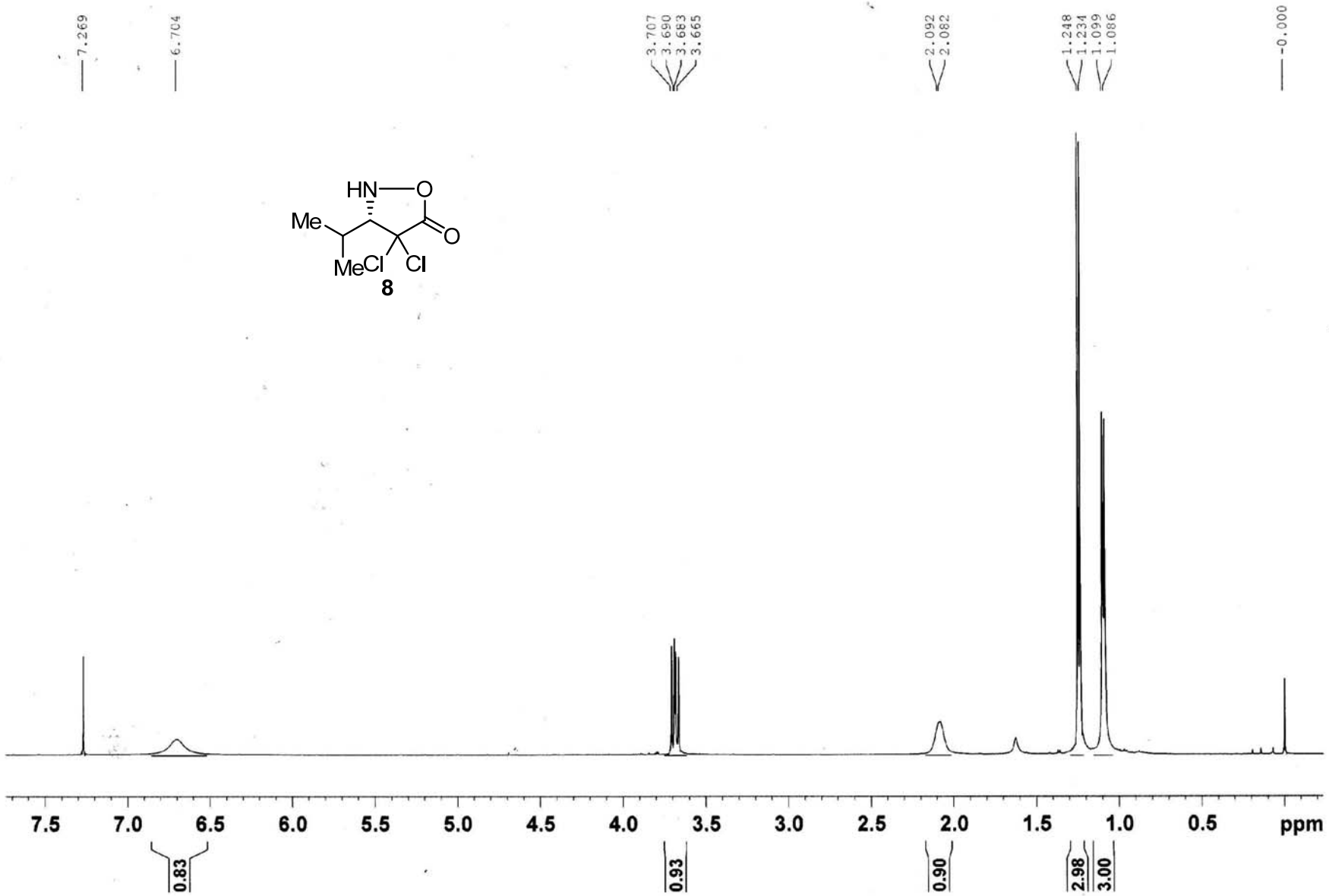
TNR165 L-Val monomer



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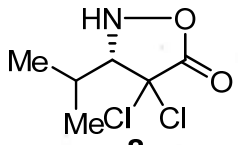

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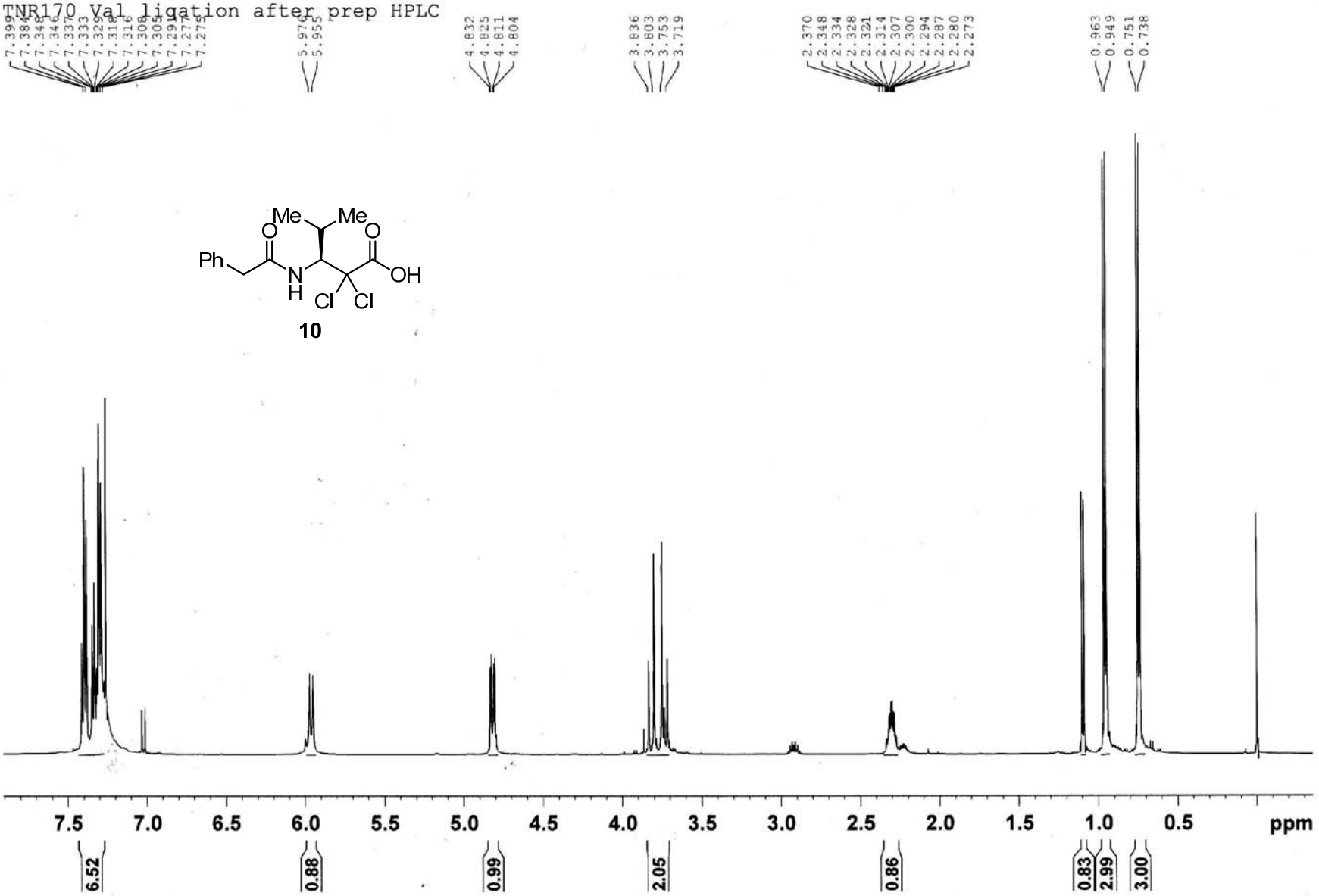
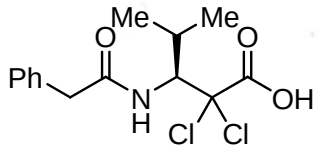


TNR170 Val ligation after prep HPLC 13C



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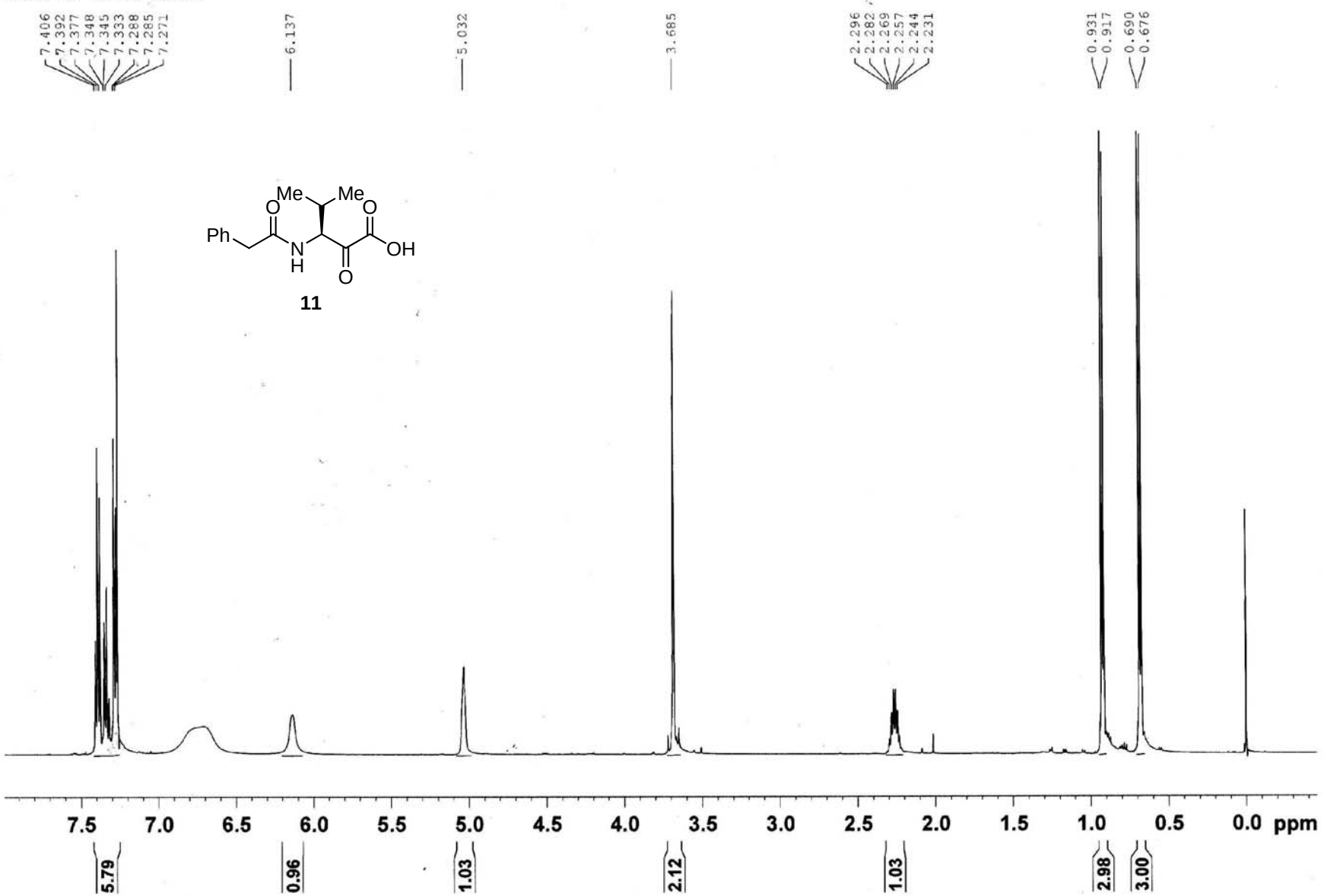
TNR173 Keto acid



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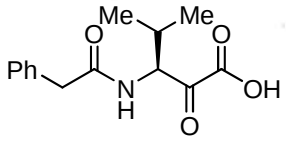

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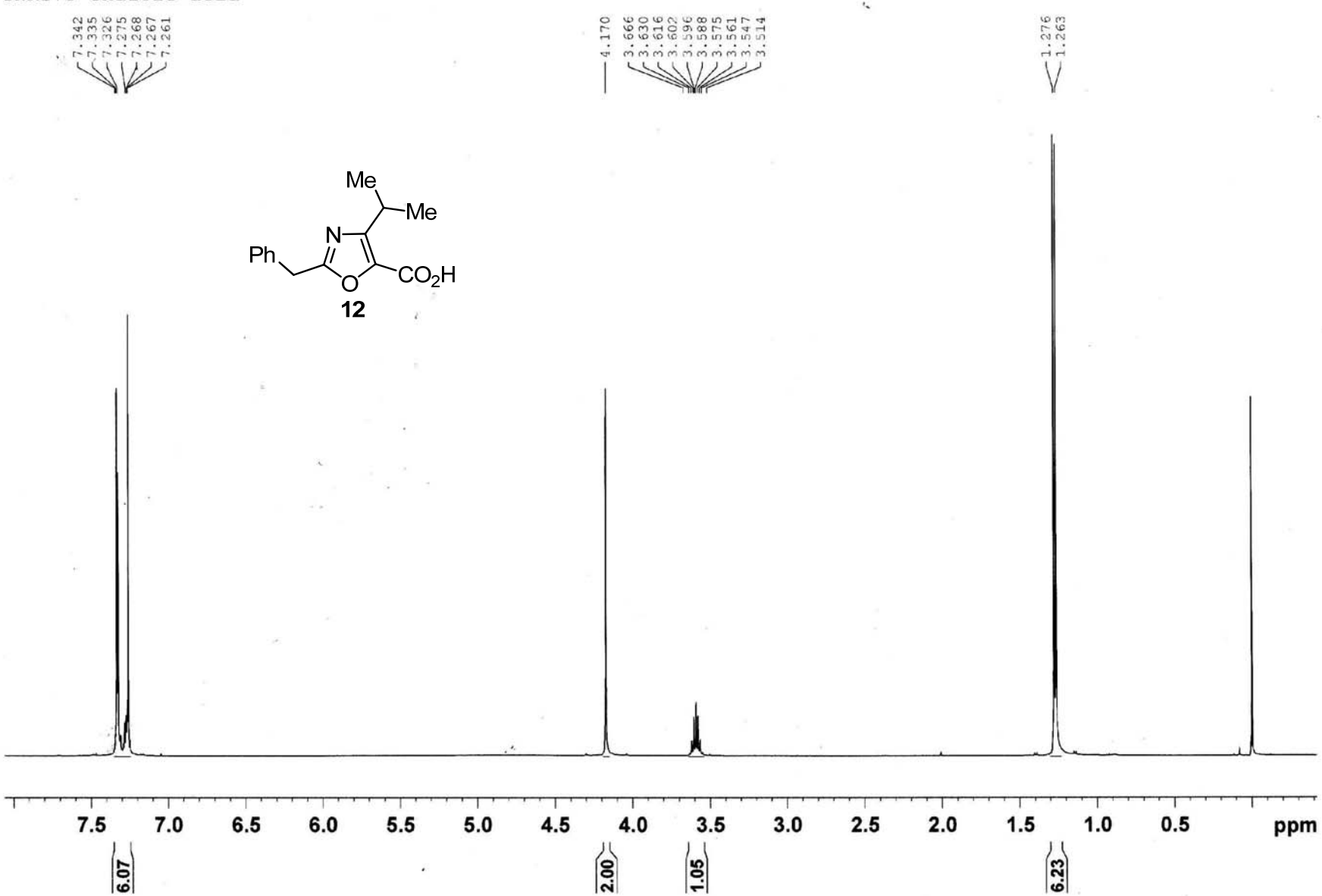
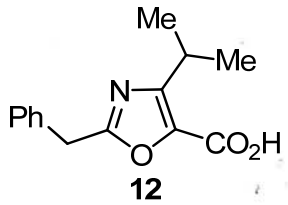


TNR173 oxazole acid 13C



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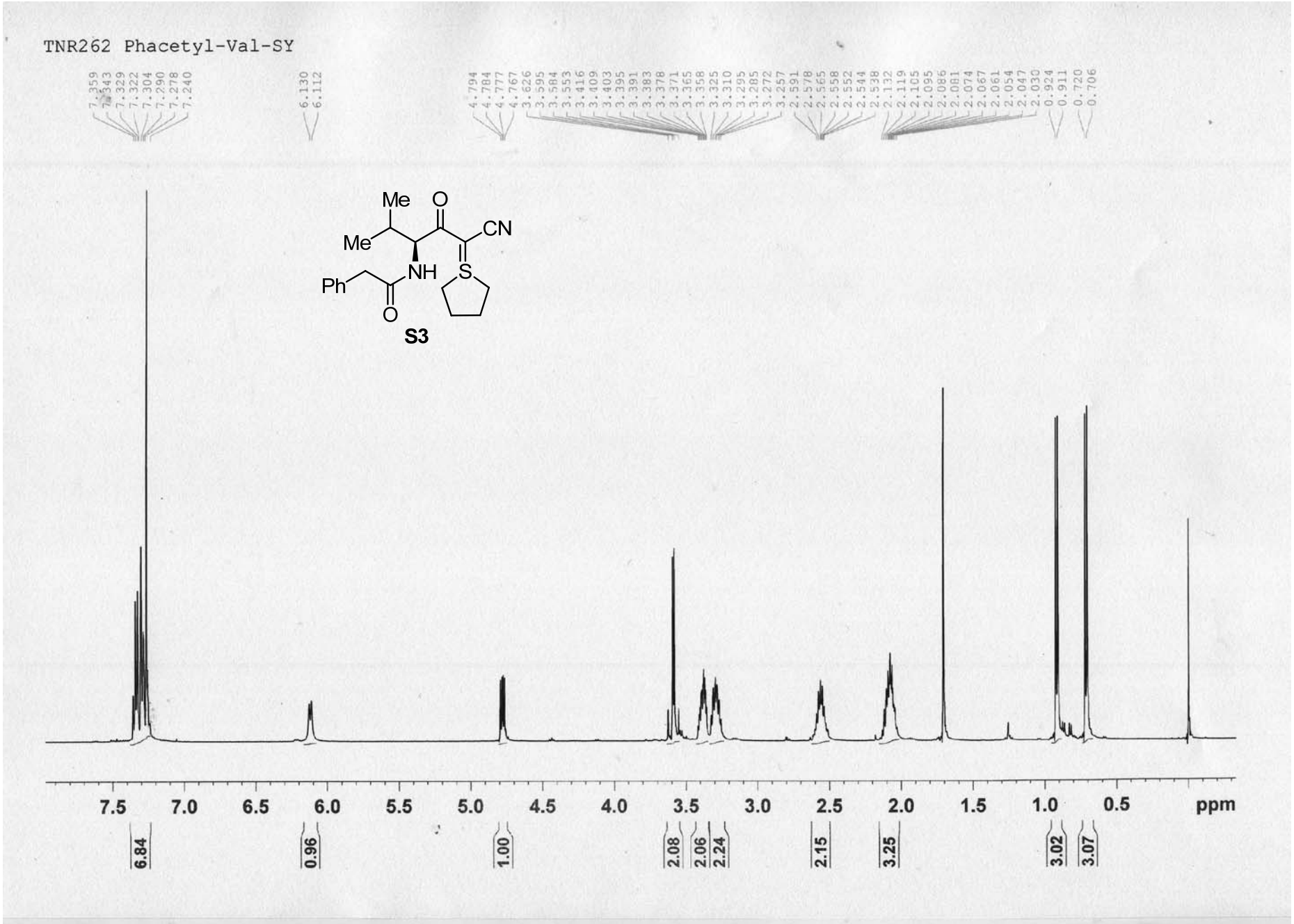
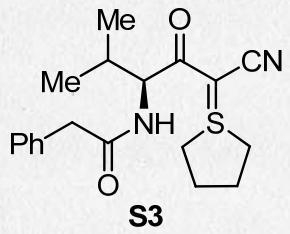
TNR262 Phacetyl-Val-SY



TNR262 Phacetyl-Val-SY 13C

189.19
170.48
135.13
129.40
128.86
127.16
119.26
59.01
55.20
45.65
45.07
43.97
31.99
28.50
28.47
19.68
17.16



S3

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm