# Supporting Information for

# Aza-SAHA Derivatives are Selective Histone Deacetylase 10 Chemical Probes That Inhibit Polyamine Deacetylation and Phenocopy HDAC10 Knockout

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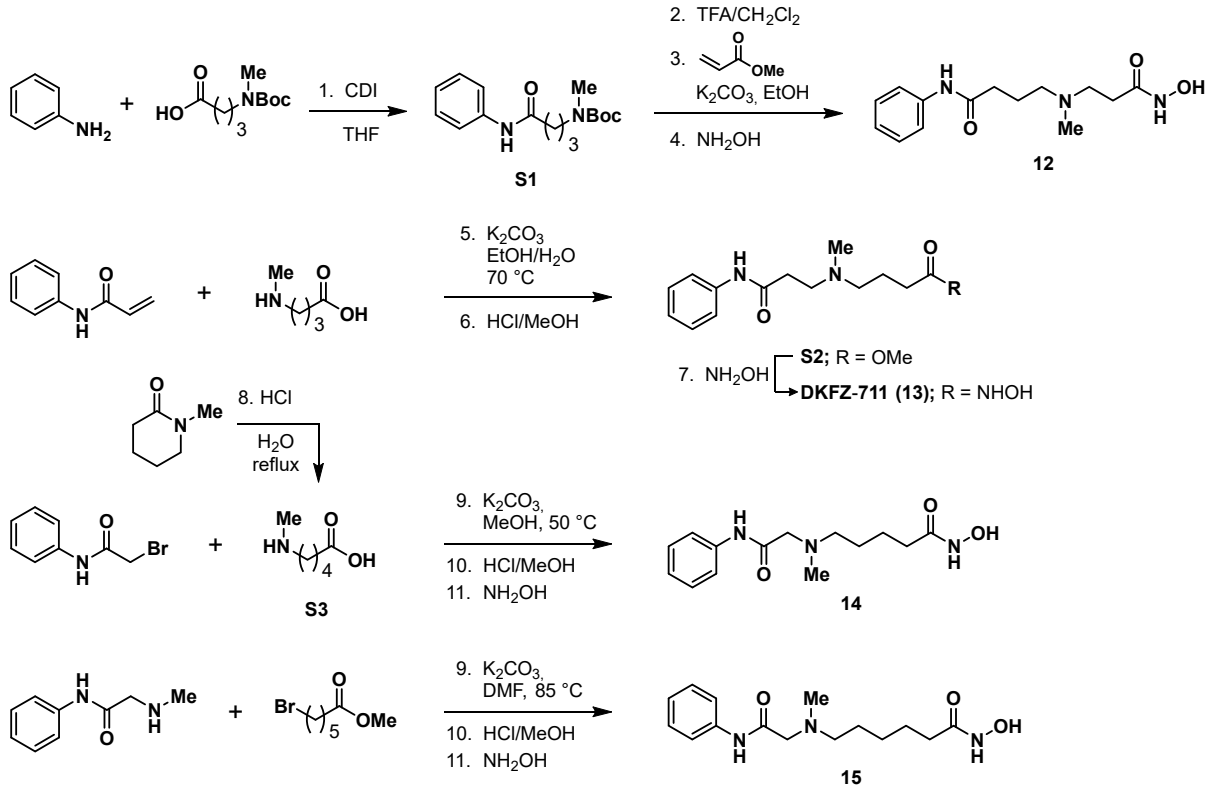
# Contents

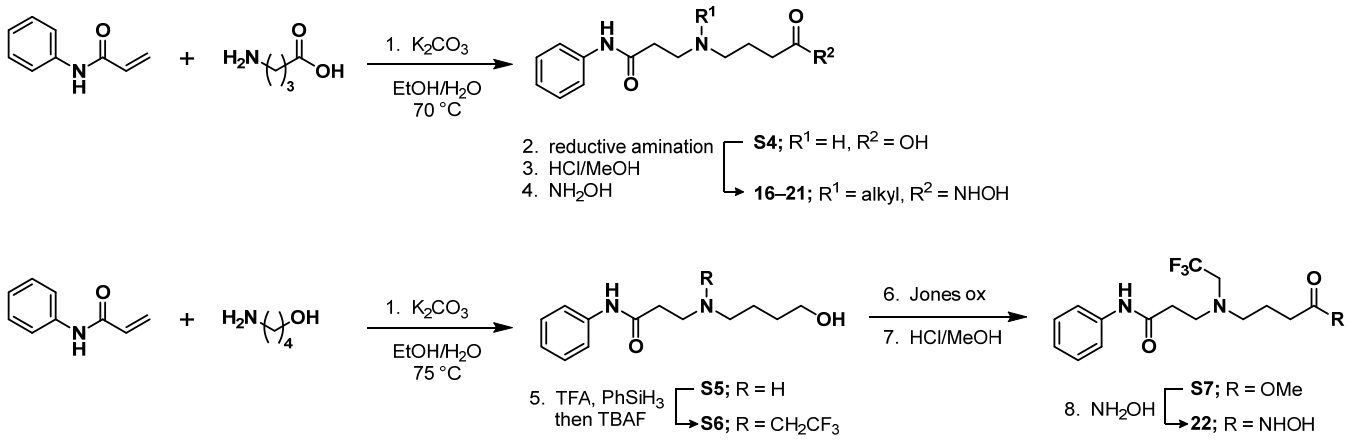
# Supplementary schemes, tables and figures

## Scheme S1. Synthesis of aza-SAHA compounds 12–15[a]



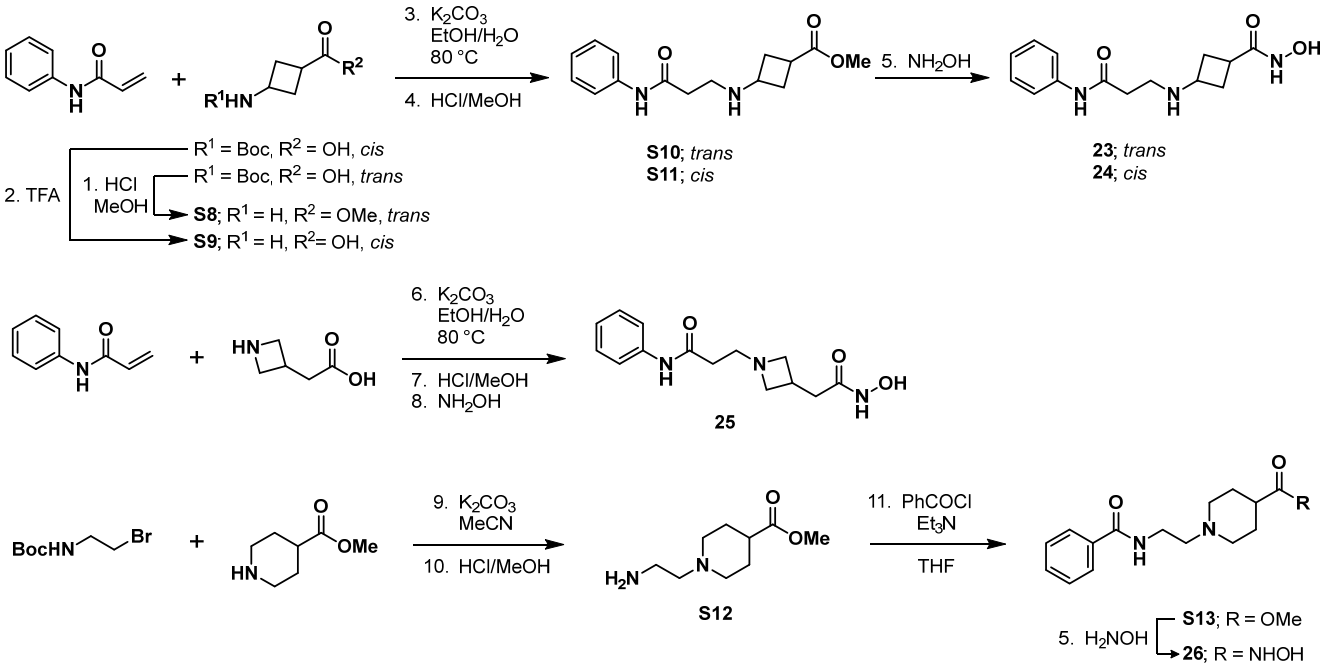

[a] Reagents and conditions: 1. CDI, THF, rt, 8 h, 86%; 2. 30% TFA in $CH_2Cl_2$, rt, 1 h; 3. methyl acrylate $K_2CO_3$, EtOH, rt, 66 h; 4. KCN, dioxane, rt, 1 h, then $NH_2OH$ (50 wt% in $H_2O$), 24 h, rt, 8% over three steps; 5. $K_2CO_3$, EtOH/$H_2O$, 70 °C, 7 h; 6. HCl/MeOH, rt, 72 h, 82% (2 steps); 7. KCN, dioxane, rt, 1 h, then $NH_2OH$ (50 wt% in $H_2O$), rt, 24 h, 66%; 8. 6.4 M HCl, reflux, quant.; 9. $K_2CO_3$, MeOH, 50 °C, 45 h or DMF, 85 °C, 40 h; 10. HCl/MeOH, rt, 18 h; 11. KCN, dioxane, rt, 1 h, then $NH_2OH$ (50 wt% in $H_2O$), rt, 24 h, 62% for **14** (3 steps), 14% for **15** (3 steps).

## Scheme S2. Synthesis of compounds 16–22[a]



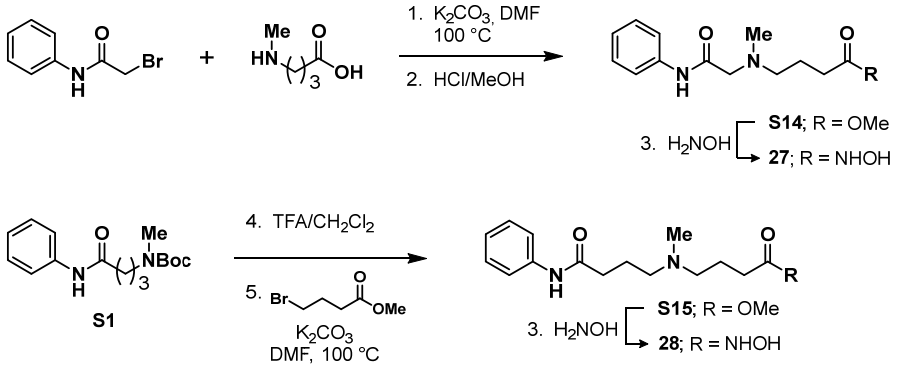

[a] Reagents and conditions 1. $K_2CO_3$, EtOH/$H_2O$, 70 °C, 3.5 h, 32% for **S4** or 75 °C, 2 h, 83% for **S5**; 2. aldehyde or ketone, $Et_3N$, $Na(OAc)_3BH$, THF, 0 °C–rt, 2–3 h or cyclopropanone ethyl trimethylsilyl acetal, HCl, $NaCNBH_3$, $H_2O$, rt, 7 d; 3. HCl/MeOH, rt, 18 h; 4. KCN, dioxane, rt, 1 h, then $NH_2OH$ (50 wt% in $H_2O$), 24 h, rt, 15–56% (3 steps); 5. TFA, $PhSiH_3$, THF, reflux, 4 h, then $CH_2Cl_2$, 1 M aq. NaOH, TBAF, 76%; 6. Jones reagent, acetone, 0 °C; 7. HCl/MeOH, rt, 16 h, 63% (2 steps); 8. KCN, dioxane, rt, 1 h, then $NH_2OH$ (50 wt% in $H_2O$), 24 h, rt, 39%.

## Scheme S3. Synthesis of cyclic linker compounds 23–26[a]



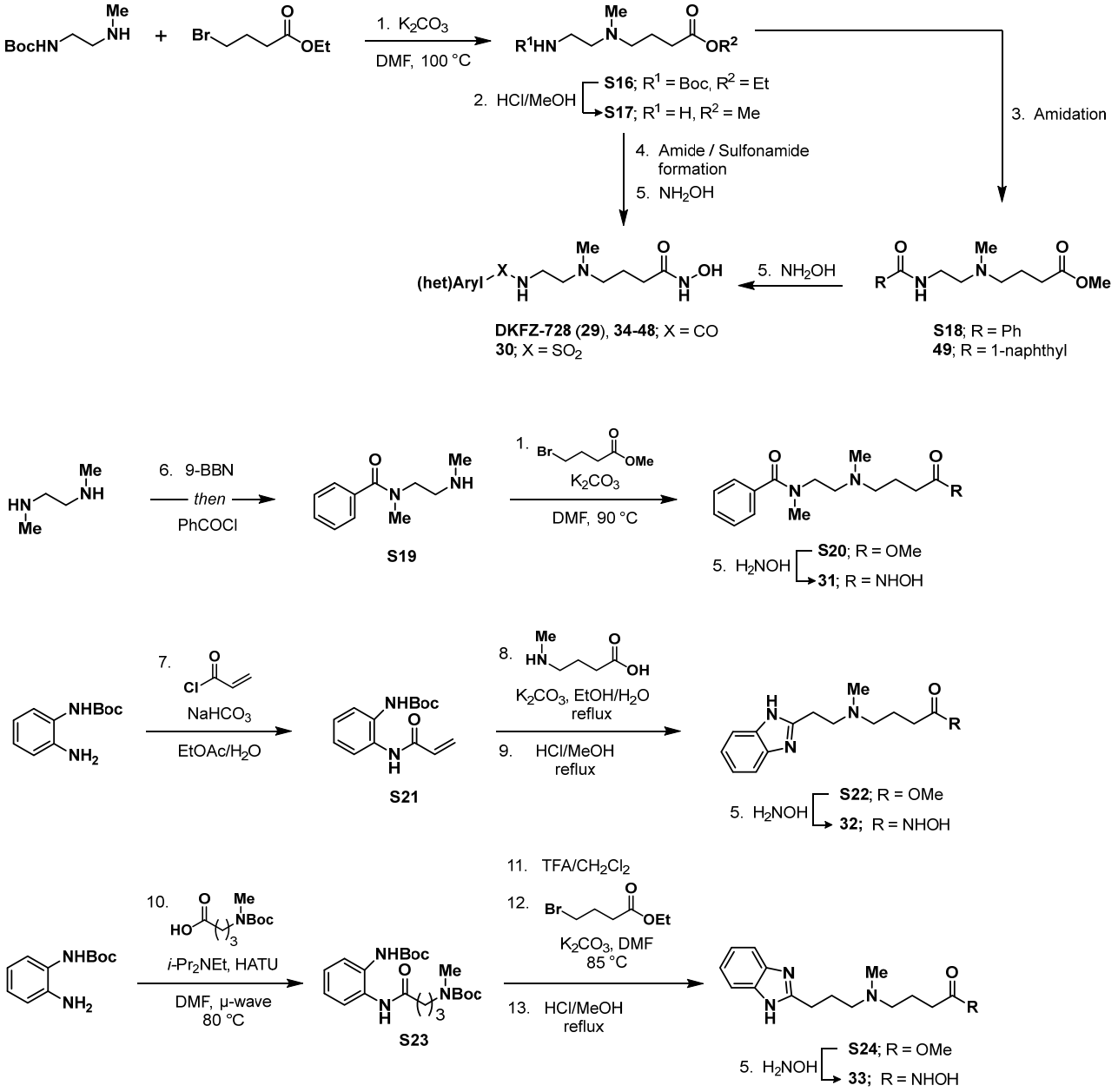

[a] Reagents and conditions: 1. 2M in HCl in MeOH, quant.; 2. 20% TFA in $CH_2Cl_2$, quant.; 3. $K_2CO_3$, EtOH/$H_2O$, 80 °C, 24–38 h; 4. HCl/MeOH, rt, 4 h, 58% for **S10** (2 steps), 38% for **S11** (2 steps); 5. KCN, dioxane, rt, 1 h, then $NH_2OH$ (50 wt% in $H_2O$), 24 h, rt, 43% for **23**, 72% for **24**, 36% for **26**; 6. $K_2CO_3$, EtOH/$H_2O$, 80 °C, 5 h; 7. HCl/MeOH, rt, 3 h; 8. KCN, dioxane, rt, 1 h, then $NH_2OH$ (50 wt% in $H_2O$), 24 h, rt, 6% (3 steps); 9. $K_2CO_3$, MeCN, reflux, 3 h; 10. HCl/MeOH, rt, 1.5 h, 65% (2 steps); 11. PhCOCl, $Et_3N$, THF, rt, 2.5 h, 28%.

## Scheme S4. Synthesis of compounds 27–28[a]



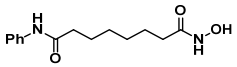
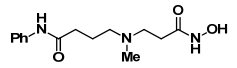
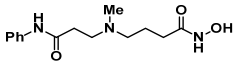
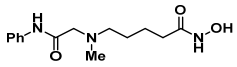
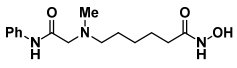

[a] Reagents and conditions: 1. $K_2CO_3$, DMF 100 °C, 1 h; 2. HCl/MeOH, rt, 18 h, 26% (2 steps); 3. KCN, dioxane, rt, 1 h, then $NH_2OH$ (50 wt% in $H_2O$), 24 h, rt, 73% for **27**, 82% for **28**; 4. 20% TFA in $CH_2Cl_2$, rt, 1 h; 5. methyl 4-bromobutyrate, $K_2CO_3$, DMF 100 °C, 4.5 h, 66% (2 steps).

## Scheme S5. Synthesis of compounds 29–48[a]



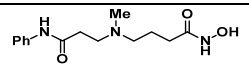
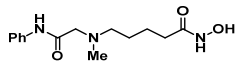
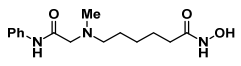
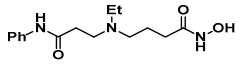
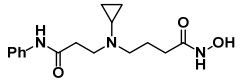
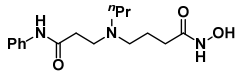
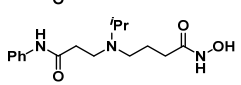
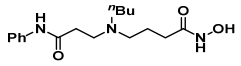
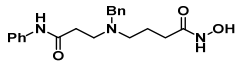
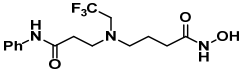
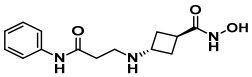
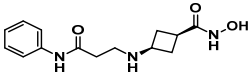
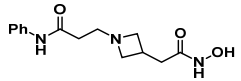
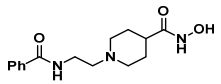
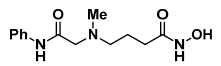
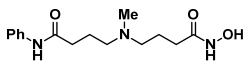

[a] Reagents and conditions: 1. $K_2CO_3$, DMF, 100 °C, 16 h, 88% for **S16** or 90 °C, 4 h, 59% for **S20**; 2. HCl/MeOH, rt, 26 h, quant; 3. PhCOCl, $Et_3N$, dioxane, rt, 2 h, 88% for **S18** or 1-naphthoic acid, *i*-$Pr_2NEt$, HOBt, EDC, $CH_2Cl_2$, rt, 18 h, 83% for **49**; 4. ArCOCl, $NaHCO_3$, EtOAc/$H_2O$, rt, 10–90 min or $PhSO_2Cl$, $K_2CO_3$, $H_2O$/MeCN, rt, 5 h or (het)aryl carboxylic acid, *i*-$Pr_2NEt$, HOBt, EDC or DCC, $CH_2Cl_2$, rt, 12–48 h; 5. KCN, dioxane, rt, 1 h, then $NH_2OH$ (50 wt% in $H_2O$), 24 h, rt, 22–80% (for individual yields see specific procedures); 6. 9-BBN, hexane/THF, rt, 1 h, then PhCOCl, rt, 2.5 h, 31%; 7. acryloyl chloride, $NaHCO_3$, EtOAc/$H_2O$, rt, 10 min, 98%; 8. *N*-methyl-GABA, $K_2CO_3$, EtOH/$H_2O$, reflux, 16 h; 9. HCl/MeOH, reflux, 18 h, 49% (2 steps); 10. *N*-Boc,*N*-MeGABA, *i*-$Pr_2NEt$, HATU, DMF, 80 °C, μ-wave, 20 min, 91%; 11. 25% TFA in $CH_2Cl_2$, rt, 90 min; 12. ethyl 4-bromobutyrate, $K_2CO_3$, DMF, 85 °C, 18 h; 13. HCl/MeOH, reflux, 21 h, 53% (3 steps).

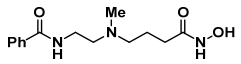
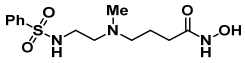
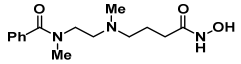
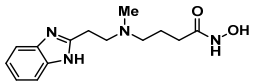
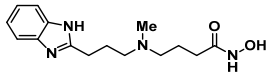
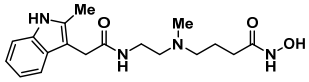
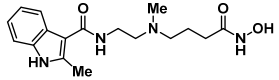
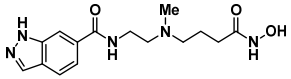
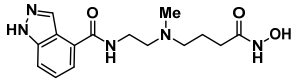
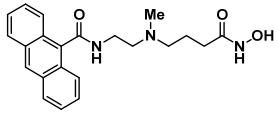
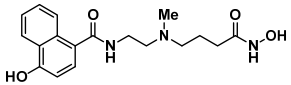
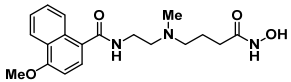
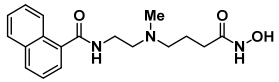
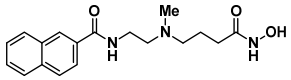
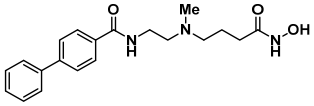
## Table S1. $pIC_{50}$ values[a] of aza-SAHA derivatives

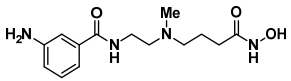
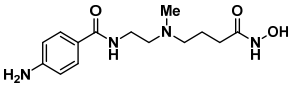
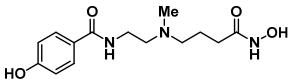
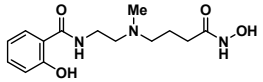
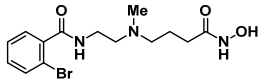
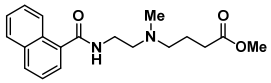
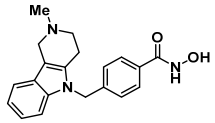
| Compound | Structure | HDAC1[b] | HDAC2[b] | HDAC3[b] | HDAC6[b] | HDAC8[b] | HDAC10[c] | HDAC10/6 selectivity | HDAC10/1 selectivity |
|---|---|---|---|---|---|---|---|---|---|
| **SAHA** |  | 7.30[d] (7.25–7.36) | 7.02 (6.97–7.07) | 6.59[d] (6.55–6.63) | 7.72[d] (7.58–7.92) | 6.16[d] (6.11–6.21) | 6.93[d] (6.85–7.02) | 0.16 | 0.43 |
| **12** |  | <4.00 | <<4.00[e] | <<4.00[e] | 4.54 (4.46–4.65) | 4.74 (4.66–4.81) | 5.65 (5.44–5.83) | 13 | 117 |
| **DKFZ-711** |  | 4.68 (4.59–4.75) | 4.13 (2.20–??) | 4.11 (1.99–??) | 5.44 (5.38–5.51) | 5.27 (5.19–5.34) | 7.48[d] (7.38–7.59) | 108 | 624 |
| **14** |  | 6.21 (6.18–6.24) | 5.74 (5.69–5.80) | 5.36 (5.30–5.42) | 6.50 (6.40–6.60) | 5.86 (5.78–5.94) | 7.05 (6.93–7.16) | 3.6 | 6.8 |
| **15** |  | 7.09 (7.06–7.12) | 6.88 (6.84–6.93) | 6.06 (6.04–6.26) | 7.32 (7.24–7.40) | 6.08 (6.00–6.16) | 8.73 (8.54–9.05) | 26 | 44 |

[a] Determined by four-parameter dose-response fit from two separate experiments each with eight dose levels in triplicates, reported as the mean, with 95% confidence intervals in parenthesis. [b] HDAC Glo assay. [c] FRET assay. [d] Mean of three separate experiments. [e] The non-linear regression did not converge at a maximum dose of 100 μM. "??" indicates that confidence intervals could not be calculated for low activity compounds.

## Table S2. $pIC_{50}$ values[a] of γ-amino hydroxamic acids and reference compounds

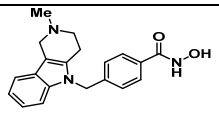
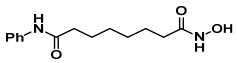
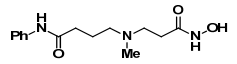
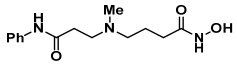
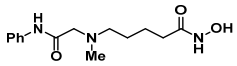
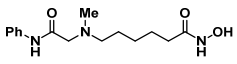
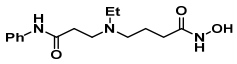
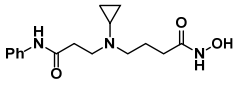
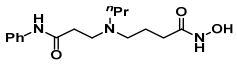
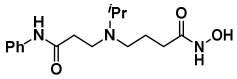
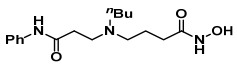
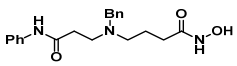
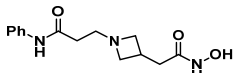
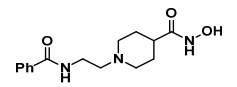
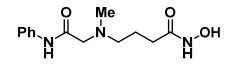
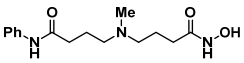
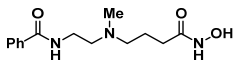
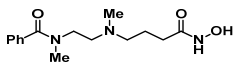
| Compound | Structure | HDAC1[b] | HDAC2[b] | HDAC3[b] | HDAC6[b] | HDAC8[b] | HDAC10[c] | SF 10/6 | SF 10/1 | LE |
|---|---|---|---|---|---|---|---|---|---|---|
| **DKFZ-711** |  | 4.68 (4.59–4.75) | 4.13 (2.20–??) | 4.11 (1.99–??) | 5.44 (5.38–5.51) | 5.27 (5.19–5.34) | 7.48[d] (7.38–7.59) | 108 | 624 | 0.51 |
| **14** |  | 6.21 (6.18–6.24) | 5.74 (5.69–5.80) | 5.36 (5.30–5.42) | 6.50 (6.40–6.60) | 5.86 (5.78–5.94) | 7.05 (6.93–7.16) | 3.6 | 6.8 | 0.48 |
| **15** |  | 7.09 (7.06–7.12) | 6.88 (6.84–6.93) | 6.06 (6.04–6.26) | 7.32 (7.24–7.40) | 6.08 (6.00–6.16) | 8.73 (8.54–9.05) | 26 | 44 | 0.57 |
| **16** |  | 4.30 (4.27–4.36) | 4.13 (??–??) | <4 | 5.26 (5.14–5.36) | 5.07 (5.00–5.14) | 6.93 (6.81–7.05) | 47 | 425 | 0.45 |
| **17** |  | 4.41 (4.35–4.53) | <4 (??–4.23) | <4 | 5.38 (5.26–5.50) | 5.58 (5.49–5.67) | 6.69 (6.61–6.77) | 20 | 188 | 0.42 |
| **18** |  | 4.09 (4.05–4.22) | <4 (??–4.01) | <4 | 5.11 (4.94–5.25) | 5.00 (4.89–5.10) | 6.56 (6.50–6.63) | 29 | 294 | 0.41 |
| **19** |  | <4 | <4 | <<4[e] | 4.68 (4.60–4.75) | 5.14 (5.05–5.22) | 6.44 (6.31–6.56) | 57 | 645 | 0.40 |
| **20** |  | 4.09 (??–4.22) | <4 (??–4.01) | <4 | 5.29 (5.23–5.34) | 5.05 (4.94–5.14) | 6.37 (6.28–6.44) | 12 | 188 | 0.38 |
| **21** |  | 4.63 (4.56–4.69) | 4.21 (0.00–4.54) | <4 | 5.98 (5.90–6.06) | 5.65 (5.55–5.76) | 5.85 (5.54–5.94) | 0.74 | 16 | 0.31 |
| **22** |  | 4.64 (4.61–4.67) | 4.46 (4.43–4.46) | <4 | 5.36 (5.30–5.42) | 5.20 (5.13–5.28) | 5.84 (5.75–5.92) | 3.0 | 16 | 0.33 |
| **23** |  | 4.69 (4.51–4.73) | 4.25 (0.00–4.64) | <4 | 5.60 (5.37–5.76) | 4.84 (4.77–??) | 6.25 (6.08–6.38) | 4.4 | 36 | 0.43 |
| **24** |  | 4.78 (4.73–4.82) | 4.57 (4.52–4.57) | <4 | 5.68 (5.55–5.82) | 5.05 (4.97–5.11) | 6.89 (6.79–6.99) | 16 | 129 | 0.47 |
| **25** |  | 4.24 (4.07–4.34) | 4.05 (3.85–4.08) | <<4[e] | 5.05 (3.99–5.28) | 4.67 (??–4.83) | 6.87 (6.76–7.00) | 66 | 423 | 0.47 |
| **26** |  | <4 | <4 | <<4[e] | 5.04 (4.99–5.10) | 5.08 (4.96–5.19) | 7.06 (6.96–7.16) | 105 | 1998 | 0.46 |
| **27** |  | 4.70 (4.57–4.79) | 4.55 (4.46–4.59) | <4 | 6.05 (5.95–6.17) | 5.48 (5.41–5.56) | 7.39 (7.28–7.49) | 21 | 483 | 0.53 |
| **28** |  | 4.29 (4.21–4.33) | <4 | <<4[e] | 4.65 (4.59–4.71) | 4.61 (4.44–??) | 8.08 (7.96–8.21) | 2679 | 6173 | 0.53 |

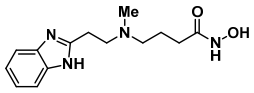
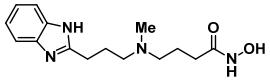
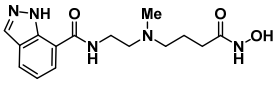
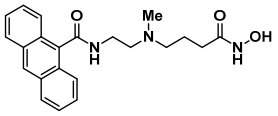
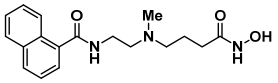
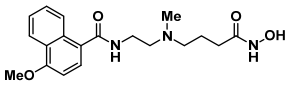
| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **DKFZ-728** |  | 4.50[d] (4.46–4.59) | 4.07[d] (3.90–4.43) | ≪4[de] | 5.02[d] (4.74–5.22) | 4.96[d] (4.76–5.09) | 7.97[d] (7.90–8.10) | 889 | 2919 | 0.55 |
| **30** |  | 4.50 (4.40–4.55) | 4.06 (0.00–4.38) | <<4[e] | 5.30 (5.03–5.45) | 5.14 (4.99–5.28) | 7.44 (7.25–7.61) | 137 | 865 | 0.49 |
| **31** |  | 4.19 (4.11–4.20) | 4.00 (0.00–4.16) | <<4[e] | 5.03 (4.92–5.14) | 4.80 (4.74–4.86) | 6.86 (6.72–6.99) | 66 | 460 | 0.45 |
| **32** |  | 4.92 (4.87–4.96) | 4.67 (4.62–4.71) | <4 | 5.74 (5.56–5.91) | 5.23 (5.15–5.30) | 8.08 (7.98–8.18) | 219 | 1452 | 0.55 |
| **33** |  | 4.39 (4.32–4.37) | 4.26 (4.16–4.35) | <<4[e] | 4.79 (4.69–4.88) | 4.79 (4.74–4.84) | 8.09 (7.94–8.23) | 1970 | 4966 | 0.53 |
| **34** |  | 4.98 (4.94–5.02) | 4.35 (??–4.83) | <<4[e] | 5.43 (5.20–5.58) | 5.53 (5.43–5.62) | 7.22 (7.07–7.38) | 63 | 173 | 0.40 |
| **35** |  | 5.13 (5.07–5.19) | 4.52 (2.51–??) | 4.05 (1.93–??) | 5.82 (5.72–5.90) | 5.32 (5.22–5.42) | 7.68 (7.53–7.82) | 72 | 352 | 0.44 |
| **36** |  | 5.00 (4.94–5.05) | 4.43 (0.00–4.76) | <4 | 5.44 (5.24–5.72) | 4.95 (4.84–5.06) | 8.12 (7.98–8.26) | 481 | 1323 | 0.48 |
| **37** |  | 4.91 (4.88–4.94) | 4.34 (0.00–4.68) | <4 | 5.44 (5.28–5.60) | 5.08 (4.98–5.18) | 8.17 (7.98–8.38) | 531 | 1789 | 0.49 |
| **38** |  | 5.70 (5.67–5.74) | 5.58 (5.53–5.62) | 4.60 (4.49–4.67) | 6.05 (5.99–6.12) | 5.93 (5.79–6.06) | 8.47 (8.29–8.69) | 263 | 597 | 0.41 |
| **39** |  | 5.02 (4.99–5.07) | 4.82 (4.76–4.87) | <4 | 5.88 (5.80–5.97) | 5.98 (5.88–6.07) | 8.19 (8.03–8.34) | 201 | 1460 | 0.45 |
| **40** |  | 5.22 (5.16–5.27) | 5.01 (4.93–5.08) | <4 (??–4.10) | 5.71 (5.54–5.86) | 5.64 (5.54–5.74) | 8.34 (8.18–8.52) | 426 | 1324 | 0.44 |
| **DKFZ-748** |  | 4.89 (4.86–4.93) | 4.29 (2.35–4.76) | <4 | 5.49 (5.28–5.65) | 5.87 (5.81–5.94) | 8.29[d] (8.12–8.56) | 636 | 2497 | 0.47 |
| **42** |  | 5.63 (??–5.66) | 5.39 (5.29–5.47) | 4.38 (4.12–4.42) | 5.81 (5.75–5.88) | 5.70 (5.60–5.79) | 8.22 (8.11–8.33) | 256 | 391 | 0.47 |
| **43** |  | 5.58 (5.55–5.61) | 4.95 (??–5.52) | 4.44 (4.36–4.49) | 5.52 (5.44–5.60) | 5.15 (5.06–5.21) | 7.90 (7.68–8.12) | 239 | 208 | 0.42 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **44** |  | 4.50 (4.42–4.56) | <4 | ≪4[e] | 5.23 (??–5.49) | 4.77 (3.70–4.94) | 7.62 (7.44–7.82) | 246 | 1315 | 0.50 |
| **45** |  | 4.78 (4.75–4.81) | 4.26 (2.30–??) | <4 | 5.47 (5.39–5.54) | 4.63 (4.56–4.71) | 7.81 (7.68–7.94) | 219 | 1060 | 0.51 |
| **46** |  | 4.75 (4.71–4.78) | 4.23 (2.30–??) | <4 | 5.84 (5.79–5.90) | 4.65 (4.56–4.75) | 8.05 (7.92–8.19) | 161 | 1998 | 0.53 |
| **47** |  | 4.12 (3.88–4.16) | <4 (0.00–4.06) | ≪4[e] | 4.92 (4.74–??) | 4.19 (4.10–4.31) | 7.41 (7.30–7.53) | 313 | 1986 | 0.48 |
| **48** |  | 4.50 (4.30–4.61) | <4 (0.00–4.40) | ≪4[e] | 5.26 (5.15–5.37) | 5.03 (4.92–5.13) | 7.76 (7.65–7.87) | 314 | 1822 | 0.51 |
| **49** |  | ≪4[e] | ≪4[e] | ≪4[e] | <4 | <4 | ≪4[e] | – | – | – |
| **tubastatin A (1)** |  | 5.66 (5.61–5.71) | 4.78 (??–4.82) | 4.76 (0.00–??) | 7.88 (7.69–8.38) | 5.84 (5.75–5.93) | 8.28[d] (8.11–8.44) | 2.5 | 416 | 0.45 |

[a] Determined by four-parameter dose-response fit from two separate experiments each with eight dose levels in triplicates, reported as the mean, with 95% confidence intervals in parenthesis. [b] HDAC Glo assay. [c] FRET assay. [d] Mean of at least three separate experiments. [e] Curve fit analysis not convergent at 100 μM. SF (selectivity factor) = $10^{(pIC50(HDAC10) - pIC50(HDAC6\ or\ 1))}$. LE (ligand efficiency) = 1.37 x $pIC_{50}$(HDAC10) / number of heavy atoms.[1] 95% confidence intervals of dose-response fits are shown in parenthesis. "??" indicates that confidence intervals could not be calculated for low activity compounds.

## Table S3. HDAC10 and 6 BRET $pIC_{50}$ values[a] of selected inhibitors and reference compounds

| Compound | Structure | HDAC6[b] | HDAC10[b] | SF 10/6 | LE | HDAC10 BRET/FRET activity |
|---|---|---|---|---|---|---|
| **tubastatin A** |  | 7.16 (6.89–7.41) | 8.04 (7.80–8.15) | 8 | 0.44 | 58% |
| **SAHA** |  | 7.13[c] (6.96–7.31) | 6.41[c] (6.14–6.68) | 0.19 | 0.46 | 30% |
| **12** |  | 2.65 (1.13–??) | 4.63 (4.02–5.01) | 96 | 0.32 | 11% |
| **DKFZ-711** |  | 4.41[d] (??–4.79) | 6.73[d] (6.56–6.88) | 208 | 0.46 | 18% |
| **14** |  | 5.73 (5.18–6.00) | 6.53 (6.36–6.68) | 6.3 | 0.45 | 40% |
| **15** |  | 6.66 (6.53–6.79) | 8.30 (8.02–8.71) | 44 | 0.54 | 35% |
| **16** |  | <4.4 | 6.13 (5.85–6.35) | >54 | 0.40 | 24% |
| **17** |  | <4.4 | 6.44 (6.26–6.60) | >109 | 0.40 | 66% |
| **18** |  | ≪4.4[e] | 6.06 (5.87–6.21) | >45 | 0.38 | 48% |
| **19** |  | ≪4.4[e] | 5.73 (5.08–6.04) | >21 | 0.36 | 37% |
| **20** |  | 4.40 (3.46–5.39) | 5.80 (5.58–5.98) | 25 | 0.35 | 48% |
| **21** |  | 4.79 (??–5.41) | 6.34 (6.16–6.50) | 35 | 0.33 | 551% |
| **25** |  | <4.4 | 5.70 (4.99–6.03) | >20 | 0.39 | 7% |
| **26** |  | <4.4 | 6.47 (6.22–6.73) | >117 | 0.42 | 27% |
| **27** |  | 5.50 (4.02–5.84) | 6.53 (6.32–6.73) | 11 | 0.47 | 10% |
| **28** |  | <4.4 | 6.89 (6.52–7.23) | >305 | 0.45 | 5% |
| **DKFZ-728** |  | <4.4[d] | 6.93[d] (6.79–7.06) | >338 | 0.47 | 9% |
| **31** |  | 4.00 (3.34–4.37) | 6.31 (5.97–6.70) | 81 | 0.41 | 22% |

| | | | | | | |
|---|---|---|---|---|---|---|
| **32** |  | 4.43 (4.18–5.12) | 6.93 (6.68–7.15) | 315 | 0.47 | 6% |
| **33** |  | <4.4 | 6.62 (6.53–6.72) | 167 | 0.43 | 4% |
| **37** |  | 4.55 (??–4.76) | 7.18 (6.95–7.38) | 422 | 0.43 | 12% |
| **38** |  | 5.41 (5.26–5.57) | 7.91 (7.70–8.12) | 316 | 0.39 | 22% |
| **DKFZ-748** |  | 4.84 (??–5.02) | 7.66 (7.47–7.83) | 662 | 0.44 | 23% |
| **40** |  | 4.92 (0.00–??) | 7.52 (7.24–7.79) | 397 | 0.40 | 10% |

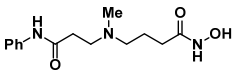
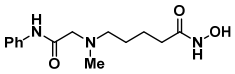
[a] Determined from pooled data of two separate BRET assay[b] experiments by four-parameter dose-response fit, each with ten dose levels in triplicates. [c] Data pooled from six separate experiments. [d] Data pooled from four separate experiments. [e] Not convergent at 40 µM. SF (selectivity factor) = $10^{(pIC50(HDAC10) - pIC50(HDAC6))}$. LE (ligand efficiency) = 1.37 x $pIC_{50}$(HDAC10) / number of heavy atoms.[1] 95% confidence intervals of dose-response fits are shown in parenthesis. "??" indicates that confidence intervals could not be calculated for low activity compounds.

## Table S4. Pharmacokinetic profile of selected HDAC10i

| Compound | MDR1-MDCKII permeability[a] | | | Mouse liver microsomal stability | | Mouse plasma stability[d] | Mouse plasma binding[e] | |
|---|---|---|---|---|---|---|---|---|
| | A to B | B to A | efflux ratio[b] | $t_{1/2}$ | $CL_{int(mic)}$[c] | $t_{1/2}$ | bound | recovery |
| **17** | 1.00 | 7.93 | 7.91 | >145 min | <9.6 | >289.1 min | 52% | 66% |
| **DKFZ-728** | 0.56 | 1.12 | 2.00 | 74.0 min | 18.7 | >289.1 min | 4.8% | 86% |
| **DKFZ-748** | 0.55 | 2.97 | 5.43 | 59.3 min | 23.4 | >289.1 min | 48% | 88% |

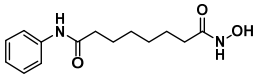
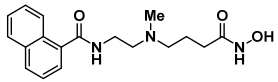
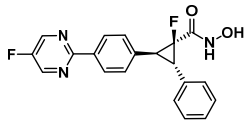
[a] MDR1-MDCKII permeability as mean apparent permeability coefficient $P_{app}$ in $10^{-6}$ cm/s. A: apical, B: basolateral membrane. [b] efflux ratio = $P_{app}$(B to A)/$P_{app}$(A to B). [c] intrinsic clearance in µL/min/mg, normalized on amount of microsomal protein. [d] no time-dependent compound decomposition observed after 120 min. [e] determined by high-throughput equilibrium dialysis of CD-1 mouse plasma (donor side) vs. buffer (acceptor side).

## Table S5. MMP-13 and MMP-9 inhibitory activity of selected aza-SAHA derivatives

| Compound | Structure | MMP-13 Inhibition[a] at 100 µM | MMP-9 Inhibition[a] at 100 µM |
|---|---|---|---|
| **DKFZ-711** |  | 6.5% | 6.9% |
| **14** |  | 11.3% | 0% |

[a] Percent inhibition of matrix metalloproteinases (MMPs) at 100 µM concentration of the test compounds. Assays were performed in triplicate as described previously[2,3].

**Table S6. Apparent p$K_D$ values[a] ± standard deviation of DKFZ-748 and reference compounds by chemical proteomics profiling.**

| Compound | Structure | HDAC1 | HDAC2 | HDAC5 | HDAC6 | HDAC8 | HDAC10 | ISOC1 | ISOC2 | ALDH1B1 | ALDH2 | GAT3DA | MBLAC2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **SAHA** |  | 6.1 ± 0.1 | 5.9 ± 0.1 | <4 | 6.4 ± 0.2 | <4 | 5.7 ± 0.6 | 6.6 ± 0.2 | 7.1 ± 0.2 | 5.8 ± 0.2 | 6.0 ± 0.1 | 5.6 ± 0.6 | 4.7 ± 0.1 |
| **DKFZ-748** |  | <4 | <4 | <4 | <4 | <4 | 6.8 ± 0.6 | 4.4 ± 0.3 | 4.9 ± 0.1 | <4 | <4 | <4 | <4 |
| **CHDI00465983**[b] |  | <4 | <4 | 6.2 ± 0.2 | <4 | ~4.8 | <4 | <4 | <4 | <4 | <4 | <4 | 6.8 ± 0.1 |

[a] Determined by LC-MS/MS after dose-dependent competition pulldown assay from three or two[b] separate experiments, each performed with eleven dose levels. Reported as mean p$K_D$ value ± standard deviation.

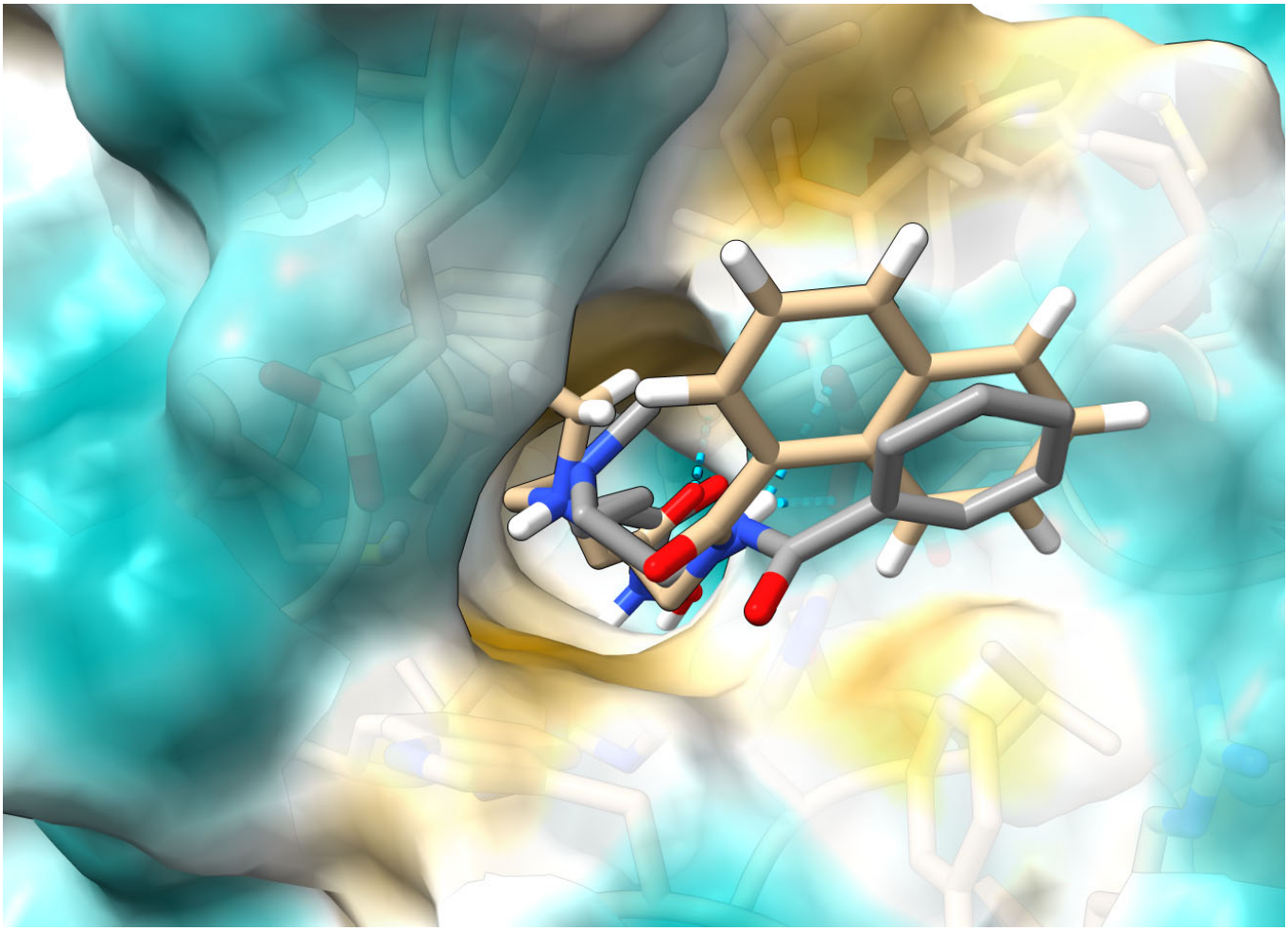


**Figure S1**. Representative pose of **DKFZ-748** (in ochre) docked into the crystal structure of the HDAC10–**DKFZ-728** complex. **DKFZ-728** position from crystal structure shown in gray. Surface colors: ochre: hydrophobic, turquois: hydrophilic. Methylene hydrogens of the docked ligand are omitted for clarity. Hydrogen bonds to **DKFZ-748** are depicted as dotted lines. For docking model coordinates, see SI file: HDAC10-728_complex_docked_748.pdb

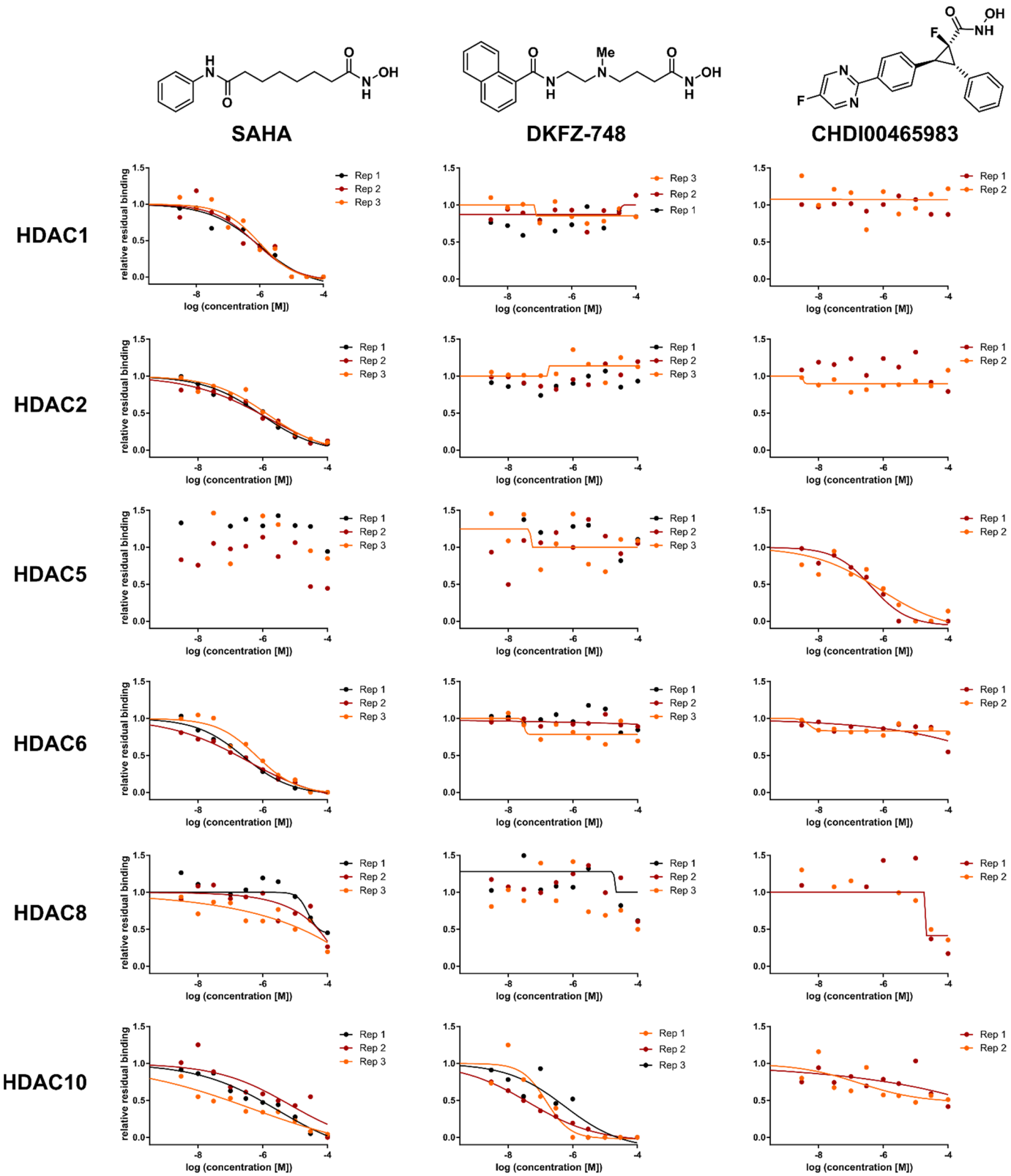



**Figure S2**. Continued on next page.

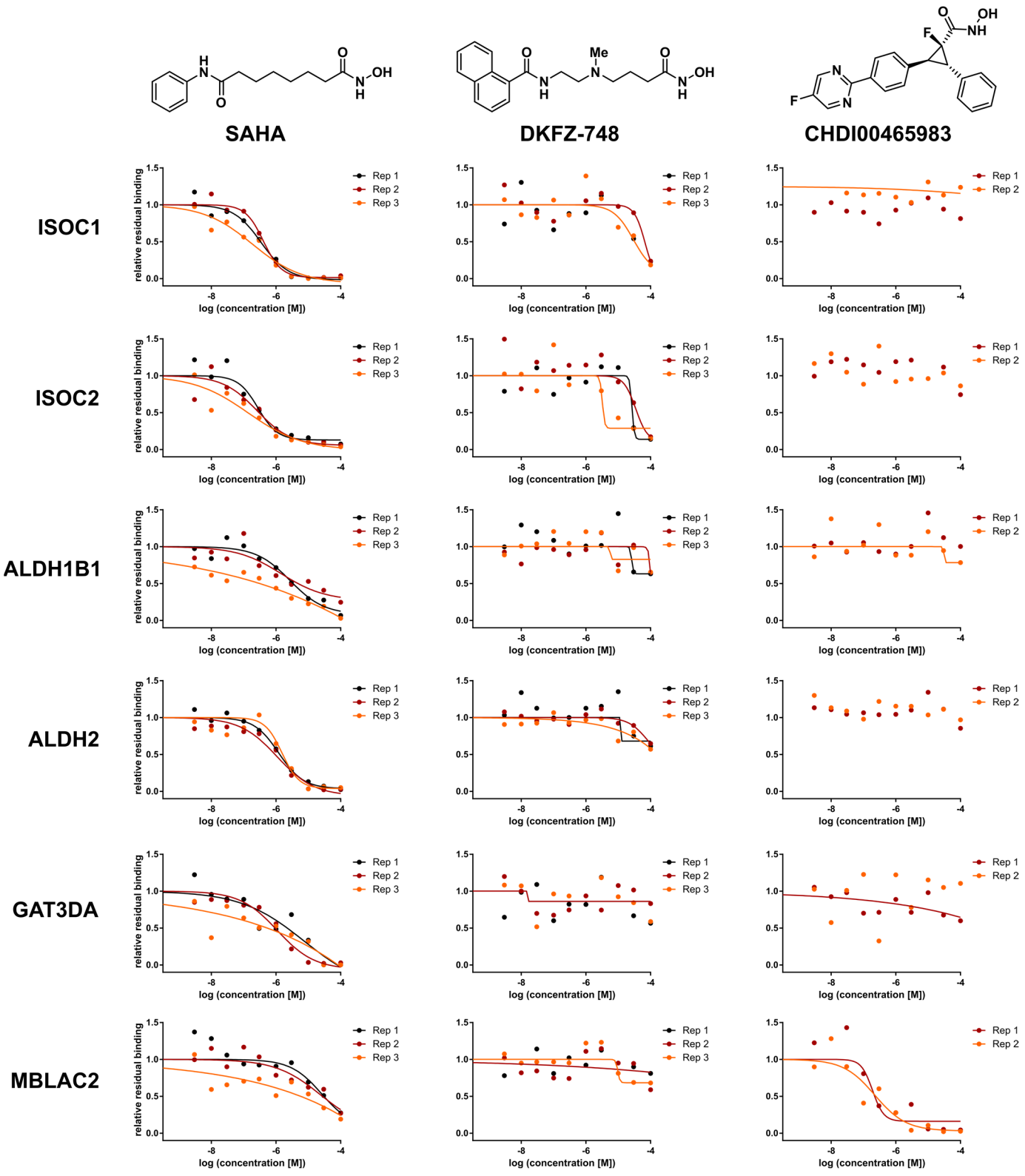



**Figure S2 (continuation).** Dose-response binding curves of SAHA, **DKFZ-748** and CHDI00465983 determined in BE(2)-C cell lysates by LC-MS/MS competition pulldown assay in 11 dose-levels. Rep 1, Rep 2, Rep 3 represent three independent experiments.

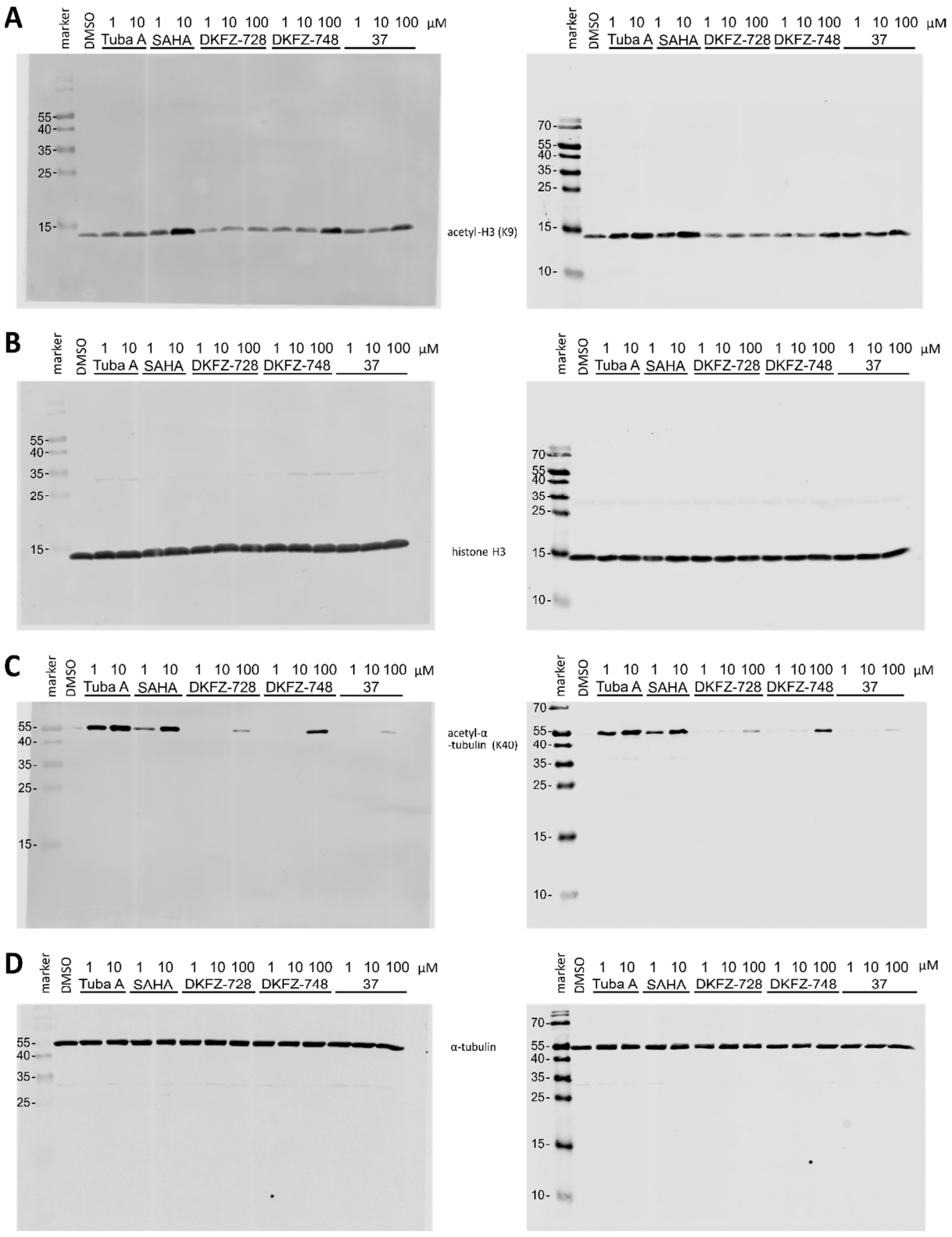



**Figure S3**. Uncropped Western blots of two independent biological replicates of BE(2)-C cells treated with inhibitors. A: acetyl-histone H3 (K9). B: histone H3. C: acetyl-α-tubulin (K40). D: α-tubulin.

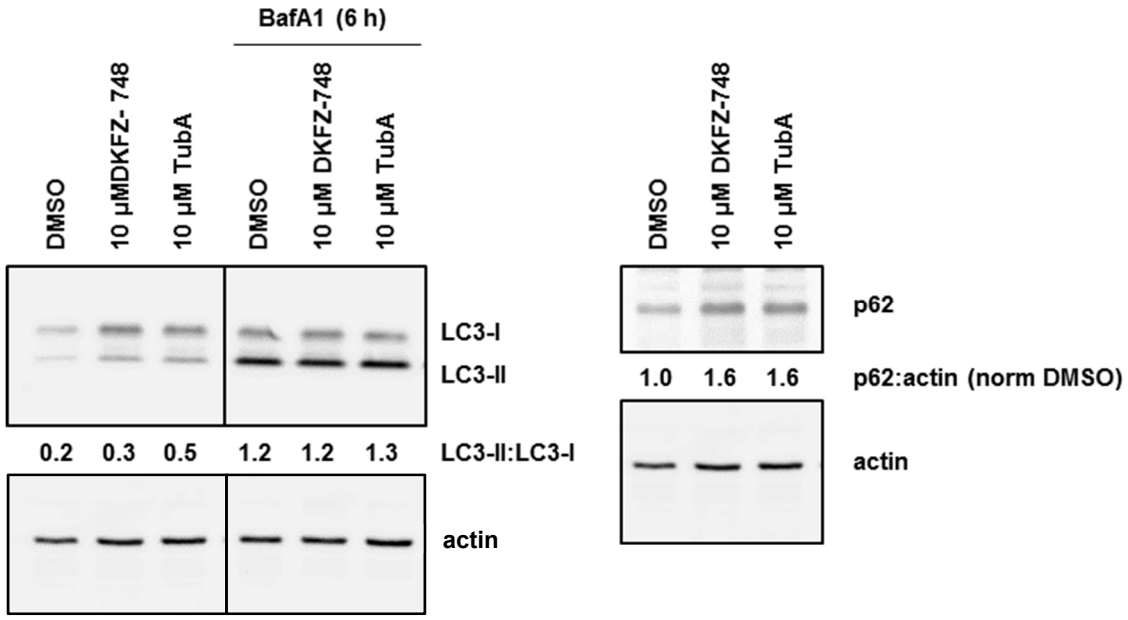



**Figure S4**. Western blots against autophagy markers LC3-I/II and p62 of BE(2)-C cells treated with the selective HDAC10i **DKFZ-748**, HDAC6/10i tubastatin A (TubaA) and late-stage autophagic flux inhibitor bafilomycin A1 (BafA1). Bands were quantified and ratios are shown below the blots. An increase in the LC3-II/I ratio is an indication for blocked autophagy. If the LC3-II/I ratio increases although autophagic flux is blocked by BafA1, this indicates induction of autophagy. An increase in p62 levels indicates that autophagy is blocked. All autophagy markers were visualized on the same blot using different antibodies. Therefore, the actin controls of the three leftmost lanes are identical in the two images. Representative blot of technical replicates.

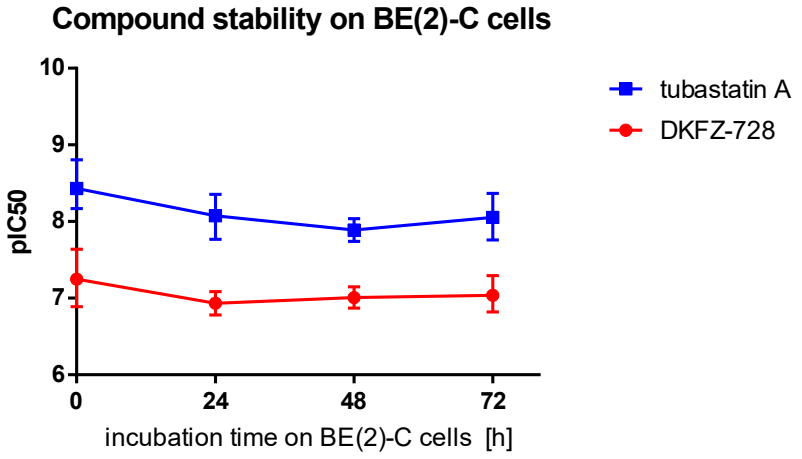



**Figure S5**. Cellular compound stability determined by HDAC10 BRET assay using test compounds diluted in medium and incubated on BE(2)-C cells for 0, 24, 48, or 72 h. Each $pIC_{50}$ was determined from a separate dose-response experiment with eight dose-levels performed in triplicates. $pIC_{50}$s are represented with 95% confidence intervals.

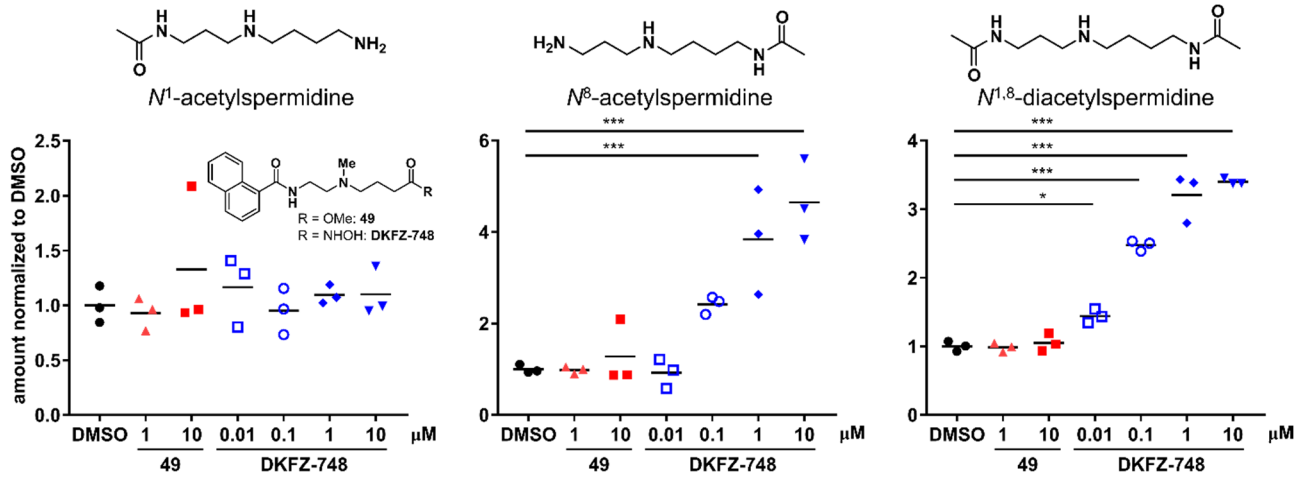



**Figure S6**. Biological replicate of data in Figure 8. Targeted metabolomics quantification of $N^1$-acetylspermidine, $N^8$-acetylspermidine and $N^{1,8}$-diacetylspermidine in BE(2)-C cells after 24 h of inhibitor treatment. Significance of inhibitor treatment was calculated between DMSO controls and samples by one-way ANOVA, following the Dunnett test for multiple comparison. *: p-value < 0.05, ***: <0.001.

# Compound synthesis

## General information for compound synthesis

Chemicals and solvents were purchased from commercial sources (Sigma-Aldrich, Merck, Acros Organics, VWR, Roth, Alfa Aesar, Th. Geyer, Fisher Chemical and abcr) at the highest level of purity and used without purification unless stated otherwise. Anhydrous tetrahydrofuran was dispensed with an MBraun SPS800 Solvent Purification System. All reactions were stirred magnetically and external bath temperatures are reported for heating.

**Thin layer chromatography (TLC)** was carried out on glass silica plates (TLC Silica gel 60 $F_{254}$; Merck). TLC visualization was accomplished using 254 and 366 nm UV light, iodine saturated silica gel ($I_2$), ninhydrin or permanganate stain.

**High resolution mass spectrometry** was recorded on a Bruker ApexQe FT-ICR instrument, (Department of Organic Chemistry, Heidelberg University).

**NMR** spectra were recorded on Bruker Avance 14.1 T and Avance III 9.4 T (German Cancer Research Center) or Bruker Avance III 14.1 T (Department of Organic Chemistry, Heidelberg University) NMR spectrometers operating at 600 or 400 MHz for $^1$H nuclei and 150 and 100 MHz for $^{13}$C nuclei. Spectra were recorded at 298.1 K.

All final compounds were found to have ≥95% purity, controlled by analytical HPLC/UV/ELSD/MS with the below described methods, and confirmed by $^1$H NMR and $^{13}$C NMR.

## Analytical HPLC-methods

Analytical HPLC/MS was performed on an Agilent 1260 Infinity system equipped with a 6120 Quadrupole mass detector and evaporative light scattering detector (ELSD).

| method name | column | solvent system | gradient |
|---|---|---|---|
| Acidic Method | Kinetex 2.6 µm C18 100 Å, LC column 50 x 2.1 mm; 40 °C; flow rate: 0.06 mL/min | A: $H_2O$, 0.01% HCOOH<br>B: MeCN | 1% B → 90% B over 6 min, then 90% B to 99% B over 2 min |
| Basic Method | Gemini 5 µm C18 110 Å, LC column 50 x 2 mm; 40 °C; flow rate: 0.06 mL/min | A: $H_2O$, 0.1% $NH_4OH$ (25% aqueous solution);<br>B: MeCN | 1% B → 90% B over 6 min, then 90% B to 99% B over 2 min |

UV-detection at 254 and 230 nm

## Medium pressure column chromatography (MPLC)

MPLC was performed in normal or reverse phase (RP) with a RediSep Rf system (Teledyne Isco) and RediSep Rf columns (Teledyne Isco). For RP purification, solvent A was $H_2O$ and B was MeCN. Normal phase separations used eluents as described.

## Preparative HPLC methods

Preparative HPLC was performed on an Agilent 1260 Infinity system.

| method name | column | solvent system |
|---|---|---|
| Acidic Method | Kinetex 5 µm C18 100 Å, AXIA packed column 250 x 21.2 mm; 20 °C; flow rate: 15.0 mL/min | A: $H_2O$, 0.05% TFA;<br>B: MeCN |
| Basic Method | Gemini 5 µm C18 110 Å, AXIA packed column 250 x 21.2 mm; 20 °C; flow rate: 15.0 mL/min | A: $H_2O$, 0.1% $NH_3$;<br>B: MeCN |

UV-detection at 254 and 230 nm

Water was removed by lyophilization with a Christ alpha 2-4 LD plus freeze-dryer.

## General Procedure A: preparation of hydroxamic acids with hydroxylamine

To a stirred solution of “methyl ester” (typically 50–200 mg, 1.0 equiv) in 1,4-dioxane (0.5–2 mL), was added KCN (0.6 equiv) and the resulting mixture was stirred at rt for 1 h. Then an aqueous solution of hydroxylamine (50%, 0.5–2 mL, minimum of 30 equiv) was added. The solution was stirred at rt for up to 24 hours, until TLC indicated complete conversion. The reaction mixture was concentrated in vacuo,

re-concentrated from MeOH to dryness, and then dissolved in a minimal volume of water/MeOH. Purification was carried out by preparative RP-HPLC or RP-MPLC.

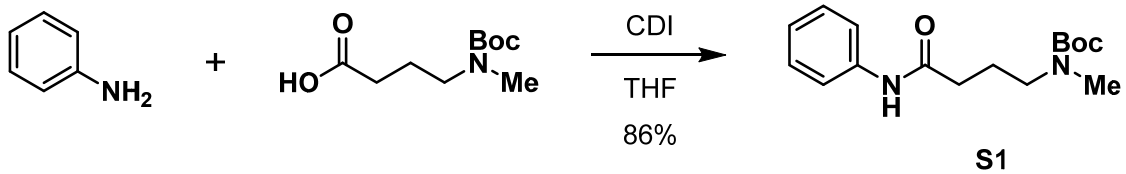
## General Procedure B: amide coupling with EDC

To a suspension of diamine **S17**·2HCl (typically 80–200 mg, 1.0 equiv), "carboxylic acid" (1.1 equiv), HOBt (catalytic amounts; only for sterically hindered acids) and EDC (1.3 equiv) in $CH_2Cl_2$ (5–10 mL) was added *i*-$Pr_2NEt$ (3.5 equiv). The reaction mixture was stirred at rt for 12–48 h, until TLC indicated complete consumption of the amine, then concentrated in vacuo, redissolved in EtOAc (50 mL) and washed with saturated aqueous $NaHCO_3$ (2 × 40 mL), brine (40 mL), then dried ($MgSO_4$) and concentrated. The residue was used without further purification unless stated otherwise.

## General Procedure C: reductive amination and esterification of gamma-amino acids

To a suspension of amino acid **S4**·HCl (100–150 mg, 1.0 equiv) and $Na(OAc)_3BH$ (1.5 equiv) in anhydrous THF (2.0 mL) was added "aldehyde" or "ketone" (10 equiv) and $Et_3N$ (2.0 equiv) at 0 °C under nitrogen. After addition, the cooling bath was removed and the reaction mixture was stirred at rt for 2–3 h, then diluted with water (10 mL), basified with saturated aqueous $NaHCO_3$ to pH 12.0, then concentrated to dryness. The residue was re-dissolved in water (10 mL) and acidified with HCl (25%) to pH 1.0, then the solvent was removed in vacuo. MeOH (25 mL) was added to the residue, stirred for 12–18 h, and then concentrated. The residue was partitioned between saturated aqueous $NaHCO_3$ (30 mL) and $CH_2Cl_2$ (20 mL) and the layers were separated. The aqueous layer was then extracted with $CH_2Cl_2$ (2 × 20 mL). The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo.

## Synthesis procedures




**S1**

***tert*-Butyl methyl(4-oxo-4-(phenylamino)butyl)carbamate** (**S1**): To a suspension of carbonyldiimidazole (0.616 g, 3.80 mmol, 1.1 equiv) in THF (5 mL) was added *N*-Boc,*N*-MeGABA[4] (0.750 g, 3.45 mmol, 1.0 equiv). After 3 h of stirring at rt, aniline (0.299 mL, 3.28 mmol, 0.95 equiv) was added and the reaction mixture was stirred for 5 h at rt, then diluted with EtOAc (50 mL) and water (200 mL). The phases were separated and the aqueous layer was extracted with EtOAc (2 × 50 mL).

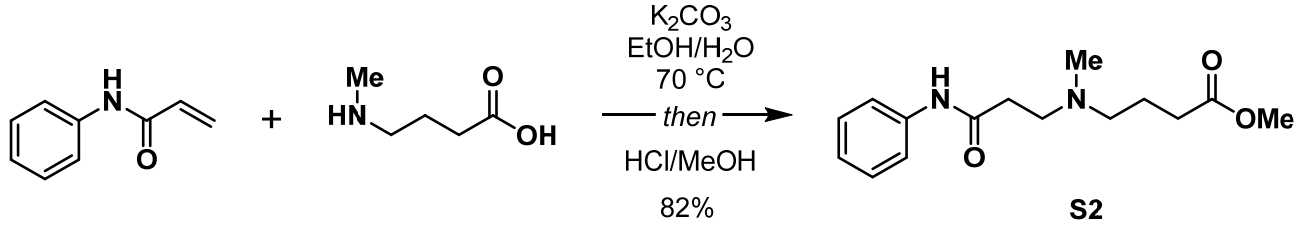
The combined organic layers were washed with ice cold 0.1 M HCl (2 × 100 mL), saturated aqueous $Na_2CO_3$ (100 mL), then dried ($MgSO_4$), filtered, and concentrated in vacuo to provide **S1** as a yellow oil, which solidified into an off-white solid (0.821 g, 2.81 mmol, 86% yield).

**TLC** $R_f$ 0.47 (10% MeOH in $CH_2Cl_2$)

**$^1$H NMR** (600 MHz, $CDCl_3$) δ 9.37 (br s, 1H), 7.64 (br s, 2H), 7.31 (t, *J* = 7.8 Hz, 2H), 7.07 (t, *J* = 7.5 Hz, 1H), 3.36 (br s, 2H), 2.86 (s, 3H), 2.31 (t, *J* = 6.3 Hz, 2H), 1.91 (app p, *J* = 6.5 Hz, 2H), 1.49 (s, 9H) ppm; $^1$H NMR-signals appeared very broad, likely caused by hindered bond rotation.

**$^{13}$C NMR** (151 MHz, $CDCl_3$) δ 171.5, 157.4, 138.9, 129.0, 123.8, 119.7, 80.3, 47.0, 34.6, 34.3, 28.6, 24.4 ppm

**LC/MS** (*m/z*): $[M+Na]^+$ calcd for $C_{16}H_{24}N_2NaO_3^+$: 315.2; found: 315.2




**S2**

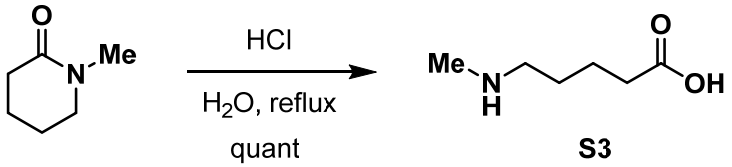
**Methyl 4-(methyl(3-oxo-3-(phenylamino)propyl)amino)butanoate** (**S2**): *N*-phenylacrylamide (2.00 g, 13.59 mmol, 1.0 equiv), *N*-MeGABA·HCl (2.30 g, 14.95 mmol, 1.1 equiv) and $K_2CO_3$ (3.76 g, 27.18 mmol, 2.0 equiv) were suspended in water/EtOH (1:1, 40 mL) and heated to 70 °C. After 7 h, the reaction mixture was cooled to rt, acidified to pH ~1 with 3 M HCl, and evaporated to dryness. The residue was suspended in MeOH (25 mL) and stirred at rt for 3 days, then concentrated in vacuo and purified by MPLC (80 g silica, gradient: 0 → 10% MeOH in $CH_2Cl_2$) to provide the HCl salt of **S2** as a yellow amorphous solid, which crystallized over time into a yellow/green solid (3.49 g, 11.09 mmol, 82% yield).

**TLC** $R_f$ 0.4 (10% MeOH in $CH_2Cl_2$)

**$^1$H NMR** (400 MHz, MeOD-$d_4$) δ 7.64 – 7.54 (m, 2H), 7.34 – 7.28 (m, 2H), 7.13 – 7.07 (m, 1H), 3.69 (s, 3H), 3.60 (br s, 1H), 3.45 (br s, 1H), 3.26 (br s, 2H), 2.96 (t, *J* = 6.6 Hz, 2H), 2.93 (s, 3H), 2.52 (t, *J* = 7.0 Hz, 2H), 2.13 – 2.02 (m, 2H) ppm

**$^{13}$C NMR** (101 MHz, MeOD-$d_4$) δ 172.3, 167.9, 137.4, 127.7, 123.3, 119.0, 54.8, 51.2, 50.2, 38.9, 29.1 ($2CH_2$), 18.3 ppm

**LC/MS** (*m/z*): $[M+H]^+$ calcd for $C_{15}H_{23}N_2O_3^+$: 279.2; found: 279.2




**S3**

**5-(Methylamino)pentanoic acid** (**S3**)[5]: A mixture of 1-methylpiperidin-2-one (1.95 g, 17.2 mmol), HCl (8 M, 16 mL) and water (4 mL) was heated to reflux for 18 h, then cooled to rt, diluted with $H_2O$ (20 mL), and concentrated in vacuo. The residue was then re-concentrated from water (20 mL) and then acetone (2 × 20 mL) to provide the HCl salt of **S3** as an off-white solid (3.02 g, quant. yield).

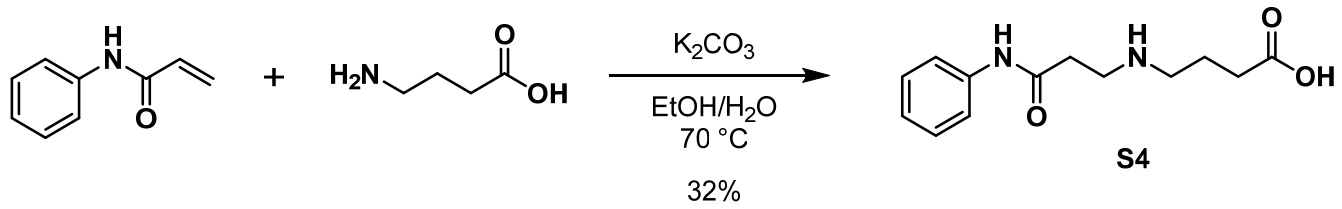
**TLC** $R_f$ 0.57 (20% MeOH and 0.5% $NH_4OH$ in $CH_2Cl_2$)

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 9.26 – 8.80 (m, 2H), 2.87 – 2.75 (m, 2H), 2.46 (t, $J$ = 5.5 Hz, 3H), 2.24 (t, $J$ = 7.1 Hz, 2H), 1.68 – 1.56 (m, 2H), 1.56 – 1.46 (m, 2H) ppm

**$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 174.1, 47.8, 33.1, 32.2, 24.8, 21.5 ppm

**LC/MS** (*m/z*): $[M+H]^+$ calcd for $C_6H_{14}NO_2^+$: 132.1; found: 132.2

Analytical data is in agreement with literature.[5]




**S4**

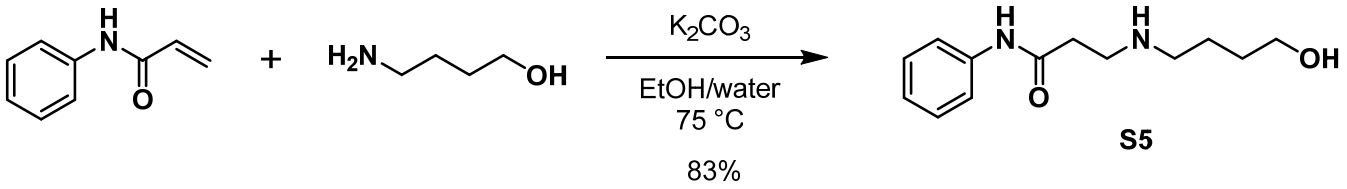
**4-((3-Oxo-3-(phenylamino)propyl)amino)butanoic acid** (**S4**): To a stirred solution of gamma-aminobutyric acid (GABA) (3.14 g, 29.90 mmol, 2.2 equiv) and $K_2CO_3$ (2.68 g, 19.40 mmol, 1.4 equiv) in water (27 mL)/EtOH (17 mL), was added dropwise a solution of *N*-phenylacrylamide (2.00 g, 13.59 mmol, 1.0 equiv) in EtOH (10 mL) at 70 °C. The reaction was stirred at 70 °C for 3.5 h, partially concentrated to remove EtOH and the remaining aqueous mixture was extracted with $CH_2Cl_2$ (5 × 20 mL). The organic layers were discarded and the aqueous phase was acidified with HCl, then evaporated to dryness to yield a white solid, which was recrystallized by dissolving in a minimal amount of water/MeOH at 50 °C and then concentrating under reduced pressure at 50 °C until precipitation was visible, followed by cooling to –20 °C overnight in a freezer. After melting the mixture at rt, crystals were filtered and washed once with ice cold water, then with acetone, to provide the HCl salt of **S4** as white crystals (1.239 g, 4.32 mmol, 32% yield).

**TLC** $R_f$ 0.05 (10% water in MeCN)

**$^1$H NMR** (400 MHz, MeOD-$d_4$) δ 7.61 – 7.55 (m, 2H), 7.34 – 7.27 (m, 2H), 7.15 – 7.05 (m, 1H), 3.36 (t, $J$ = 6.4 Hz, 2H), 3.18 – 3.09 (m, 2H), 2.87 (t, $J$ = 6.4 Hz, 2H), 2.49 (t, $J$ = 7.1 Hz, 2H), 2.00 (app p, $J$ = 7.1 Hz, 2H) ppm

**$^{13}$C NMR** (101 MHz, MeOD-$d_4$) δ 176.0, 170.1, 139.6, 129.8, 125.3, 121.1, 48.4, 44.8, 32.8, 31.6, 22.4 ppm

**LC/MS** (*m/z*): $[M+H]^+$ calcd for $C_{13}H_{19}N_2O_3^+$: 251.1; found: 251.2, $[M–H]^–$ calcd for $C_{13}H_{17}N_2O_3^–$: 249.1; found: 249.2



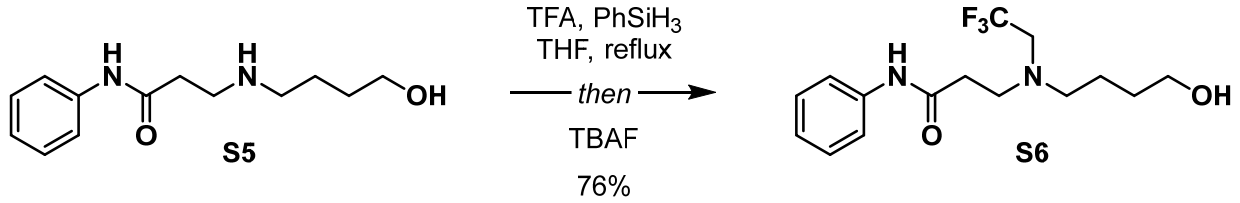

**3-((4-Hydroxybutyl)amino)-*N*-phenylpropanamide** (**S5**): To a stirred solution of 1,4-aminobutanol (1.576 g, 17.68 mmol, 2.2 equiv) and $K_2CO_3$ (1.666 g, 12.05 mmol, 1.5 equiv) in water (15 mL), was added dropwise a solution of *N*-phenylacrylamide (1.183 g, 8.036 mmol, 1.0 equiv) in EtOH (15 mL) at 75 °C. After 2 h, the reaction mixture was concentrated in vacuo until the clear solution became opaque. Saturated aqueous $Na_2CO_3$ (20 mL) was added and the mixture was extracted with EtOAc (2 × 25 mL). The combined organic layers were washed with saturated aqueous $Na_2CO_3$ (1 × 50 mL) then dried ($MgSO_4$), filtered, and concentrated. The product was purified by MPLC (40 g silica, gradient: 5 → 10% MeOH and 0.5% $NH_4OH$ in $CH_2Cl_2$ over 5 CV, 10 → 15% over 14 CV, then 15% → 30% over 8 CV) to provide **S5** as a slightly yellow oil (1.576 g, 6.67 mmol, 83% yield).

**TLC** $R_f$ 0.19 (20% MeOH and 0.5% $NH_4OH$ in $CH_2Cl_2$)

**$^1$H NMR** (400 MHz, $CDCl_3$) δ 9.66 (br s, 1H), 7.56 – 7.48 (m, 2H), 7.33 – 7.24 (m, 2H), 7.11 – 7.02 (m, 1H), 3.65 – 3.56 (m, 2H), 3.47 – 3.44 (m, 1H), 2.99 – 2.90 (m, 3H), 2.72 – 2.62 (m, 2H), 2.55 – 2.47 (m, 2H), 1.72 – 1.57 (m, 4H) ppm

**$^{13}$C NMR** (151 MHz, $CDCl_3$) δ 170.8, 138.5, 129.0, 124.0, 119.9, 62.5, 49.5, 45.2, 36.4, 31.7, 27.9 ppm

**LC/MS** (*m/z*): $[M+H]^+$ calcd for $C_{13}H_{21}N_2O_2^+$: 237.2; found: 237.2




**3-((4-Hydroxybutyl)(2,2,2-trifluoroethyl)amino)-*N*-phenylpropanamide** (**S6**): To a solution of **S5** (0.613 g, 2.594 mmol, 1.0 equiv) in dry THF (3 mL) was added $PhSiH_3$ (0.640 mL, 5.188 mmol, 2.0 equiv), then TFA (0.397 mL, 5.188 mmol, 2.0 equiv) at 70 °C under an argon atmosphere.[6] Vigorous bubbling ($H_2$ evolution) was immediately observed. The reaction mixture was heated to reflux for 4 h,

then concentrated in vacuo, redissolved in $CH_2Cl_2$ (50 mL), then quenched with 1 M NaOH (50 mL) and TBAF (2.60 mL, 1 M solution in THF, 1.0 equiv). The phases were separated and the organic layer was washed with 1 M NaOH (2 × 50 mL), dried ($MgSO_4$), filtered, and concentrated. The product was purified by FCC (160 g silica, eluent: 5% MeOH in $CH_2Cl_2$) to provide **S6** as a pale yellow oil (0.701 g, 1.982 mmol based on 90% purity, 76% yield).

**TLC** $R_f$ 0.28 (5% MeOH in $CH_2Cl_2$)

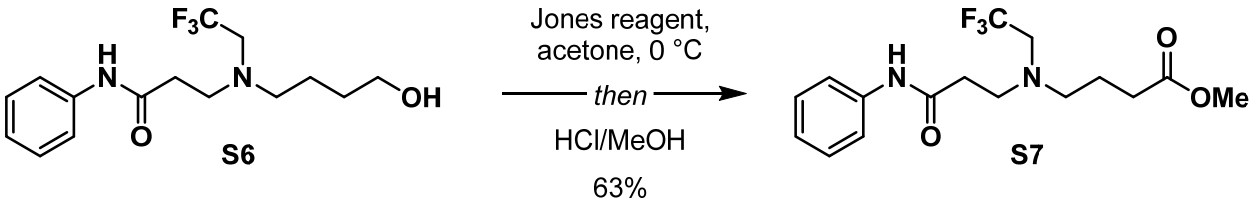
**$^1$H NMR** (400 MHz, $CDCl_3$) $\delta$ 9.17 (s, 1H), 7.56 – 7.47 (m, 2H), 7.36 – 7.27 (m, 2H), 7.15 – 7.03 (m, 1H), 3.62 (t, *J* = 5.9 Hz, 2H), 3.16 (app q, *J* = 9.4 Hz, 2H), 3.01 (t, *J* = 6.1 Hz, 2H), 2.76 (t, *J* = 7.2 Hz, 2H), 2.54 (t, *J* = 6.1 Hz, 2H), 2.16 (s, 1H), 1.68 – 1.50 (m, 4H) ppm

**$^{13}$C NMR** (151 MHz, $CDCl_3$) $\delta$ 170.0, 138.3, 129.1, 125.5 (q, *J* = 280 Hz), 124.2, 119.9, 62.4, 55.1 (q, *J* = 31 Hz), 54.9, 51.6, 34.9, 30.1, 23.2 ppm

**$^{19}$F NMR** (376 MHz, $CDCl_3$) $\delta$ –72.08 ppm

**LC/MS** (*m/z*): $[M+H]^+$ calcd for $C_{15}H_{22}F_3N_2O_2^+$: 319.2; found: 319.2

Note: The compound was obtained with 90% purity, but was sufficiently pure for the next reaction and was used without further purification.




**Methyl 4-((3-oxo-3-(phenylamino)propyl)(2,2,2-trifluoroethyl)amino)butanoate** (**S7**): To a stirred solution of **S6** (0.403 g, 1.138 mmol based on 90% purity, 1.0 equiv) in acetone (7 mL), cooled in an ice bath, was added Jones reagent (2.00 M $CrO_3$ and 3.16 M $H_2SO_4$ in water, 1.26 mL, 2.0 equiv) dropwise until conversion to the carboxylic acid was complete as indicated by TLC (~2.5 h). The reaction was carefully quenched by addition of MeOH (10 mL), then concentrated in vacuo, and re-dissolved in 1 M HCl in MeOH (50 mL). To this solution was added $MgSO_4$ (approx. 1 g) and the mixture stirred for 16 h. Upon complete esterification, solid $NaHCO_3$ was added until the reaction became neutral then the suspension was concentrated to dryness and redissolved in saturated aqueous $NaHCO_3$ (150 mL), then extracted with EtOAc (2 × 60 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (100 mL), dried ($MgSO_4$), filtered, and concentrated. The crude product was purified by MPLC (12 g silica, gradient: 20 → 60% EtOAc in hexane) to provide **S7** as a clear, brown oil (246.4 mg, 0.711 mmol, 63% yield).

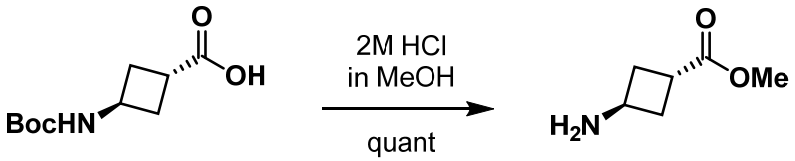
**TLC** $R_f$ 0.51 (60% EtOAc in hexane)

**$^1$H NMR** (400 MHz, $CDCl_3$) δ 9.03 (s, 1H), 7.56 – 7.47 (m, 2H), 7.33 – 7.27 (m, 2H), 7.11 – 7.05 (m, 1H), 3.65 (s, 3H), 3.13 (q, *J* = 9.4 Hz, 2H), 3.00 (t, *J* = 6.1 Hz, 2H), 2.73 (dd, *J* = 8.5, 6.3 Hz, 2H), 2.50 (t, *J* = 6.1 Hz, 2H), 2.35 (t, *J* = 7.0 Hz, 2H), 1.83 (app p, *J* = 7.0 Hz, 2H) ppm

**$^{13}$C NMR** (151 MHz, $CDCl_3$) δ 173.8, 170.1, 138.2, 129.1, 124.7 (q, *J* = 280.7 Hz), 124.2, 119.9, 55.2 (q, *J* = 30.9 Hz), 53.7, 51.9, 51.8, 35.3, 31.1, 21.9

**$^{19}$F NMR** (376 MHz, $CDCl_3$) δ –72.5 ppm

**LC/MS** (*m/z*): $[M+H]^+$ calcd for $C_{16}H_{22}F_3N_2O_3^+$: 347.2; found: 347.2, $[M+Na]^+$ calcd for $C_{16}H_{21}F_3N_2NaO_3^+$: 369.1; found: 369.2




**S8**

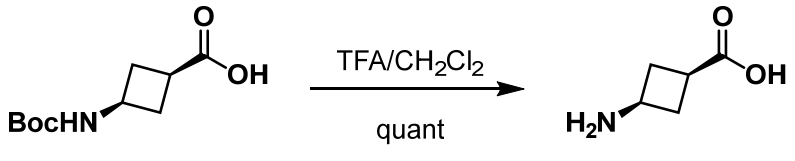
***trans*-Methyl 3-aminocyclobutanecarboxylate** (**S8**): *N*-Boc-*trans*-3-aminocyclobutane-1-carboxylic acid (0.600 g, 2.79 mmol, 1.0 equiv) was dissolved in 2 M HCl in MeOH (20 mL, 40 mmol, 14.4 equiv) and stirred at rt overnight, then concentrated to dryness and concentrated from MeOH to provide the HCl salt of **S8** as slightly yellow solid (0.483 g, quant yield).

**TLC** $R_f$ 0.18 (20% MeOH in $CH_2Cl_2$)

**$^1$H NMR** (400 MHz, MeOD-$d_4$) δ 3.99 – 3.87 (m, 1H), 3.72 (s, 3H), 3.30 – 3.22 (m, 1H), 2.67 – 2.58 (m, 2H), 2.52 – 2.42 (m, 2H) ppm

**$^{13}$C NMR** (151 MHz, MeOD-$d_4$) δ 176.5, 52.6, 45.2, 33.8, 30.9 ppm

**LC/MS** (*m/z*): $[M+H]^+$ calcd for $C_6H_{12}NO_2^+$: 130.1; found: 130.2




**S9**

***cis*-3-Aminocyclobutanecarboxylic acid** (**S9**): To a stirred suspension of *N*-Boc-*cis*-3-aminocyclobutane-1-carboxylic acid (0.402 g, 1.87 mmol, 1.0 equiv) in $CH_2Cl_2$ (12 mL) was added TFA (3.0 mL, 39.17 mmol, 21 equiv) at 0 °C. After addition, the ice-bath was removed and the reaction mixture was allowed to warm to rt. After 3.5 h, the reaction mixture was concentrated, dissolved in $CH_2Cl_2$/MeOH, and concentrated provide the TFA salt of **S9** as colorless crystals (0.434 g, quant yield).

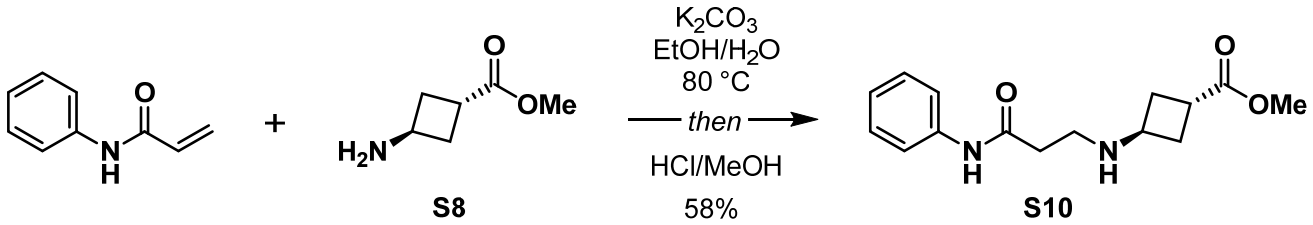
**TLC** $R_f$ 0.18 (20% MeOH in $CH_2Cl_2$)

**$^1$H NMR** (600 MHz, $D_2O$) δ 3.79 (app p, *J* = 8.3 Hz, 1H), 3.07 (tt, *J* = 9.8, 8.3 Hz, 1H), 2.67 – 2.58 (m, 2H), 2.40 – 2.31 (m, 2H) ppm

**$^{13}$C NMR** (151 MHz, $D_2O$) δ 178.0, 163.0 (q, *J* = 34 Hz), 116.3 (q, *J* = 292 Hz), 41.0, 31.1, 30.1 ppm

**$^{19}$F NMR** (376 MHz, $D_2O$) δ –75.65

**LC/MS** (*m/z*): $[M+H]^+$ calcd for $C_5H_{10}NO_2^+$: 116.1; found: 116.2



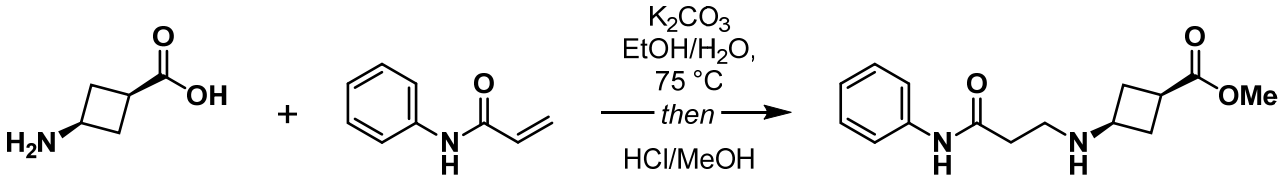

***trans*-Methyl 3-((3-oxo-3-(phenylamino)propyl)amino)cyclobutanecarboxylate** (**S10**): A mixture of amino ester **S8**·HCl (0.165 g, 1.00 mmol, 1.0 equiv), *N*-phenylacrylamide (0.155 g, 1.05 mmol, 1.05 equiv) and $K_2CO_3$ (0.276 g, 2.00 mmol, 2.0 equiv) in water/EtOH (1:1, 10 mL) was heated to 80 °C. After 38 h, the reaction mixture was cooled to rt, diluted with 1 M HCl (40 mL), washed with EtOAc (2 × 30 mL), and the aqueous layer was then evaporated to dryness. The residue was suspended in MeOH (25 mL), treated with 2 M HCl in MeOH (5 mL), stirred at rt for 3 h, and then concentrated. The residue was partitioned between half saturated aqueous $K_2CO_3$ (40 mL) and $CH_2Cl_2$ and the two layers were separated. The aqueous layer was extracted with $CH_2Cl_2$ (2 × 25 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The product was purified by MPLC (24 g silica, gradient: $CH_2Cl_2$ for 1 CV, then 0 → 10% MeOH in $CH_2Cl_2$ over 10 CV) to provide **S10** as a slightly yellow oil (0.160 g, 0.578 mmol, 58% yield).

**TLC** $R_f$ 0.68 (20% MeOH in $CH_2Cl_2$)

**$^1$H NMR** (600 MHz, $CDCl_3$) δ 10.07 (s, 1H), 7.51 – 7.47 (m, 2H), 7.31 – 7.26 (m, 2H), 7.05 (tt, *J* = 7.3, 1.1 Hz, 1H), 3.69 (s, 3H), 3.61 – 3.50 (m, 1H), 3.11 – 3.03 (m, 1H), 2.87 – 2.83 (m, 2H), 2.59 – 2.49 (m, 2H), 2.49 – 2.43 (m, 2H), 2.13 – 2.01 (m, 2H), 1.80 (br s, 1H) ppm

**$^{13}$C NMR** (151 MHz, $CDCl_3$) δ 176.3, 170.9, 138.5, 129.0, 123.9, 119.8, 52.0, 51.3, 42.5, 36.3, 33.0, 32.8 ppm

**LC/MS** (*m/z*): $[M+H]^+$ calcd for $C_{15}H_{21}N_2O_3^+$: 277.2; found: 277.2




**S9** **S11**

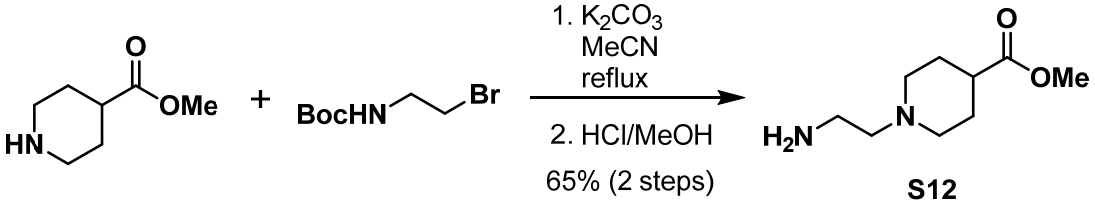
***cis*-Methyl 3-((3-oxo-3-(phenylamino)propyl)amino)cyclobutanecarboxylate** (**S11**): Amino acid **S9**·TFA (0.243 g, 1.06 mmol, 1.0 equiv), *N*-phenylacrylamide (0.172 g, 1.17 mmol, 1.1 equiv) and $K_2CO_3$ (0.330 g, 2.39 mmol, 2.25 equiv) were dissolved in water/EtOH (1:1, 8 mL) and stirred at 75 °C for 24 h, then cooled to rt, diluted with 1 M HCl (30 mL), extracted with EtOAc (2 × 25 mL) and the aqueous layer evaporated to dryness. The residue was suspended in MeOH (25 mL), acidified with HCl (2 M in MeOH, 1 mL), stirred at rt for 4 h, then concentrated to dryness. The residue was dissolved in half saturated aqueous $K_2CO_3$ (30 mL) and extracted with $CH_2Cl_2$ (4 × 20 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The product was purified by MPLC (24 g silica, gradient: 0 → 5% MeOH in $CH_2Cl_2$ over 9 CV, then 5% MeOH over 8 CV) to provide **S11** as a slightly colorless oil (0.111 g, 0.402 mmol, 38% yield).

**TLC** $R_f$ 0.40 (20% MeOH in $CH_2Cl_2$)

**$^1$H NMR** (600 MHz, $CDCl_3$) δ 10.15 (br s, 1H), 7.54 – 7.49 (m, 2H), 7.33 – 7.26 (m, 2H), 7.06 (tt, *J* = 7.4, 1.2 Hz, 1H), 3.68 (s, 3H), 3.29 (tt, *J* = 8.5, 7.2 Hz, 1H), 2.89 (br t, *J* = 5.7 Hz, 2H), 2.83 (tt, *J* = 9.2, 8.0 Hz, 1H), 2.59 – 2.53 (m, 2H), 2.46 (br t, *J* = 5.7 Hz, 2H), 2.10 – 2.01 (m, 2H), 1.67 (br s, 1H) ppm

**$^{13}$C NMR** (151 MHz, $CDCl_3$) δ 175.3, 170.9, 138.6, 129.0, 123.9, 119.8, 52.0, 49.3, 42.4, 36.3, 34.0, 31.2 ppm

**LC/MS** (*m/z*): $[M+H]^+$ calcd for $C_{15}H_{21}N_2O_3^+$: 277.2; found: 277.2




**S12**

**Methyl 1-(2-aminoethyl)piperidine-4-carboxylate** (**S12**): A suspension of methyl piperidine-4-carboxylate hydrochloride (2.50 g, 13.92 mmol, 1.0 equiv), *tert*-butyl (2-bromoethyl)carbamate (3.28 g, 14.61 mmol, 1.05 equiv) and $K_2CO_3$ (5.77 g, 41.75 mmol, 3.0 equiv) in acetonitrile (60 mL) was stirred for 20 h at rt, and then heated to reflux for 3 h, until TLC indicated complete consumption of starting material. The white suspension was filtered, the filter cake washed with $CH_2Cl_2$ (2 × 30 mL) and the filtrate concentrated. The residue was purified by FCC (300 g silica, eluent: 10% MeOH and 0.5%

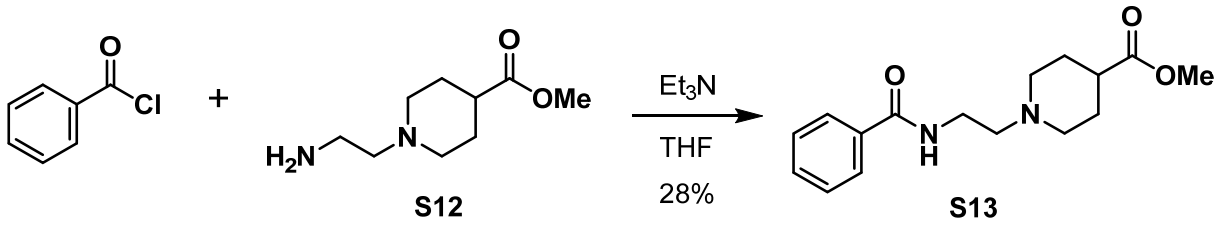
$NH_4OH$ in $CH_2Cl_2$) to provide Boc-protected **S12** as a yellow oil that solidified into off-white crystals (2.594 g, 9.059 mmol, 65% yield, *m/z*: $[M+H]^+$: 287.2, TLC $R_f$ 0.65 in 10% MeOH and 0.5% $NH_4OH$ in $CH_2Cl_2$), which were dissolved in 2M HCl in MeOH (113 mL) and stirred at rt for 1.5 h, then concentrated to dryness and further concentrated from MeOH (3 x 100 mL) to provide **S12**·2HCl as an off-white solid (2.350 g, 9.059 mmol, quant yield).

**TLC** $R_f$ 0.11 (50% MeOH and 0.5% $NH_4OH$ in $CH_2Cl_2$)

**$^1$H NMR** (400 MHz, DMSO) $\delta$ 11.16 (br s, 1H), 8.60 (br s, 3H), 3.63 (s, 3H), 3.60 – 3.48 (m, 2H), 3.37 – 3.25 (m, 4H), 3.05 (br t, *J* = 12.7 Hz, 2H), 2.68 (br t, *J* = 12.5 Hz, 1H), 2.19 – 1.81 (m, 4H) ppm

**$^{13}$C NMR** (151 MHz, MeOD-*d*$_4$) $\delta$ 174.7, 54.7, 53.7, 52.6, 39.3, 35.2, 26.8 ppm

**LC/MS** (*m/z*): $[M+H]^+$ calcd for $C_9H_{19}N_2O_2^+$: 187.1; found: 187.2



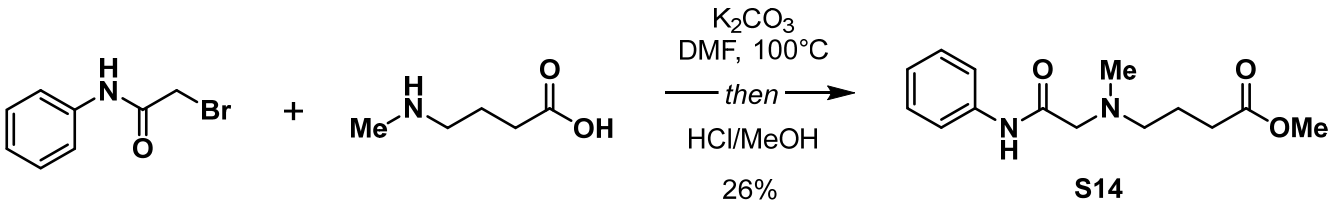

**Methyl 1-(2-benzamidoethyl)piperidine-4-carboxylate** (**S13**): To a solution of **S12**·2HCl (1.00 g, 3.858 mmol, 1.0 equiv) and $Et_3N$ (2.69 mL, 19.29 mmol, 5.0 equiv) in anhydrous THF (30 mL) was added benzoyl chloride (0.493 mL, 4.244 mmol, 1.1 equiv) at rt under argon. After 2.5 h, the reaction mixture was concentrated under reduced pressure, and partitioned between saturated aqueous $NaHCO_3$ (20 mL) and EtOAc (15 mL). The layers were separated and the aqueous layer was extracted with EtOAc (15 mL). The combined organic layers were dried ($MgSO_4$), filtered, concentrated and purified by FCC (125 g silica, eluent: 4% MeOH and 0.5% $NH_4OH$ in $CH_2Cl_2$) to provide **S13** as an off-white solid (343 mg, 1.181 mmol, 28% yield).

**TLC** $R_f$ 0.23 (5% MeOH and 0.5% $NH_4OH$ in $CH_2Cl_2$)

**$^1$H NMR** (400 MHz, DMSO-*d*$_6$) $\delta$ 8.36 (t, *J* = 5.6 Hz, 1H), 7.85 – 7.78 (m, 2H), 7.55 – 7.49 (m, 1H), 7.48 – 7.41 (m, 2H), 3.59 (s, 3H), 3.39 – 3.34 (m, 2H), 2.83 (dt, *J* = 11.6, 3.5 Hz, 2H), 2.44 (t, *J* = 7.0 Hz, 2H), 2.34 – 2.24 (m, 1H), 2.03 (td, *J* = 11.4, 2.6 Hz, 2H), 1.82 – 1.75 (m, 2H), 1.62 – 1.48 (m, 2H) ppm

**$^{13}$C NMR** (151 MHz, DMSO-*d*$_6$) $\delta$ 174.9, 166.1, 134.6, 131.1, 128.3, 127.1, 57.1, 52.4, 51.4, 40.2, 37.0, 28.0 ppm

**LC/MS** (*m/z*): $[M+H]^+$ calcd for $C_{16}H_{23}N_2O_3^+$: 291.2; found: 291.2



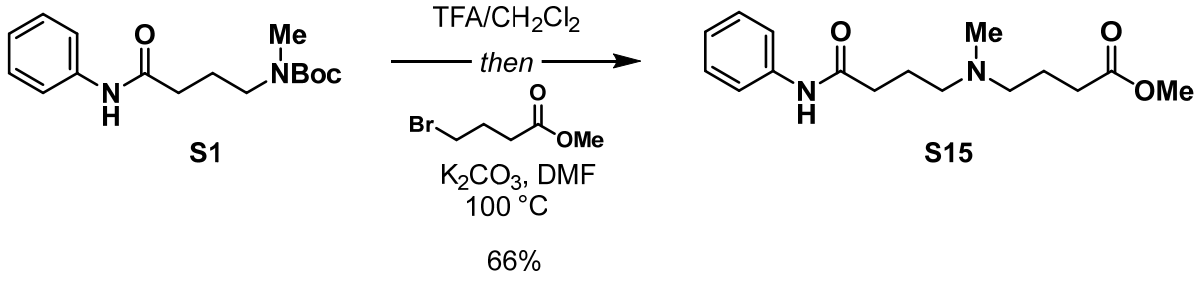

**Methyl 4-(methyl(2-oxo-2-(phenylamino)ethyl)amino)butanoate** (**S14**): A suspension of 2-bromo-*N*-phenylacetamide (0.600 g, 2.80 mmol, 1.0 equiv), *N*-MeGABA·HCl (0.450 g, 2.94 mmol, 1.05 equiv) and $K_2CO_3$ (0.775 g, 5.61 mmol, 2.0 equiv) in DMF (10 mL) was stirred at 100 °C for 1 h, then acidified with 2 M HCl (10 mL), and evaporated to dryness. The residue was suspended in MeOH (15 mL), acidified with HCl (2 M in MeOH, 0.5 mL), stirred at rt for 18 h, then concentrated. The residue was partitioned between half saturated aqueous $K_2CO_3$ (25 mL) and $CH_2Cl_2$ (20 mL) and the two layers were separated. The aqueous layer was extracted with $CH_2Cl_2$ (2 × 20 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo. The product was purified by MPLC (24 g silica, gradient: 0 → 10% MeOH in $CH_2Cl_2$ over 20 CV) to provide **S14** as a colorless oil (0.192 g, 0.726 mmol, 26% yield).

**TLC** $R_f$ 0.69 (10% MeOH in $CH_2Cl_2$)

**$^1$H NMR** (600 MHz, $CDCl_3$) δ 9.06 (br s, 1H), 7.66 – 7.61 (m, 2H), 7.35 – 7.29 (m, 2H), 7.09 (tt, *J* = 7.5, 1.2 Hz, 1H), 3.65 (s, 3H), 3.12 (s, 2H), 2.51 (t, *J* = 7.0 Hz, 2H), 2.39 (t, *J* = 7.0 Hz, 2H), 2.32 (s, 3H), 1.87 (app p, *J* = 7.0 Hz, 2H) ppm

**$^{13}$C NMR** (151 MHz, $CDCl_3$) δ 174.0, 169.1, 137.9, 129.1, 124.2, 119.5, 62.6, 57.5, 51.8, 42.8, 32.2, 22.9 ppm

**LC/MS** (*m/z*): $[M+H]^+$ calcd for $C_{14}H_{21}N_2O_3^+$: 265.2; found: 265.2




**Methyl 4-(methyl(4-oxo-4-(phenylamino)butyl)amino)butanoate** (**S15**): To a solution of **S1** (0.810 g, 2.77 mmol, 1.0 equiv) in $CH_2Cl_2$ (8 mL) was added TFA (2.1 mL, 27.7 mmol, 10 equiv). After 1 h, the reaction mixture was concentrated to dryness. The residue was dissolved in DMF (5 mL), and $K_2CO_3$ (1.15 g, 8.31 mmol, 3.0 equiv) and methyl 4-bromobutyrate (0.37 mL, 2.91 mmol, 1.05 equiv) were added. This suspension was heated to 100 °C for 4.5 h, then concentrated and partitioned between half

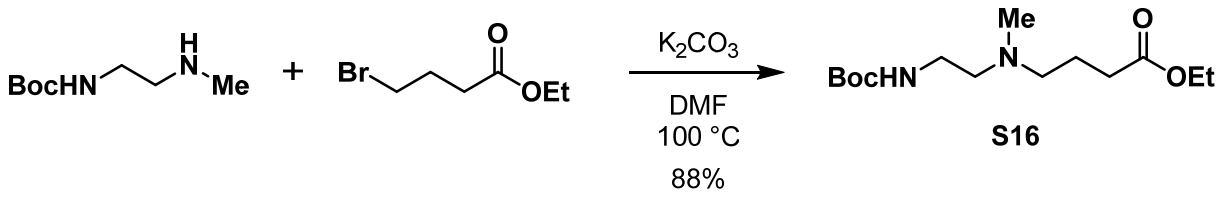
saturated aqueous $K_2CO_3$ (100 mL) and $CH_2Cl_2$ (60 mL). The two layers were separated and the aqueous layer was extracted with $CH_2Cl_2$ (3 × 60 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (100 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude product was purified by FCC (78 g silica, eluent: 5% then 10% MeOH and 0.5% $NH_4OH$ in $CH_2Cl_2$) to provide **S15** as a yellow oil (0.537 g, 1.84 mmol, 66% yield).

**TLC** $R_f$ 0.17 (10% MeOH and 0.5% $NH_4OH$ in $CH_2Cl_2$)

**$^1$H NMR** (600 MHz, $CDCl_3$) δ 9.08 (br s, 1H), 7.56 (d, *J* = 8.0 Hz, 2H), 7.29 (t, *J* = 7.3 Hz, 2H), 7.06 (t, *J* = 7.3 Hz, 1H), 3.66 (s, 3H), 2.48 – 2.40 (m, 6H), 2.35 (t, *J* = 7.0 Hz, 2H), 2.24 (s, 3H), 1.90 – 1.80 (m, 4H) ppm

**$^{13}$C NMR** (151 MHz, $CDCl_3$) δ 174.3, 171.8, 138.8, 129.0, 123.8, 119.6, 57.0, 56.9, 51.8, 41.6, 36.0, 32.3, 23.0, 22.4 ppm

**LC/MS** (*m/z*): $[M+H]^+$ calcd for $C_{16}H_{25}N_2O_3^+$: 293.2; found: 293.2



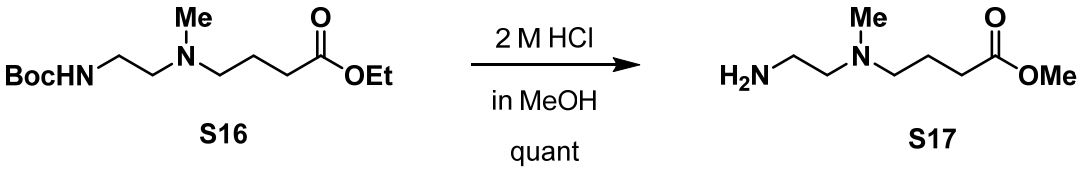

**Ethyl 4-((2-((*tert*-butoxycarbonyl)amino)ethyl)(methyl)amino)butanoate** (**S16**): To a suspension of *tert*-butyl (2-(methylamino)ethyl)carbamate (1.52 g, 8.74 mmol, 1.0 equiv) and $K_2CO_3$ (2.42 g, 17.49 mmol, 2.0 equiv) in DMF (9 mL) was added ethyl 4-bromobutyrate (1.38 mL, 9.62 mmol, 1.1 equiv). The reaction mixture was stirred at 100 °C for 16 h, then cooled to rt, poured into water (100 mL) and extracted with EtOAc (3 × 100 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo. The product was purified by FCC (280 g silica, eluent: 8% then 20% MeOH in $CH_2Cl_2$) to provide **S16** as brown oil (2.21 g, 7.67 mmol, 88% yield).

**TLC** $R_f$ 0.41 (10% MeOH in $CH_2Cl_2$)

**$^1$H NMR** (600 MHz, $CDCl_3$) δ 5.06 (br s, 1H), 4.12 (q, *J* = 7.1 Hz, 2H), 3.18 (app q, *J* = 5.9 Hz, 2H), 2.44 (t, *J* = 5.9 Hz, 2H), 2.37 (t, *J* = 7.2 Hz, 2H), 2.31 (t, *J* = 7.2 Hz, 2H), 2.18 (s, 3H), 1.78 (p, *J* = 7.2 Hz, 2H), 1.43 (s, 9H), 1.24 (t, *J* = 7.1 Hz, 3H) ppm

**$^{13}$C NMR** (151 MHz, $CDCl_3$) δ 173.8, 156.2, 79.1, 60.4, 56.8, 56.7, 41.6, 37.9, 32.2, 28.5, 22.6, 14.4 ppm

**LC/MS** (*m/z*): $[M+H]^+$ calcd for $C_{14}H_{29}N_2O_4^+$: 289.2; found: 289.2




S16 S17

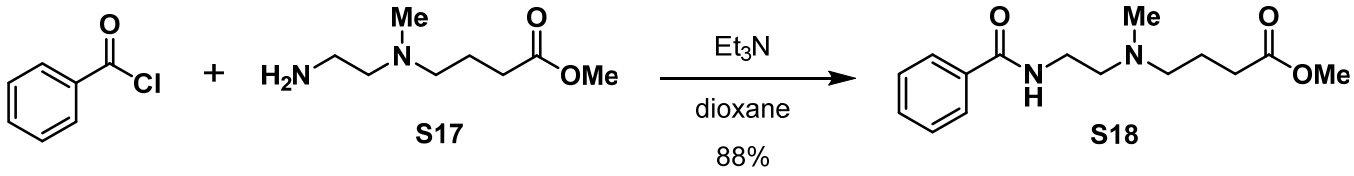
**Methyl 4-((2-aminoethyl)(methyl)amino)butanoate** (**S17**): Ethyl ester **S16** (1.80 g, 6.27 mmol, 1 equiv) was dissolved in 2 M HCl in MeOH (8 mL, 156.74 mmol, 25 equiv) and stirred at rt for 26 h. The solvent was removed in vacuo and residual acid was removed by co-evaporation with MeOH (3 × 10 mL) to provide the 2·HCl salt of **S17** as a yellow amorphous solid (1.54 g, 6.24 mmol, quant yield). The product was stored as a stock solution in MeOH and used without further purification.

**TLC** $R_f$ 0.08 (20% MeOH in $CH_2Cl_2$), 0.21 (20% MeOH and 0.5% $NH_4OH$ in $CH_2Cl_2$)

**$^1$H NMR** (400 MHz, MeOD-$d_4$) $\delta$ 3.70 (s, 3H), 3.62 – 3.43 (m, 4H, $2CH_2$), 3.39 – 3.23 (m, 2H, $CH_2$), 2.98 (s, 3H), 2.53 (t, $J$ = 7.0 Hz, 2H), 2.17 – 2.04 (m, 2H) ppm

**$^{13}$C NMR** (101 MHz, MeOD-$d_4$) $\delta$ 174.3, 57.1, 53.7, 52.4, 41.0, 35.3, 31.1, 20.4 ppm

**LC/MS** (*m/z*): $[M+H]^+$ calcd for $C_8H_{19}N_2O_2^+$: 175.1; found: 175.2




S17 S18

**Methyl 4-((2-benzamidoethyl)(methyl)amino)butanoate** (**S18**): To a stirred suspension of **S17**·2HCl (375 mg, 1.519 mmol, 1.0 equiv) and $Et_3N$ (0.85 mL, 6.07 mmol, 4.0 equiv) in dioxane (10 mL) was slowly added benzoyl chloride (0.22 mL, 1.898 mmol, 1.25 equiv) at rt. After 2 h, TLC indicated complete conversion. The resulting suspension was filtered, the filter cake washed with dioxane (100 mL) and the filtrate concentrated in vacuo. The residue was dissolved in $CH_2Cl_2$ (40 mL) and washed with saturated aqueous $NaHCO_3$ (2 × 40 mL), dried ($MgSO_4$), filtered, and concentrated. The product was purified by MPLC (12 g silica, gradient: 0 → 2.1% MeOH and 0.5% $NH_4OH$ in $CH_2Cl_2$ over 11.5 CV, then 2.1 → 3% MeOH over 10 CV) to provide **S18** as a yellow oil (373 mg, 1.338 mmol, 88% yield).

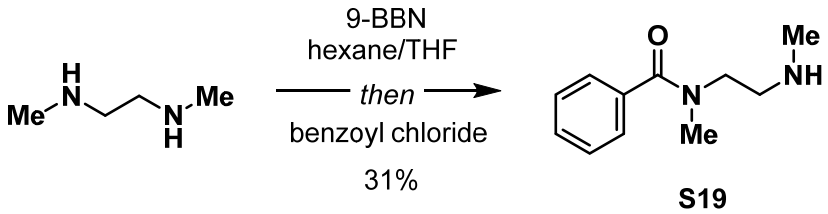
**TLC** $R_f$ 0.40 (5% MeOH and 0.5% $NH_4OH$ in $CH_2Cl_2$)

**$^1$H NMR** (400 MHz, $CDCl_3$) $\delta$ 7.88 – 7.77 (m, 2H), 7.52 – 7.37 (m, 3H), 6.95 (br s, 1H), 3.59 (s, 3H), 3.54 – 3.46 (m, 2H), 2.60 – 2.52 (m, 2H), 2.42 (t, $J$ = 6.8 Hz, 2H), 2.36 (t, $J$ = 7.1 Hz, 2H), 2.21 (s, 3H), 1.82 (app p, $J$ = 6.8 Hz, 2H) ppm

**$^{13}$C NMR** (101 MHz, $CDCl_3$) $\delta$ 174.3, 167.5, 134.8, 131.4, 128.6, 127.2, 56.8, 56.5, 51.7, 41.6, 37.3, 32.2, 22.8 ppm

**LC/MS** (*m/z*): $[M+H]^+$ calcd for $C_{15}H_{23}N_2O_3^+$: 279.2; found: 279.2

**HR-MS** (*m/z*): $[2M+Na]^+$ calcd for $C_{30}H_{44}N_4NaO_6^+$: 579.3153; found: 579.3152




**S19**

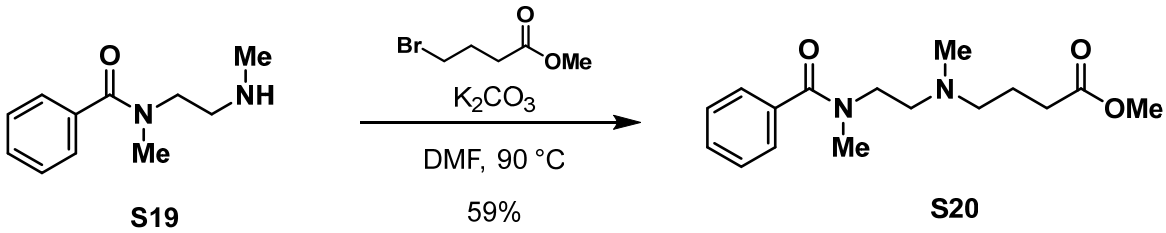
***N*-methyl-*N*-(2-(methylamino)ethyl)benzamide** (**S19**)[7]: To a stirred solution of 9-BBN in hexanes (0.40 M, 15.95 mL, 6.379 mmol, 1.05 equiv) was added a solution of 1,2-dimethylethylenediamine (0.69 mL, 6.379 mmol, 1.05 equiv) in anhydrous THF (25 mL) at rt. After 1 h, benzoyl chloride (0.70 mL, 6.075 mmol, 1.0 equiv) was added. After 2.5 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ (300 mL) and extracted with EtOAc (3 × 120 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated. The crude product was purified by MPLC (24 g silica, gradient: 0 → 9% MeOH and 0.5% $NH_4OH$ in $CH_2Cl_2$ over 5 CV, 9% for 2 CV, 9 → 15% over 2.5 CV, then 15% for 3 CV) to provide **S19** as a yellow oil (357 mg, 1.857 mmol, 31% yield).

**TLC** $R_f$ 0.27 (20% MeOH and 0.5% $NH_4OH$ in $CH_2Cl_2$)

**$^1$H NMR** (400 MHz, $CDCl_3$) δ 7.55 – 7.28 (m, 5H), 3.66 (br s, 2H, rotamer A), 3.37 (br s, 2H, rotamer B), 3.09 (br s, 3H. rotamer B), 2.99 (s, 3H, rotamer A), 2.88 (s, 2H, rotamer A), 2.72 (s, 2H, rotamer B), 2.49 (s, 3H, rotamer A), 2.29 (s, 3H, rotamer B) ppm

**LC/MS** *m/z*: $[M+H]^+$: calcd for $C_{11}H_{17}N_2O^+$: 193.1; found: 193.2

Analytical data is in agreement with literature.[7]




**S19** **S20**

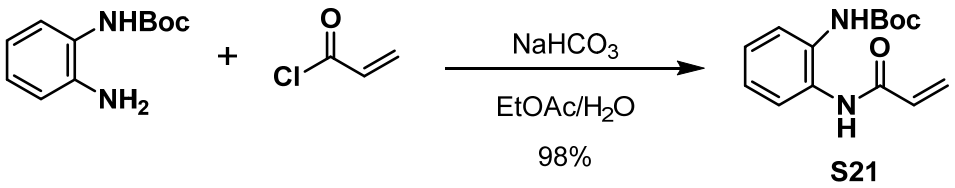
**Methyl 4-(methyl(2-(*N*-methylbenzamido)ethyl)amino)butanoate** (**S20**): To a solution of amine **S19** (357 mg, 1.857 mmol) in DMF (4.5 mL) was added $K_2CO_3$ (0.763 g, 5.521 mmol, 3.0 equiv) and methyl 4-bromobutyrate (0.37 mL, 2.899 mmol, 1.56 equiv). The suspension was stirred at 90 °C for 4 h, then poured into saturated aqueous $NaHCO_3$ (150 mL) and extracted with $CH_2Cl_2$ (3 × 60 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated. The product was purified by MPLC (12 g silica, gradient: 1 → 4.5% MeOH and 0.5% $NH_4OH$ in $CH_2Cl_2$ over 7 CV, then 4.5 → 5% over 9 CV) to provide **S20** as a yellow oil (320 mg, 1.093 mmol, 59%).

**TLC** $R_f$ 0.49 (10% MeOH and 0.5% $NH_4OH$ in $CH_2Cl_2$).

**$^1$H NMR** (400 MHz, $CDCl_3$) $\delta$ 7.38 (s, 5H, Ar H), 3.65 (s, 4H, $–COOCH_3$ and $CH_2$ rotamer A), 3.30 (t, $J$ = 5.9 Hz, 1H, $CH_2$ rotamer B), 3.17 – 2.89 (m, 3H, $–CONCH_3–$ both rotamers), 2.67 (br s, 1H, $CH_2$ rotamer A), 2.57 – 2.14 (m, 6.5H, $CH_2$ rotamer B, $2CH_2$ both rotamers and $–NCH_3$ rotamer A), 1.98 (s, 1.5H, $–NCH_3$ rotamer B), 1.90 – 1.73 (m, 1H, $CH_2$ rotamer A), 1.73 – 1.57 (m, 1H, $CH_2$ rotamer B) ppm.

**$^{13}$C NMR** (151 MHz, $CDCl_3$) $\delta$ 174.2 (*C*OOMe rotamer A), 174.1 (*C*OOMe rotamer B), 172.3(Ar–*C*ONMe rotamer A), 171.4 (Ar–*C*ONMe rotamer B), 136.8 (Ar*C*–CO), 129.5 (ArCH rotamer A), 129.4 (ArCH rotamer B), 128.5 (ArCH), 127.1 (ArCH rotamer A), 126.8 (ArCH rotamer B), 57.2 (N–*C*$H_2$–$CH_2$–$CH_2$– rotamer A), 57.1 (N–*C*$H_2$–$CH_2$–$CH_2$– rotamer B), 55.6 (CON–$CH_2$–*C*$H_2$–N rotamer A), 54.9 (CON–$CH_2$–*C*$H_2$–N rotamer B), 51.7 (COO*C*$H_3$), 49.5 (CON–*C*$H_2$–$CH_2$–N rotamer A), 45.5 (CON–*C*$H_2$–$CH_2$–N rotamer B), 42.2 (–N*C*$H_3$), 38.4 (–CON*C*$H_3$– rotamer A), 33.5(–CON*C*$H_3$– rotamer B), 31.8 (–*C*$H_2$COOMe rotamer A), 31.7 (–*C*$H_2$COOMe rotamer B), 22.8 (–$CH_2$–*C*$H_2$–$CH_2$– rotamer A), 22.6 (–$CH_2$–*C*$H_2$–$CH_2$– rotamer B) ppm; The NMR spectra show two rotamers with overlapping signals in $^1$H NMR. The EXSY experiment confirmed that each set of two signals originates from the same nucleus. Overlapping rotamer signals were differentiated by an HSQC edit experiment and are listed separately, if they could be distinguished. The approximate ratio of rotamer A to B is 1.2:1, but they are treated as if they are in a 1:1 ratio for the signals reported above.

**LC/MS** (*m/z*): $[M+H]^+$ calcd for $C_{16}H_{25}N_2O_3^+$: 293.2; found: 293.2




**S21**

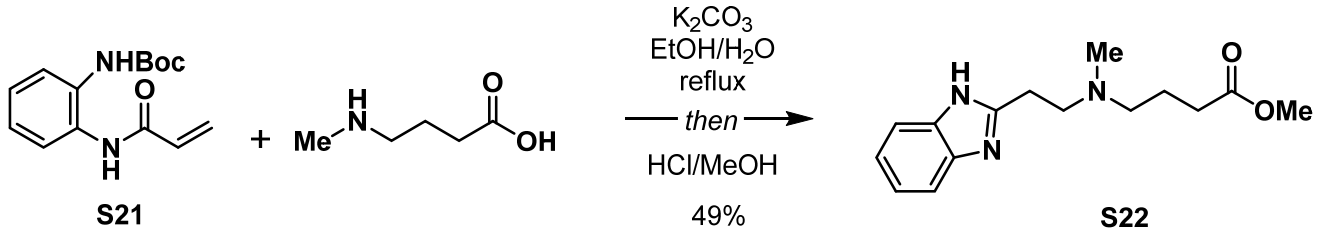
***tert*-Butyl (2-acrylamidophenyl)carbamate** (**S21**): To a vigorously stirred mixture of *N*-Boc-1,2-phenylenediamine (500 mg, 2.40 mmol, 1.0 equiv) in saturated aqueous $NaHCO_3$ (10 mL) and EtOAc (10 mL) was added acryloyl chloride (0.217 mL, 2.64 mmol, 1.1 equiv). After stirring for 5 min, the reaction mixture was diluted with EtOAc (20 mL), the phases were separated, and the organic layer was washed with saturated aqueous $NaHCO_3$ (2 × 20 mL) and water (20 mL), then dried ($MgSO_4$), filtered, and concentrated to provide **S21** as an off–white solid (619 mg, 2.36 mmol, 98% yield).

**TLC** $R_f$ 0.24 (30% EtOAc in hexane)

**$^1$H NMR** (600 MHz, $CDCl_3$) $\delta$ 8.45 (s, 1H), 7.55 (t, $J$ = 4.9 Hz, 1H), 7.31 (dd, $J$ = 6.1, 3.5 Hz, 1H), 7.19 – 7.09 (m, 2H), 6.92 (s, 1H), 6.40 (d, $J$ = 17.0 Hz, 1H), 6.24 (dd, $J$ = 17.0, 10.3 Hz, 1H), 5.76 (d, $J$ = 10.3 Hz, 1H), 1.51 (s, 9H) ppm

**$^{13}$C NMR** (151 MHz, $CDCl_3$) δ 164.3, 154.5, 131.3, 130.5, 130.1, 127.7, 126.3, 125.7, 125.6, 124.6, 81.3, 28.4 ppm

**LC/MS** (*m/z*): $[M+Na]^+$ calcd for $C_{14}H_{18}N_2NaO_3^+$: 285.1; found: 285.1




**S21** **S22**

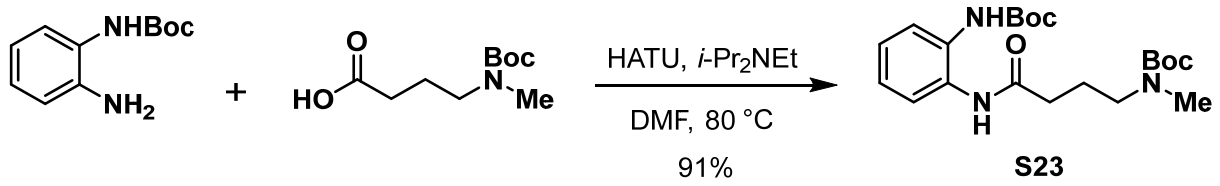
**Methyl 4-((2-(benzimidazol-2-yl)ethyl)(methyl)amino)butanoate** (**S22**): A mixture of acrylamide **S21** (0.609 g, 2.32 mmol, 1.0 equiv), *N*-MeGABA·HCl (0.373 g, 2.44 mmol, 1.05 equiv) and $K_2CO_3$ (0.642 g, 4.64 mmol, 2.0 equiv) in water/EtOH (1:1, 10 mL) was heated to reflux. After 16 h, the reaction mixture was cooled to rt, diluted with 2 M HCl (10 mL) and evaporated to dryness. Residual water was removed by successive concentration from MeOH and toluene (2 × 20 mL each). The residue was dissolved in anhydrous methanolic HCl (0.5 M, 30 mL) and heated to reflux. After 18 h, the reaction mixture was concentrated, and partitioned between half saturated aqueous $K_2CO_3$ (30 mL) and $CH_2Cl_2$ (30 mL). The layers were separated and the aqueous layer was extracted with $CH_2Cl_2$ (2 × 25 mL). The combined organics were dried ($MgSO_4$), filtered, and concentrated in vacuo. The product was purified by MPLC (24 g silica, gradient: 0 → 8% MeOH in $CH_2Cl_2$ over 8 CV, then 8% MeOH over 10 CV) to provide **S22** as a brown oil (0.312 g, 1.133 mmol, 49% yield).

**TLC** $R_f$ 0.28 (20% MeOH in $CH_2Cl_2$)

**$^1$H NMR** (600 MHz, $CDCl_3$) δ 7.59 – 7.51 (m, 2H), 7.21 – 7.15 (m, 2H), 3.65 (s, 3H), 3.08 (t, *J* = 6.2 Hz, 2H), 2.78 (t, *J* = 6.2 Hz, 2H), 2.47 (t, *J* = 7.1 Hz, 2H), 2.37 (t, *J* = 7.1 Hz, 2H), 2.29 (s, 3H), 1.87 (app p, *J* = 7.1 Hz, 2H) ppm

**$^{13}$C NMR** (151 MHz, $CDCl_3$) δ 174.1, 154.7, 122.0, 56.9, 55.7, 51.8, 41.0, 32.0, 26.1, 22.5 ppm; Due to tautomerism of the benzimidazole NH, two benzimidazole carbon signals are too broad to be identified in $^{13}$C spectra.

**LC/MS** (*m/z*): $[M+H]^+$ calcd for $C_{15}H_{22}N_3O_2^+$: 276.2; found: 276.2



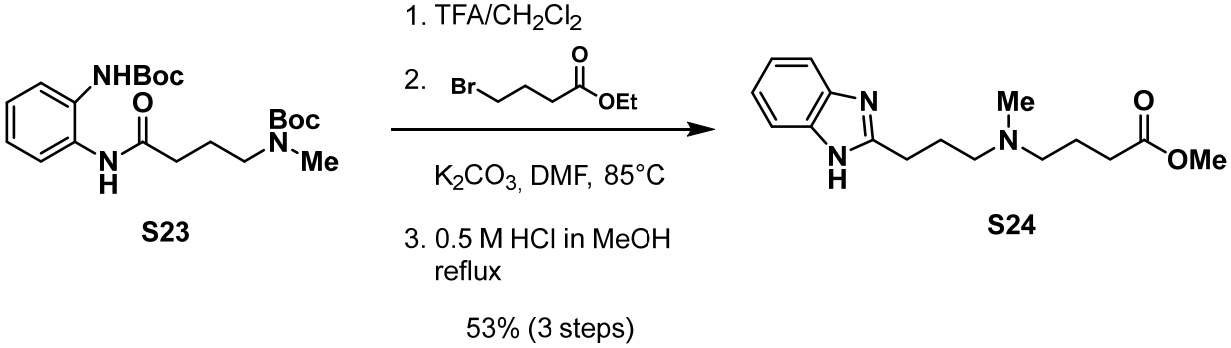

***tert*-Butyl (4-((2-((*tert*-butoxycarbonyl)amino)phenyl)amino)-4-oxobutyl)(methyl)carbamate** (**S23**): A solution of *N*-Boc-1,2-phenylenediamine (0.200 g, 0.960 mmol, 1.0 equiv), *N*-Boc,*N*-MeGABA[4] (0.209 g, 0.960 mmol, 1.0 equiv) HATU (0.438 g, 1.15 mmol, 1.2 equiv) , and *i*-$Pr_2$NEt (0.384 mL, 2.88 mmol, 3.0 equiv) in DMF (2.5 mL) was heated by microwave irradiation to 80 °C for 20 min. The reaction mixture was diluted with EtOAc (50 mL), washed with half saturated aqueous $K_2CO_3$ (30 mL) and brine (2 × 30 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The product was purified by MPLC (10 g silica, gradient: 20 → 60% EtOAc in hexane) to provide **S23** as a white foam (355 mg, 0.870 mmol, 91% yield)

**TLC** $R_f$ 0.57 (60% EtOAc in hexane)

**$^1$H NMR** (600 MHz, MeOD) δ 7.63 – 7.50 (m, 1H), 7.40 (br s, 1H), 7.20 (td, *J* = 7.7, 1.6 Hz, 1H), 7.13 (t, *J* = 7.7 Hz, 1H), 3.33 (t, *J* = 7.1 Hz, 2H), 2.89 (br s, 3H), 2.42 (t, *J* = 7.4 Hz, 2H), 1.93 (app p, *J* = 7.2 Hz, 2H), 1.52 (s, 9H), 1.47 (s, 9H) ppm

**$^{13}$C NMR** (151 MHz, MeOD) δ 172.2 and 172.1 (–$CH_2$–<u>C</u>ONHAr), 155.6 and 155.4 (MeN<u>C</u>O$O^t$Bu), 153.6 (ArN<u>C</u>O$O^t$Bu), 131.1 and 130.9 (ArCN), 128.9 and 128.8 (ArCN), 125.2 (ArCH), 124.5 (ArCH, broad), 123.7 and 123.6 (ArCH), 123.2 (ArCH, very broad), 79.3 (–O<u>C</u>$Me_3$, broad), 78.9 and 78.8 (–O<u>C</u>$Me_3$), 47.4 (–<u>C</u>$H_2$NMe, overlapped with solvent signal), 32.7 and 32.5 (–$NCH_3$), 32.4 (–$CH_2$N–), 26.7 (–OC<u>C</u>$H_3$), 26.6 (–OC<u>C</u>$H_3$), 22.9 and 22.5 (–$CH_2$–<u>C</u>$H_2$–$CH_2$–) ppm; The NMR spectra show rotamers due to hindered rotation around amide and carbamate C–N bonds, resulting in two signals per nucleus or very broad signals. Rotamers were confirmed by the EXSY experiment.

**LC/MS** (*m/z*): $[M+Na]^+$ calcd for $C_{21}H_{33}N_3NaO_5^+$: 430.2; found: 430.2



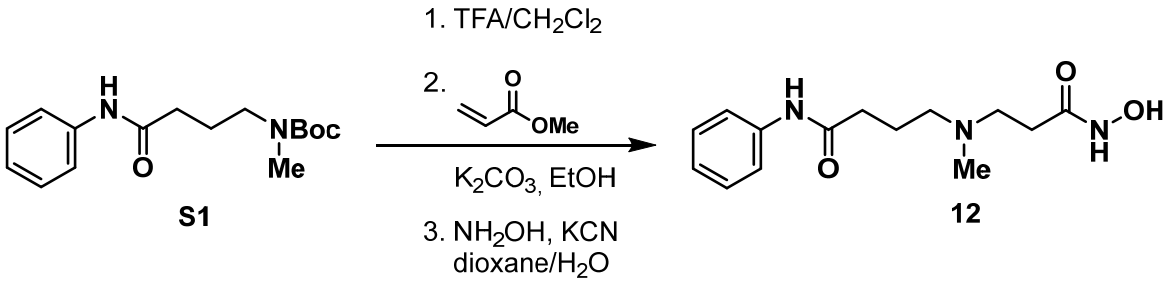
**Methyl 4-((3-(benzimidazol-2-yl)propyl)(methyl)amino)butanoate** (**S24**): To a solution of Boc-protected amine **S23** (355 mg, 0.870 mmol, 1.0 equiv) in $CH_2Cl_2$ (7.5 mL) was added TFA (2.5 mL) at rt. After 90 min, the reaction mixture was concentrated to dryness. The residue was dissolved in DMF (2.0 mL), ethyl 4-bromobyturate (0.131 mL, 0.914 mmol, 1.05 equiv) and $K_2CO_3$ (0.481 g, 3.48 mmol, 4.0 equiv) were added, and the mixture stirred at 85 °C for 18 h, then acidified with 1 M aqueous HCl (10 mL), and the solution concentrated to dryness. The residue was then heated to reflux in methanolic HCl (0.5 M, 30 mL). After 21 h the reaction mixture was concentrated in vacuo, and partitioned between aqueous $K_2CO_3$ (30 mL) and $CH_2Cl_2$ (30 mL). The two layers were separated and the aqueous layer was extracted with $CH_2Cl_2$ (3 × 30 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo. The product was purified by MPLC (12 g silica, gradient: 0 → 20% MeOH in $CH_2Cl_2$) to provide **S24** as a brown oil, which solidified upon standing (0.133 g, 0.460 mmol, 53% yield over three steps).

**TLC** $R_f$ 0.19 (20% MeOH in $CH_2Cl_2$)

**$^1$H NMR** (600 MHz, $CDCl_3$) δ 9.99 (br s, 1H), 7.56 – 7.50 (m, 2H), 7.20 – 7.15 (m, 2H), 3.65 (s, 3H), 3.02 (t, *J* = 6.7 Hz, 2H), 2.48 (t, *J* = 6.2 Hz, 2H), 2.43 (t, *J* = 7.3 Hz, 2H), 2.35 (t, *J* = 7.2 Hz, 2H), 2.24 (s, 3H), 1.98 (p, *J* = 6.4 Hz, 2H), 1.85 (app p, *J* = 7.2 Hz, 2H) ppm

**$^{13}$C NMR** (151 MHz, $CDCl_3$) δ 174.1, 155.5, 138.9, 121.9, 114.7, 57.6, 56.8, 51.8, 41.4, 31.9, 27.9, 24.8, 22.3 ppm

**LC/MS** (*m/z*): $[M+H]^+$ calcd for $C_{16}H_{24}N_3O_2^+$: 290.2; found: 290.2




**4-((3-(Hydroxyamino)-3-oxopropyl)(methyl)amino)-*N*-phenylbutanamide** (**12**): To a solution of Boc-amine **S1** (0.359 g, 1.229 mmol, 1.0 equiv) in $CH_2Cl_2$ (5 mL), was added TFA (2.15 mL, 27.04 mmol, 22 equiv) at rt. After 1 h, the reaction mixture was concentrated to dryness and re-concentrated from MeOH (3 × 10 mL) to remove excess acid. To a solution of the resulting residue in EtOH (5 mL) was added $K_2CO_3$ (0.340 g, 2.459 mmol, 2.0 equiv) and methyl acrylate (0.123 mL, 1.352 mmol, 1.1 equiv) at rt. After stirring for 66 h, the mixture was diluted with water (20 mL) and saturated aqueous $NaHCO_3$ (50 mL) and extracted with $CH_2Cl_2$ (3 × 30 mL). The combined organic layers were dried ($MgSO_4$),

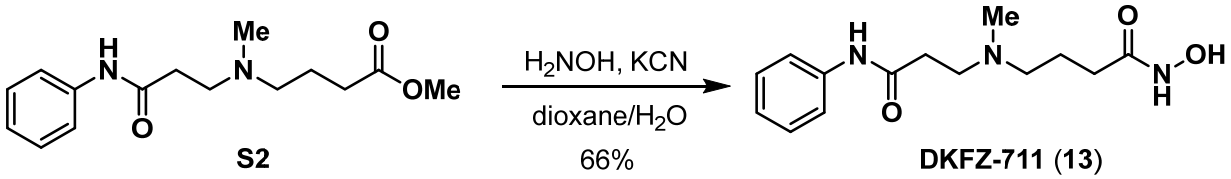
filtered, and concentrated in vacuo. The residue was then stirred in 1 M HCl in MeOH (10 mL) at rt for 16 h (significant transesterification to the ethyl ester had occurred during the Michael addition) and concentrated. The product was purified by MPLC (4 g silica, gradient: 0 → 10% MeOH and 0.5% $NH_4OH$ in $CH_2Cl_2$ over 32 CV, 10 → 14% MeOH over 2.5 CV, then 14% for 3 CV) to provide the corresponding ester to the title compound as a yellow oil (121 mg, *m/z*: $[M+H]^+$: 279.2), which was directly converted to hydroxamic acid **12** according to General Procedure A. The product was purified by RP-MPLC (15.5 g C18Aq Teledyne Isco #69-2203-559, gradient: 0 → 21% B over 24 CV) to provide **12** as a colorless amorphous solid (27.0 mg, 0.097 mmol, 8% yield (3 steps)).

**TLC** $R_f$ 0.12 (10% MeOH and 0.5% $NH_4OH$ in $CH_2Cl_2$)

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 10.38 (br s, 1H*), 9.85 (s, 1H), 8.74 (br s, 1H*), 7.64 – 7.54 (m, 2H), 7.33 – 7.21 (m, 2H), 7.06 – 6.95 (m, 1H), 2.54 (t, *J* = 7.1 Hz, 2H), 2.36 – 2.23 (m, 4H), 2.13 (s, 3H), 2.12 – 2.02 (m, 2H), 1.70 (app p, *J* = 7.2 Hz, 2H) ppm; *partially exchanged with adventitious water.

**$^{13}$C NMR** (151 MHz, DMSO-$d_6$) δ 171.3, 168.1, 139.4, 128.6, 122.9, 119.0, 56.1, 53.1, 41.5, 34.3, 30.5, 22.9 ppm

**HR-MS** (*m/z*): $[2M+Na]^+$ calcd for $C_{28}H_{42}N_6NaO_6^+$: 581.3058; found: 581.3058




**S2** **DKFZ-711** (**13**)

***N*-(2-((4-(Hydroxyamino)-4-oxobutyl)(methyl)amino)ethyl)benzamide** (**DKFZ-711, 13**): Ester **S2** (187.3 mg, 0.673 mmol, 1.0 equiv) was converted to hydroxamic acid **13** according to General Procedure A. The product was purified by HPLC Acidic Method (gradient: 1 → 10% B in 3 min, then 10 → 45% B in 11 min) to provide the TFA salt of **DKFZ-711** as an orange, hygroscopic, amorphous solid (174.2 mg, 0.443 mmol, 66% yield).

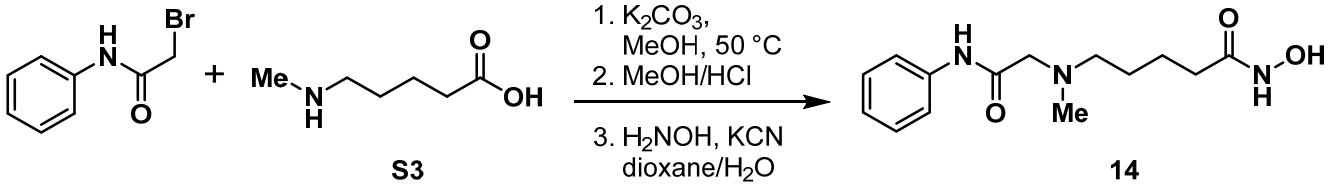
**TLC** $R_f$ 0.21 (10% MeOH and 0.5% $NH_4OH$ in $CH_2Cl_2$)

**$^1$H NMR** (400 MHz, $D_2O$) δ 7.49 – 7.40 (m, 4H), 7.33 – 7.22 (m, 1H), 3.70 – 3.58 (m, 1H), 3.51 – 3.37 (m, 1H), 3.33 – 3.18 (m, 2H), 2.97 (t, *J* = 6.7 Hz, 2H), 2.93 (s, 3H), 2.32 (t, *J* = 7.2 Hz, 2H), 2.14 – 2.03 (m, 2H) ppm

**$^{13}$C NMR** (101 MHz, $D_2O$) δ 171.1, 170.1, 162.9 (q, *J* = 34 Hz), 136.5, 129.2, 125.7, 121.8, 116.3 (q, *J* = 292 Hz), 55.5, 51.8, 40.0, 30.0, 29.0, 19.6 ppm

**$^{19}$F NMR** (376 MHz, $D_2O$) δ –78.4 ppm

**HR-MS** (*m/z*): $[M+H]^+$ calcd for $C_{14}H_{22}N_3O_3^+$: 280.1656; found: 280.1656




***N*-hydroxy-5-(methyl(2-oxo-2-(phenylamino)ethyl)amino)pentanamide** (**14**): A suspension of 2-bromo-*N*-phenylacetamide (0.184 g, 0.859 mmol, 1.2 equiv), **S3**·HCl (0.120 g, 0.716 mmol, 1.00 equiv) and $K_2CO_3$ (0.317 g, 2.291 mmol, 3.2 equiv) in MeOH (9 mL) was stirred at 50 °C for 45 h. It was then acidified with 2 M HCl (50 mL), washed with $Et_2O$ (4 × 20 mL), and the aqueous layer was evaporated to dryness. The residue was re-suspended in MeOH (40 mL), acidified with HCl (2 M in MeOH, 0.5 mL), stirred at rt for 18 h, and then concentrated. The residue was dissolved in half saturated aqueous $K_2CO_3$ (25 mL) and extracted with $CH_2Cl_2$ (4 × 20 mL), dried ($MgSO_4$) and concentrated in vacuo. The crude methyl ester was converted to hydroxamic acid **14** according to General Procedure A. The product was purified by HPLC Acidic Method (gradient: 1 → 25% B in 3 min, then 25 → 45% B in 11 min) to provide the TFA salt of **14** as an off-white solid (0.175 g, 0.445 mmol, 62% yield (3 steps)).

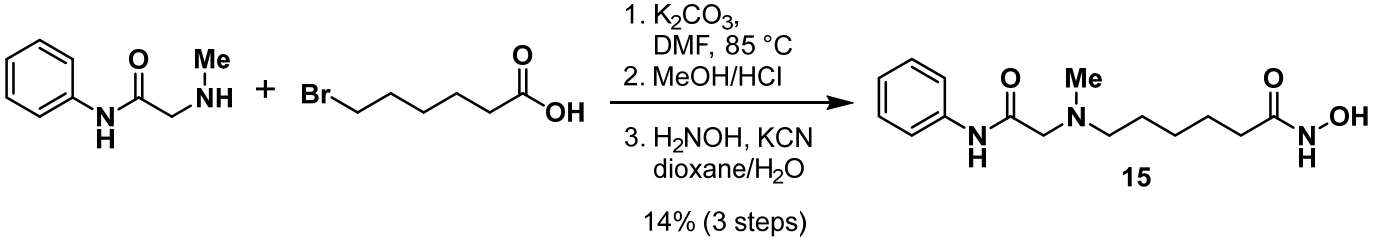
**TLC** $R_f$ 0.24 (10% MeOH and 0.5% $NH_4OH$ in $CH_2Cl_2$)

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 10.63 (s, 1H), 10.42 (s, 1H*), 9.83 (br s, 1H*), 8.72 (br s, 1H*), 7.63 – 7.54 (m, 2H), 7.40 – 7.32 (m, 2H), 7.12 (tt, *J* = 7.4, 1.1 Hz, 1H), 4.11 (s, 2H), 3.21 – 3.08 (m, 2H), 2.85 (s, 3H), 2.00 (t, *J* = 7.2 Hz, 2H), 1.72 – 1.60 (m, 2H), 1.52 (app p, *J* = 7.4 Hz, 2H) ppm; *partially exchanged with adventitious water

**$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 168.6, 163.2, 158.0 (q, *J* = 31 Hz), 137.9, 129.0, 124.2, 119.5, 117.3 (q, *J* = 301 Hz), 56.7, 55.9, 41.0, 31.6, 23.1, 22.1 ppm

**$^{19}$F NMR** (376 MHz, DMSO-$d_6$) δ –76.3

**HR-MS** (*m/z*): $[M+H]^+$ calcd for $C_{14}H_{22}N_3O_3^+$: 280.1656; found: 280.1655




***N*-Hydroxy-6-(methyl(2-oxo-2-(phenylamino)ethyl)amino)hexanamide** (**15**): To a suspension of 2-methylamino)-*N*-phenylacetamide[8] (0.288 g, 1.755 mmol, 1.0 equiv) and $K_2CO_3$ (0.606 g, 4.388 mmol,

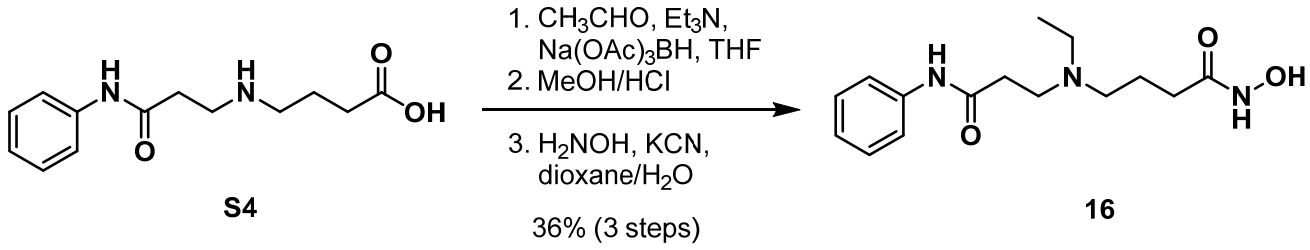
2.5 equiv) in DMF (5 mL) at 85 °C was added 6-bromohexanoic acid (0.787 g, 4.037 mmol, 2.30 equiv) in two portions, stirring at 85 °C for 20 h after each addition. After consumption of the amine, the reaction mixture was acidified with 20 mL 1 M HCl, and evaporated to dryness. The residue was re-suspended in 1 M HCl in MeOH (20 mL), stirred at rt for 16 h, then concentrated, re-dissolved in water (pH < 2) and extracted with EtOAc (2 × 40 mL). The aqueous phase was basified with $K_2CO_3$ until pH > 12, then extracted with $CH_2Cl_2$ (3 × 50 mL). The combined organic phases were dried ($MgSO_4$), filtered, and concentrated in vacuo. The product was purified by MPLC (12 g silica, gradient: 0% MeOH and 0.5% $NH_4OH$ in $CH_2Cl_2$ for 3 CV, then 0 → 1% MeOH and 0.5% $NH_4OH$ in $CH_2Cl_2$ over 10 CV, then 1 → 3% over 8 CV) to provide the methyl ester of **15** as a yellow oil (97 mg, *m/z*: $[M+H]^+$: 293.2, $[M-H]^-$: 291.2), which was directly converted to **15** according to General Procedure A. The product was purified by RP-MPLC (15.5 g C18Aq Teledyne Isco #69-2203-559, gradient: 0% B for 2 CV, 0 → 28% B over 19 CV, then 28% B over 6 CV) to provide **15** as a white solid (72.5 mg, 0.247 mmol, 14% yield (3 steps)).

**TLC** $R_f$ 0.36 (10% MeOH in $CH_2Cl_2$)

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 9.62 (br s, 3H*), 7.68 – 7.59 (m, 2H), 7.34 – 7.25 (m, 2H), 7.04 (tt, *J* = 7.3, 1.2 Hz, 1H), 3.10 (s, 2H), 2.41 (t, *J* = 7.3 Hz, 2H), 2.27 (s, 3H), 1.93 (t, *J* = 7.3 Hz, 2H), 1.56 – 1.38 (m, 4H), 1.32 – 1.19 (m, 2H) ppm; *partially exchanged with adventitious water

**$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 169.0, 168.8, 138.6, 128.7, 123.3, 119.3, 61.4, 57.0, 42.4, 32.3, 26.4 ($2CH_2$ overlapped), 25.1 ppm

**HR-MS** (*m/z*): $[2M+Na]^+$ calcd for $C_{30}H_{46}N_6NaO_6^+$: 609.3371; found: 609.3371




**S4** **16**

**4-(Ethyl(3-oxo-3-(phenylamino)propyl)amino)-*N*-hydroxybutanamide** (**16**): The corresponding methyl ester of the title compound was prepared from **S4**·HCl (100 mg, 0.349 mmol, 1.0 equiv) and acetaldehyde (192 µL, 3.49 mmol, 10 equiv) according to General Procedure C. The crude product was converted to hydroxamic acid **16** according to General Procedure A. The product was purified by HPLC Acidic Method (gradient: 1 → 10% B in 3 min, then 10 → 45% B in 11 min) to provide the TFA salt of **16** as a yellow, hygroscopic, amorphous solid (51.8 mg, 0.127 mmol, 36% yield (3 steps)).

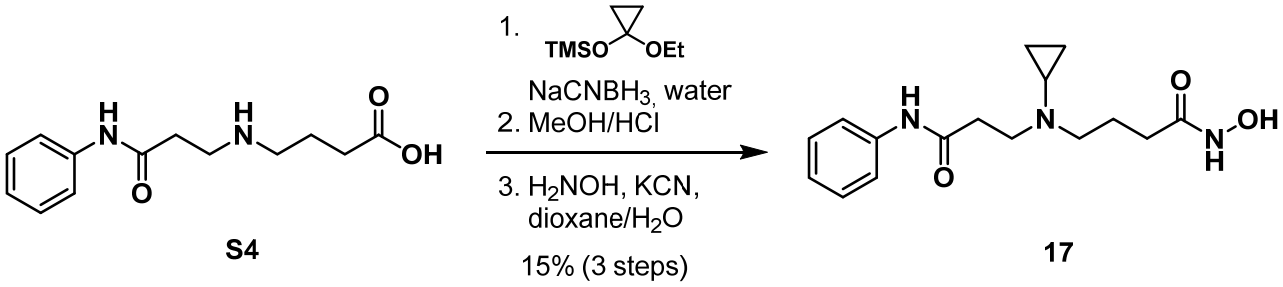
**TLC** $R_f$ 0.08 (10% MeOH in $CH_2Cl_2$)

**$^1$H NMR** (400 MHz, $D_2O$) δ 7.48 – 7.41 (m, 4H), 7.32 – 7.22 (m, 1H), 3.59 – 3.50 (m, 2H), 3.31 (q, *J* = 7.3 Hz, 2H), 3.28 – 3.18 (m, 2H), 2.95 (t, *J* = 6.8 Hz, 2H), 2.36 – 2.28 (m, 2H), 2.12 – 2.01 (m, 2H), 1.34 (t, *J* = 7.3 Hz, 3H) ppm

**$^{13}$C NMR** (101 MHz, $D_2O$) δ 174.0, 173.0, 139.3, 132.1, 128.6, 124.7, 165.8 (q, *J* = 36 Hz), 119.2 (q, *J* = 292 Hz), 54.7, 51.3, 51.1, 32.9, 31.9, 22.1, 11.0 ppm

**$^{19}$F NMR** (376 MHz, $D_2O$) δ –75.6 ppm

**HR-MS** (*m/z*): $[M+H]^+$ calcd for $C_{15}H_{24}N_3O_3^+$: 294.1812; found: 294.1816




**4-(Cyclopropyl(3-oxo-3-(phenylamino)propyl)amino)-*N*-hydroxybutanamide** (**17**): To a Schlenk tube charged with **S4**·HCl (150 mg, 0.523 mmol, 1.0 equiv) and $NaCNBH_3$ (49.3 mg, 0.785 mmol, 1.5 equiv) was added degassed water (1.5 mL), (1-ethoxycyclopropoxy)trimethylsilane (1.05 mL, 5.23 mmol, 10 equiv) and HCl (37%, 51.5 µL, 0.523 mmol, 1.0 equiv) under argon. After 1 d, additional $NaCNBH_3$ (as a freshly prepared 1 mmol solution in degassed $H_2O$) was added daily for 6 d (total 1.15 mL, 2.2 equiv) until complete consumption of the amine starting material. The reaction was evaporated to dryness. The residue was re-dissolved in water (10 mL) and acidified with HCl (25%) to pH 1.0, then the solvent was removed in vacuo. MeOH (25 mL) was added to the residue, stirred for 18 h, and then concentrated. The residue was partitioned between saturated aqueous $NaHCO_3$ (30 mL) and $CH_2Cl_2$ (20 mL) and the layers were separated. The aqueous layer was then extracted with $CH_2Cl_2$ (2 × 20 mL). The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. The crude product was converted to hydroxamic acid **17** according to General Procedure A. The product was purified by HPLC Acidic Method (gradient: 1 → 10% B in 3 min, then 10 → 45% B in 11 min) to provide the TFA salt of **17** as a white solid (32.8 mg, 0.078 mmol, 15% yield (3 steps)).

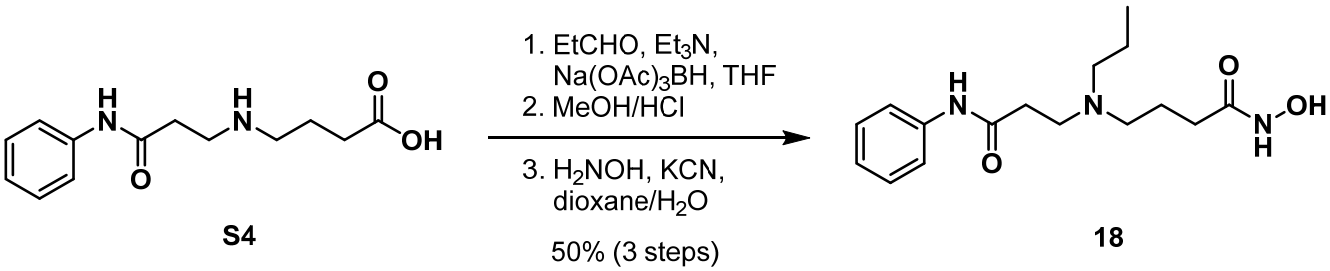
**TLC** $R_f$ 0.17 (10% MeOH in $CH_2Cl_2$)

**$^1$H NMR** (400 MHz, $D_2O$) δ 7.50 – 7.40 (m, 4H), 7.29 (qt, *J* = 5.5, 2.9 Hz, 1H), 3.71 (t, *J* = 6.7 Hz, 2H), 3.42 – 3.31 (m, 2H), 3.04 (t, *J* = 6.7 Hz, 2H), 2.86 (ddd, *J* = 11.2, 7.2, 4.6 Hz, 1H), 2.33 (t, *J* = 7.1 Hz, 2H), 2.15 (dq, *J* = 14.6, 7.2 Hz, 2H), 1.06 (dd, *J* = 13.8, 3.2 Hz, 2H), 1.05 (s, 2H) ppm

**$^{13}$C NMR** (101 MHz, $D_2O$) δ 174.1, 173.1, 165.9 (q, *J* = 36 Hz), 139.3, 132.1, 128.6, 124.7, 119.2 (q, *J* = 292 Hz), 58.1, 54.0, 40.4, 33.3, 32.0, 22.3, 7.3 (2 $CH_2$)* ppm; *broadened due to relatively slow nitrogen inversion.[9]

**$^{19}$F NMR** (376 MHz, $D_2O$) δ –78.4 ppm

**HR-MS** (*m/z*): $[M+H]^+$ calcd for $C_{16}H_{24}N_3O_3^+$: 306.1812; found: 306.1817




**S4** **18**

**4-(Propyl(3-oxo-3-(phenylamino)propyl)amino)-*N*-hydroxybutanamide** (**18**): The corresponding methyl ester of the title compound was prepared from **S4**·HCl (150 mg, 0.523 mmol, 1.0 equiv) and propanal (375 µl, 5.23 mmol, 10 equiv) according to General Procedure C. The crude product was converted to hydroxamic acid **18** according to General Procedure A. The product was purified by HPLC Acidic Method (gradient: 1 → 10% B in 3 min, then 10 → 45% B in 11 min) to provide the TFA salt of **18** as a yellow, hygroscopic, amorphous solid (110 mg, 0.260 mmol, 50% yield (3 steps)).

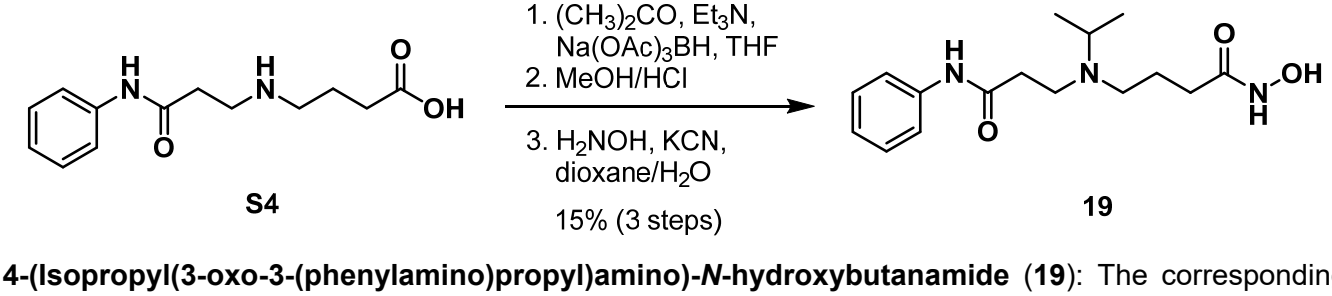
**TLC** $R_f$ 0.17 (10% MeOH in $CH_2Cl_2$).

**$^1$H NMR** (600 MHz, $D_2O$) δ 7.44 – 7.37 (m, 4H), 7.24 (tt, *J* = 5.8, 2.6 Hz, 1H), 3.51 (q, *J* = 6.5 Hz, 2H), 3.25 – 3.17 (m, 2H), 3.17 – 3.09 (m, 2H), 2.90 (t, *J* = 6.8 Hz, 2H), 2.28 (t, *J* = 7.1 Hz, 2H), 2.06 – 1.97 (m, 2H), 1.78 – 1.68 (m, 2H), 0.95 (t, *J* = 7.4 Hz, 3H) ppm

**$^{13}$C-NMR** (151 MHz, $D_2O$) δ 171.1, 170.1, 162.9 (q, *J* = 36 Hz), 136.4, 129.2, 125.7, 121.7, 116.3 (q, *J* = 292 Hz), 54.8, 52.3, 48.7, 29.9, 28.9, 19.1, 16.8, 10.0 ppm

**$^{19}$F NMR** (376 MHz, $D_2O$) δ –75.6 ppm

**HR-MS** (*m/z*): $[M+H]^+$ calcd for $C_{16}H_{26}N_3O_3^+$: 308.1969; found: 308.1974




**S4** **19**

**4-(Isopropyl(3-oxo-3-(phenylamino)propyl)amino)-*N*-hydroxybutanamide** (**19**): The corresponding methyl ester of the title compound was prepared from **S4**·HCl (150 mg, 0.523 mmol, 1.0 equiv) and

acetone (390 µL, 5.23 mmol, 10 equiv) according to General Procedure C. The crude product was converted to hydroxamic acid **19** according to General Procedure A. The product was purified by HPLC Acidic Method (gradient: 1 → 10% B in 3 min, then 10 → 45% B in 11 min) to provide the TFA salt of **19** as a yellow, hygroscopic, amorphous solid (33.0 mg, 0.078 mmol, 15% yield (3 steps)).

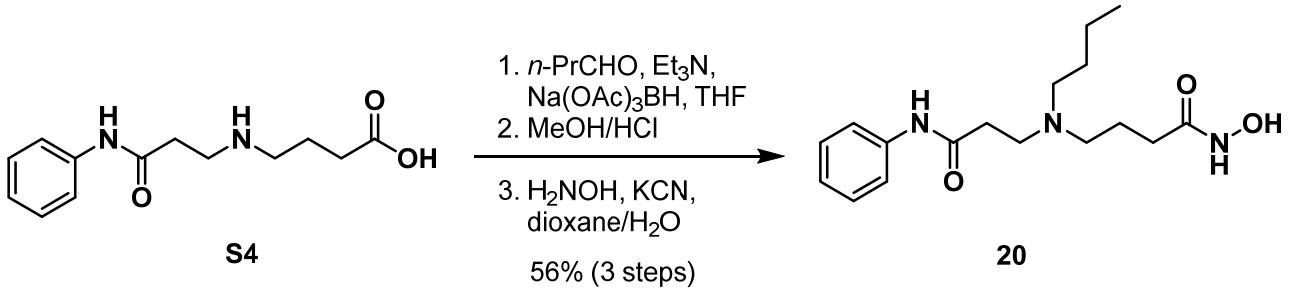
**TLC** $R_f$ 0.04 (10% MeOH in $CH_2Cl_2$)

**$^1$H NMR** (400 MHz, $D_2O$) δ 7.48 – 7.39 (m, 4H), 7.28 (dtd, *J* = 8.4, 5.0, 3.1 Hz, 1H), 3.78 (hept, *J* = 6.6 Hz, 1H), 3.59 (dt, *J* = 13.9, 7.0 Hz, 1H), 3.39 (dt, *J* = 13.4, 6.5 Hz, 1H), 3.25 (ddd, *J* = 13.3, 9.0, 6.8 Hz, 1H), 3.14 (ddd, *J* = 13.4, 9.0, 6.5 Hz, 1H), 2.94 (t, *J* = 6.8 Hz, 2H), 2.33 (t, *J* = 7.0 Hz, 2H), 2.14 – 1.96 (m, 2H), 1.35 (t, *J* = 7.1 Hz, 6H) ppm

**$^{13}$C NMR** (101 MHz, $D_2O$) δ 174.0, 173.1, 165.8 (q, *J* = 35 Hz), 139.3, 132.1, 128.6, 124.7, 119.2 (q, *J* = 292 Hz), 58.6, 52.7, 48.9, 33.5, 32.1, 23.2, 18.7, 18.3 ppm

**$^{19}$F NMR** (376 MHz, $D_2O$) δ –78.4 ppm

**HR-MS** (*m/z*): $[M+H]^+$ calcd for $C_{16}H_{26}N_3O_3^+$: 308.1969; found: 308.1974




**S4** **20**

**4-(Butyl(3-oxo-3-(phenylamino)propyl)amino)-*N*-hydroxybutanamide** (**20**): The corresponding methyl ester of the title compound was prepared from **S4**·HCl (150 mg, 0.523 mmol, 1.0 equiv) and *n*-butanal (472 µL, 5.23 mmol, 10 equiv) according to General Procedure C. The crude product was converted to hydroxamic acid **20** according to General Procedure A. The product was purified by HPLC Acidic Method (gradient: 1 → 10% B in 3 min, then 10 → 45% B in 11 min) to provide the TFA salt of **20** as a yellow, hygroscopic, amorphous solid (127 mg, 0.292 mmol, 56% yield (3 steps)).

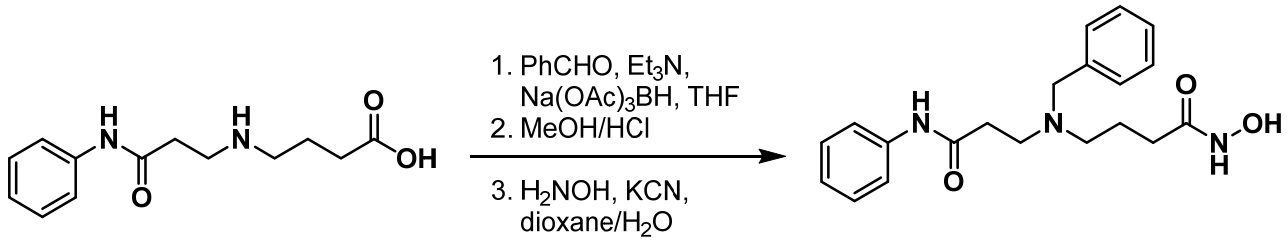
**TLC** $R_f$ 0.08 (10% MeOH in $CH_2Cl_2$).

**$^1$H NMR** (400 MHz, $D_2O$) δ 7.46 – 7.40 (m, 4H), 7.30 – 7.22 (m, 1H), 3.59 – 3.48 (m, 2H), 3.27 – 3.14 (m, 4H), 2.98 – 2.88 (m, 2H), 2.35 – 2.25 (m, 2H), 2.10 – 1.98 (m, 2H), 1.77 – 1.65 (m, 2H), 1.38 (app p, *J* = 7.5 Hz, 2H), 0.97 – 0.89 (m, 3H) ppm

**$^{13}$C NMR** (101 MHz, $D_2O$) δ 171.2, 170.2, 163.5 (q, *J* = 39 Hz), 136.5, 129.3, 125.8, 121.9, 116.4 (q, *J* = 291 Hz), 53.3, 52.4, 48.9, 30.0, 29.0, 25.2, 19.3 (2 $CH_2$), 12.8 ppm

**$^{19}$F NMR** (376 MHz, $D_2O$) δ –75.6 ppm

**HR-MS** (*m/z*): $[M+H]^+$ calcd for $C_{17}H_{28}N_3O_3^+$: 322.2125; found: 322.2127




**S4** **21**

**4-(Benzyl(3-oxo-3-(phenylamino)propyl)amino)-*N*-hydroxybutanamide** (**21**): The corresponding methyl ester of the title compound was prepared from **S4**·HCl (150 mg, 0.523 mmol, 1.0 equiv) and benzaldehyde (539 µL, 5.23 mmol, 10 equiv) according to General Procedure C. The crude product was converted to hydroxamic acid **21** according to General Procedure A. The produce was purified by HPLC Acidic Method (gradient: 1 → 10% B in 3 min, then 10 → 45% B in 11 min) to provide the TFA salt of **21** as an off-white solid (119 mg, 0.254 mmol, 49% yield (3 steps)).

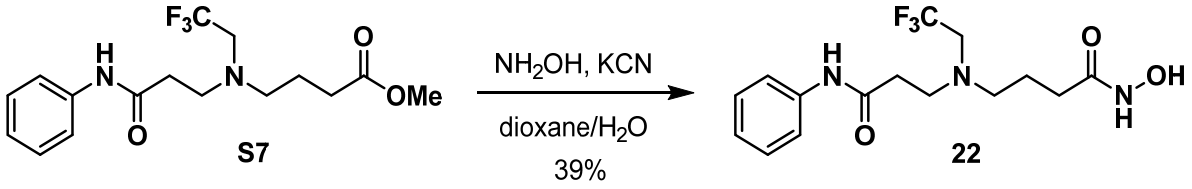
**TLC** $R_f$ 0.17 (10% MeOH in $CH_2Cl_2$)

**$^1$H NMR** (400 MHz, MeOD-$d_4$) δ 7.62 – 7.48 (m, 7H), 7.35 – 7.29 (m, 2H), 7.11 (tt, *J* = 7.4, 1.2 Hz, 1H), 4.45 (s, 2H), 3.54 (t, *J* = 6.8 Hz, 2H), 3.28 (t, *J* = 7.4 Hz, 2H), 2.91 (t, *J* = 6.8 Hz, 2H), 2.27 (t, *J* = 6.3 Hz, 2H), 2.09 (p, *J* = 6.7 Hz, 2H) ppm

**$^{13}$C NMR** (101 MHz, MeOD-$d_4$) δ 171.4, 170.1, 163.0 (q, *J* = 34 Hz), 139.4, 132.1, 131.3, 130.8, 130.6, 129.9, 125.5, 121.3, 118.2 (q, *J* = 293 Hz), 58.9, 54.6, 50.3, 31.1, 30.6, 20.7 ppm

**$^{19}$F NMR** (376 MHz, MeOD-$d_4$) δ –79.7 ppm

**HR-MS** (*m/z*): $[M+H]^+$ calcd for $C_{20}H_{26}N_3O_3^+$: 356.1969; found: 356.1972




**S7** **22**

***N*-Hydroxy-4-((3-oxo-3-(phenylamino)propyl)(2,2,2-trifluoroethyl)amino)butanamide** (**22**): Ester **S7** (164 mg, 0.473 mmol, 1.0 equiv) was converted to hydroxamic acid **22** according to General Procedure A. The product was purified by RP-MPLC (15.5 g C18Aq Teledyne Isco #69-2203-559, gradient: 15 → 23% B over 11 CV, then 23% B for 16.5 CV) to provide **22** as a white solid (63.3 mg, 0.182 mmol, 39% yield).

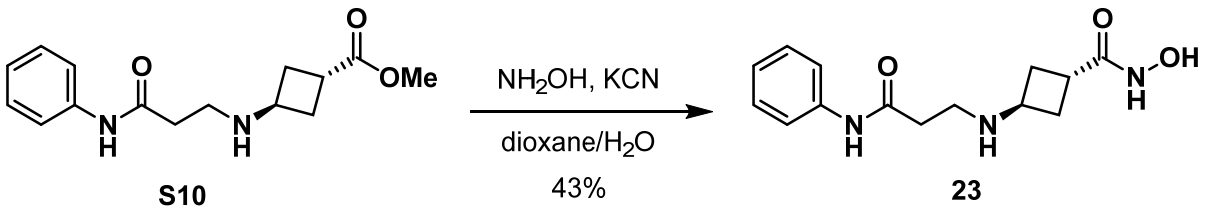
**TLC** $R_f$ 0.16 (5% MeOH in $CH_2Cl_2$)

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 10.32 (br s, 1H*), 9.44 (br s, 2H*), 7.59 (app d, *J* = 7.4 Hz, 2H), 7.27 (app t, *J* = 7.8 Hz, 2H), 7.01 (app t, *J* = 7.4 Hz, 1H), 3.22 (q, *J* = 10.1 Hz, 2H), 2.91 (t, *J* = 7.1 Hz, 2H), 2.56 (t, *J* = 7.3 Hz, 2H), 2.46 (t, *J* = 7.1 Hz, 2H), 1.95 (t, *J* = 7.2 Hz, 2H), 1.62 (app p, *J* = 7.3 Hz, 2H) ppm; *partially exchanged with adventitious water.

**$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 170.0, 168.6, 139.4, 128.6, 126.1 (q, *J* = 281 Hz), 122.9, 119.1, 53.98 (q, *J* = 29 Hz), 53.4, 51.0, 34.7, 29.6, 23.2 ppm

**$^{19}$F NMR** (376 MHz, DMSO-$d_6$) δ –71.9 ppm

**HR-MS** (*m/z*): $[2M+Na]^+$ calcd for $C_{30}H_{40}F_6N_6NaO_6^+$: 717.2806; found: 717.2807



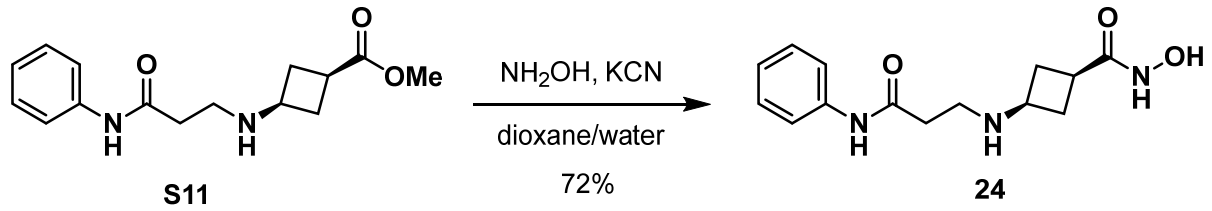

**(1*r*,3*r*)-*N*-Hydroxy-3-((3-oxo-3-(phenylamino)propyl)amino)cyclobutanecarboxamide** (**23**): Ester **S10** (131.2 mg, 0.475 mmol, 1.0 equiv) was converted to hydroxamic acid **23** according to General Procedure A. The product was purified by RP-MPLC (15.5 g C18 Teledyne Isco #69-2203-334, gradient: 0% B for 3 CV, then 0 → 20% over 20 CV). The resulting solid was triturated with EtOAc (twice) and $Et_2O$ (once) to provide **23** as a white solid (56.3 mg, 0.203 mmol, 43% yield).

**TLC** $R_f$ 0.25 (20% MeOH in $CH_2Cl_2$)

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 10.29 (br s, 1H*), 10.02 (s, 1H), 8.66 (br s, 1H*), 7.62 – 7.53 (m, 2H), 7.33 – 7.23 (m, 2H), 7.01 (tt, *J* = 7.4, 1.2, 1.2 Hz, 1H), 3.43 – 3.24 (m, overlapped with water peak), 2.79 – 2.66 (m, 3H), 2.40 (t, *J* = 6.7 Hz, 2H), 2.29 – 2.18 (m, 2H), 1.91 – 1.79 (m, 2H) ppm; *partially exchanged with adventitious water.

**$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 172.0, 170.5, 139.3, 128.7, 123.0, 119.1, 51.2, 42.6, 37.1, 32.5, 30.7 ppm

**HR-MS** (*m/z*): $[M+H]^+$ calcd for $C_{14}H_{20}N_3O_3^+$: 278.1499; found: 278.1500




S11 24

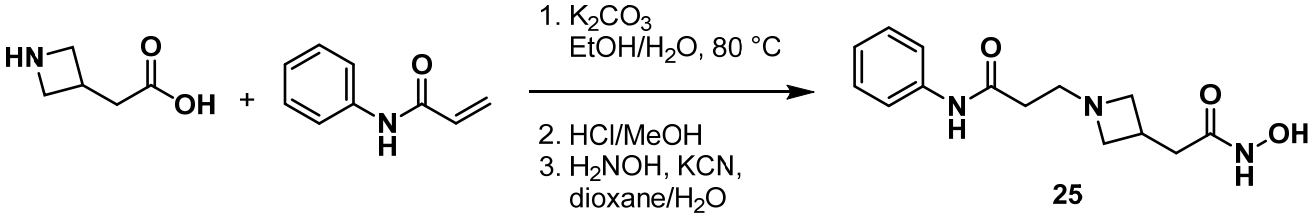
**(1*s*,3*s*)-*N*-Hydroxy-3-((3-oxo-3-(phenylamino)propyl)amino)cyclobutanecarboxamide** (**24**): Ester **S11** (138.6 mg, 0.502 mmol, 1.0 equiv) was converted to hydroxamic acid **24** according to General Procedure A. The product was purified by RP-MPLC (15.5 g C18Aq #69-2203-559, gradient: 0% B for 3 CV, 0 → 20% over 20 CV, then 20% over 10 CV) to provide **24** as a white solid (100.2 mg, 0.361 mmol, 72% yield).

**TLC** $R_f$ 0.07 (20% MeOH in $CH_2Cl_2$)

**$^1$H NMR** (600 MHz, DMSO-$d_6$) δ 10.34 (br s, 1H*), 10.03 (s, 1H), 9.12 – 8.28 (br, 1H*), 7.60 – 7.55 (m, 2H), 7.31 – 7.24 (m, 2H), 7.01 (tt, $J$ = 7.4, 1.2 Hz, 1H), 3.36 (br s, 1H*), 3.12 – 2.98 (m, 1H), 2.71 (t, $J$ = 6.8 Hz, 2H), 2.46 – 2.41 (m, 1H), 2.41 – 2.37 (m, 2H), 2.25 – 2.17 (m, 2H), 1.88 – 1.76 (m, 2H) ppm; *partially exchanged with adventitious water.

**$^{13}$C NMR** (151 MHz, DMSO-$d_6$) δ 170.5, 170.5, 139.3, 128.7, 122.9, 119.0, 49.5, 42.5, 37.1, 33.6, 29.1 ppm

**HR-MS** (*m/z*): $[M+H]^+$ calcd for $C_{14}H_{20}N_3O_3^+$: 278.1499; found: 278.1499




25

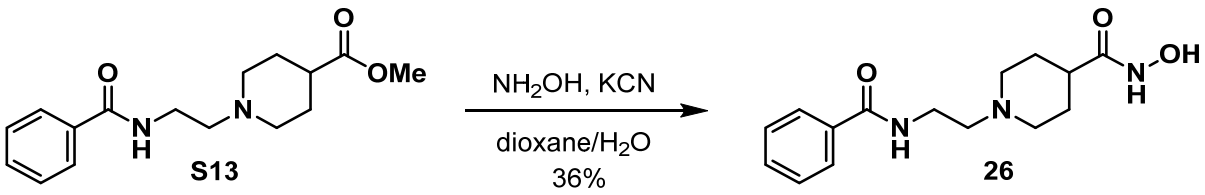
**3-(3-(2-(Hydroxyamino)-2-oxoethyl)azetidin-1-yl)-*N*-phenylpropanamide** (**25**): To a stirred solution of 2-(azetidin-3-yl)acetic acid (1.102 g, 4.809 mmol, 1.0 equiv) and *N*-phenylacrylamide (0.743 g, 5.05 mmol, 1.05 equiv) in $H_2O$/EtOH (1:1, 15 mL), was added $K_2CO_3$ (1.994 g, 14.43 mmol, 3.0 equiv) and the reaction mixture was stirred at 80 °C. After 5 h, the reaction mixture was cooled to rt, diluted with 1 M HCl (40 mL), washed with EtOAc (2 × 30 mL) and the aqueous layer was evaporated to dryness. The residue was suspended in MeOH (25 mL), acidified with 2M HCl in MeOH (5 mL), stirred at rt for 3 h, then concentrated to dryness. The residue was dissolved in half saturated aqueous $K_2CO_3$ (40 mL) and extracted with $CH_2Cl_2$ (3 × 25 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The product was purified by MPLC (40 g silica, gradient: pure $CH_2Cl_2$ for 2 CV, 0 → 7% MeOH in $CH_2Cl_2$ over 8 CV, then 7% MeOH in $CH_2Cl_2$ over 7 CV) to provide the corresponding methyl ester of the title compound as a

yellow oil (0.173 g, TLC $R_f$ 0.50 (20% MeOH in $CH_2Cl_2$), LC/MS (*m/z*): $[M+H]^+$ 277.2). The material was directly converted to hydroxamic acid **25** according to General Procedure A. The product was purified by RP-MPLC (15.5 g C18Aq Teledyne Isco #69-2203-559, gradient: 0% B for 3 CV, then 0 → 15% over 25 CV) to provide **25** as a white solid (76.0 mg, 0.274 mmol, 6% yield (3 steps)).

**$^1$H NMR** (600 MHz, DMSO-$d_6$) δ 9.98 (s, 1H), 9.90 (br s, 1H*), 9.14 (br s, 1H*), 7.58 (d, *J* = 8.1 Hz, 2H), 7.28 (t, *J* = 7.8 Hz, 2H), 7.01 (t, *J* = 7.4 Hz, 1H), 3.28 (t, *J* = 7.0 Hz, 2H), 2.75 (t, *J* = 6.6 Hz, 2H), 2.61 (t, *J* = 7.0 Hz, 2H), 2.59 – 2.52 (m, 1H), 2.27 (t, *J* = 6.9 Hz, 2H), 2.19 (d, *J* = 7.7 Hz, 2H) ppm; *partially exchanged with adventitious water.

**$^{13}$C NMR** (151 MHz, DMSO-$d_6$) δ 170.0, 167.7, 139.3, 128.7, 123.0, 119.0, 59.5, 55.0, 36.9, 35.1, 27.4 ppm

**HR-MS** (*m/z*): $[M+H]^+$ calcd for $C_{14}H_{20}N_3O_3^+$: 278.1499; found: 278.1503




**1-(2-Benzamidoethyl)-*N*-hydroxypiperidine-4-carboxamide** (**26**): Ester **S13** (183 mg, 0.629 mmol, 1.0 equiv) was converted to hydroxamic acid **26** according to General Procedure A. The product was purified by RP-MPLC (15.5 g C18Aq Teledyne Isco #69-2203-559, gradient: 0 → 9% B over 4 CV, 9% B for 4 CV, 9 → 21% B over 6 CV, then 21% B for 4 CV) to provide **26** as a white solid (65.5 mg, 0.225 mmol, 36% yield).

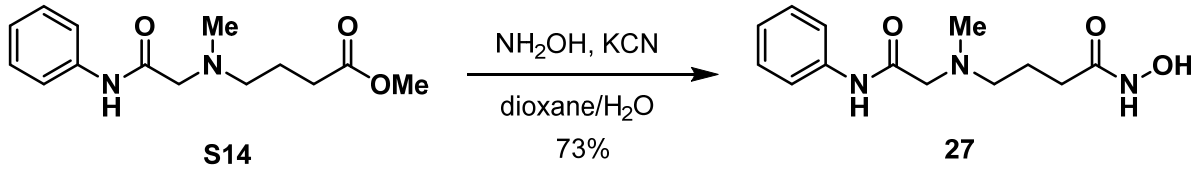
**TLC** $R_f$ 0.08 (10% MeOH and 0.5% $NH_4OH$ in $CH_2Cl_2$)

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 9.51 (br s, 2H*), 8.37 (t, *J* = 5.6 Hz, 1H), 7.89 – 7.76 (m, 2H), 7.56 – 7.49 (m, 1H), 7.48 – 7.40 (m, 2H), 3.36 (app q, *J* = 6.6 Hz, 2H), 2.91 (dt, *J* = 11.4, 3.5 Hz, 2H), 2.44 (t, *J* = 7.0 Hz, 2H), 2.05 – 1.82 (m, 3H), 1.67 – 1.46 (m, 4H) ppm; *partially exchanged with adventitious water.

**$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 171.5, 166.1, 134.6, 131.0, 128.3, 127.1, 57.2, 52.8, 39.4, 37.0, 28.5 ppm

**LC/MS** (*m/z*): $[M+H]^+$ 292.2

**HR-MS** (*m/z*): $[2M+Na]^+$ calcd for $C_{30}H_{42}N_6NaO_6^+$: 605.3064; found: 605.3062




**S14** **27**

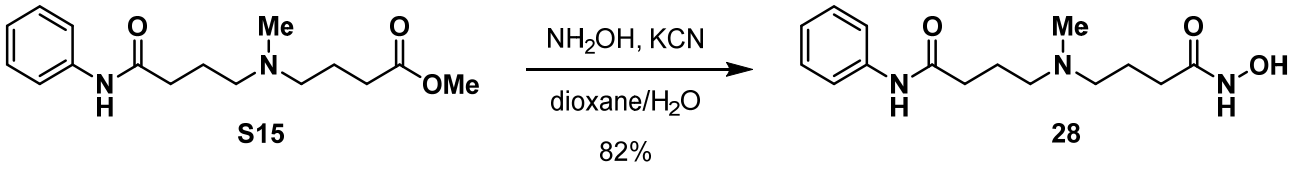
***N*-Hydroxy-4-(methyl(2-oxo-2-(phenylamino)ethyl)amino)butanamide** (**27**): Ester **S14** (165 mg, 0.625 mmol, 1.0 equiv) was converted to hydroxamic acid **27** according to General Procedure A. The product was purified by RP-MPLC (15.5 g C18Aq Teledyne Isco #69-2203-559, gradient: 0 → 20% B over 25 CV, then 20% B for 10 CV) to provide **27** as an off-white solid (121 mg, 0.457 mmol, 73% yield).

**TLC** $R_f$ 0.29 (20% MeOH in $CH_2Cl_2$)

**$^1$H NMR** (600 MHz, DMSO-$d_6$) δ 10.11 – 8.98 (br, 3H*), 7.69 – 7.63 (m, 2H), 7.32 – 7.27 (m, 2H), 7.05 (tt, *J* = 7.4, 1.2 Hz, 1H), 3.09 (s, 2H), 2.41 (t, *J* = 7.1 Hz, 2H), 2.25 (s, 3H), 2.01 (t, *J* = 7.3 Hz, 2H), 1.69 (app p, *J* = 7.0 Hz, 2H) ppm; *partially exchanged with adventitious water.

**$^{13}$C NMR** (151 MHz, DMSO-$d_6$) δ 169.3, 169.0, 138.6, 128.6, 123.3, 119.4, 61.5, 56.7, 42.4, 30.4, 23.0 ppm

**HR-MS** (*m/z*): $[M+H]^+$ calcd for $C_{13}H_{20}N_3O_3^+$: 266.1499; found: 266.1504




**S15** **28**

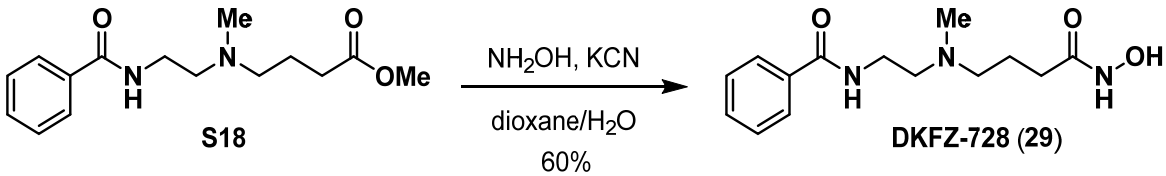
***N*-Hydroxy-4-(methyl(4-oxo-4-(phenylamino)butyl)amino)butanamide** (**28**): Ester **S15** (156 mg, 0.535 mmol, 1.0 equiv) was converted to hydroxamic acid **28** according to General Procedure A. The product was purified by RP-MPLC (15.5 g C18Aq Teledyne Isco #69-2203-559, gradient: 0% B for 4 CV, 0 → 20% B over 23 CV, then 20% B for 10 CV) to provide **28** as a white solid (129 mg, 0.440 mmol, 82% yield).

**TLC** $R_f$ 0.11 (10% MeOH and 0.5% $NH_4OH$ in $CH_2Cl_2$)

**$^1$H NMR** (600 MHz, DMSO-$d_6$) δ 11.13 – 8.04 (br, 2H*), 9.87 (s, 1H), 7.62 – 7.56 (m, 2H), 7.30 – 7.23 (m, 2H), 7.01 (tt, *J* = 7.3, 1.2 Hz, 1H), 2.36 – 2.26 (m, 4H), 2.24 (t, *J* = 7.3 Hz, 2H), 2.11 (s, 3H), 1.96 (t, *J* = 7.5 Hz, 2H), 1.70 (app p, *J* = 7.3 Hz, 2H), 1.61 (app p, *J* = 7.4 Hz, 2H) ppm; *partially exchanged with adventitious water.

**$^{13}$C NMR** (151 MHz, DMSO-$d_6$) δ 171.2, 169.2, 139.4, 128.6, 122.9, 119.0, 56.5 ($2CH_2$ overlapped), 41.7, 34.3, 30.2, 22.9, 22.8 ppm

**HR-MS** (*m/z*): $[M+H]^+$ calcd for $C_{15}H_{24}N_3O_3^+$: 294.1812; found: 294.1814




***N*-(2-((4-(Hydroxyamino)-4-oxobutyl)(methyl)amino)ethyl)benzamide** (**DKFZ-728**; **29**): Ester **S18** (259 mg, 0.931 mmol, 1.0 equiv) was converted to hydroxamic acid **29** according to General Procedure A. The product was purified by RP-MPLC (15.5 g C18Aq Teledyne Isco #69-2203-559, gradient: 0 → 17% B over 17 CV) to provide **DKFZ-728 (29)** as a white solid (155 mg, 0.55 mmol, 60% yield).

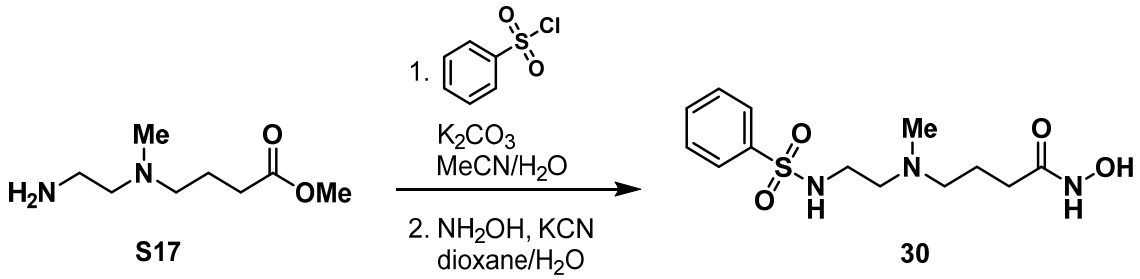
**TLC** $R_f$ 0.26 (20% MeOH in $CH_2Cl_2$)

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 9.49 (br s, 2H*), 8.38 (t, $J$ = 5.7 Hz, 1H), 7.89 – 7.77 (m, 2H), 7.54 – 7.41 (m, 3H), 3.34 (app q, $J$ = 6.4 Hz, 2H), 2.46 (t, $J$ = 7.0 Hz, 2H), 2.31 (t, $J$ = 7.2 Hz, 2H), 2.18 (s, 3H), 1.96 (t, $J$ = 7.4 Hz, 2H), 1.62 (app p, $J$ = 7.2 Hz, 2H) ppm; *partially exchanged with adventitious water.

**$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 169.1, 166.1, 134.6, 131.0, 128.3, 127.1, 56.5, 56.1, 42.0, 37.3, 30.1, 22.9 ppm

**LC/MS** (*m/z*): $[M+H]^+$ 280.2, $[M-H]^-$ 278.2

**HR-MS** (*m/z*): $[M+H]^+$ calcd for $C_{14}H_{22}N_3O_3^+$: 280.1656; found: 280.1657




***N*-Hydroxy-4-(methyl(2-(phenylsulfonamido)ethyl)amino)butanamide** (**30**): To a stirred solution of **S17**·2HCl (121.9 mg, 0.493 mmol, 1.0 equiv) and $K_2CO_3$ (239 mg, 1.726 mmol, 3.5 equiv) in MeCN/water (5:1, 12 mL) was added benzenesulfonyl chloride (82 µL, 0.641 mmol, 1.3 equiv) at rt. After 16 h, TLC indicated complete consumption of **S17**, and the reaction mixture was concentrated in vacuo. The residue was partitioned between EtOAc (25 mL) and half saturated aqueous $K_2CO_3$ (30 mL). The phases were separated and the aqueous phase was extracted with EtOAc (2 × 25 mL). The combined organic layers were washed with half saturated aqueous $K_2CO_3$ (2 × 25 mL) and brine (25 mL), dried ($MgSO_4$), filtered, and concentrated. The crude product was directly converted to hydroxamic acid **30** according to General Procedure A. The product was purified by HPLC Basic

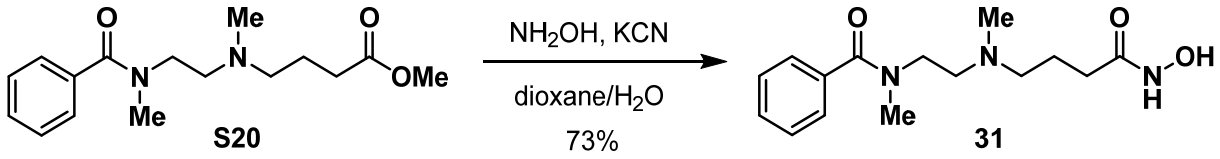
Method (gradient: 1 → 40% B in 13 min) to provide **30** as white solid (112.3 mg, 0.341 mmol, 69% yield (2 steps)).

**TLC** $R_f$ 0.33 (20% MeOH in $CH_2Cl_2$)

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 8.83 (br s, 3H*), 7.81 (d, *J* = 7.2 Hz, 2H), 7.71 – 7.52 (m, 3H), 2.81 (t, *J* = 7.0 Hz, 2H), 2.28 (t, *J* = 7.0 Hz, 2H), 2.17 (t, *J* = 7.2 Hz, 2H), 2.02 (s, 3H), 1.90 (t, *J* = 7.4 Hz, 2H), 1.52 (app p, *J* = 7.3 Hz, 2H) ppm; *partially exchanged with adventitious water.

**$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 169.0, 140.6, 132.3, 129.2, 126.5, 56.4, 56.2, 41.6, 40.6, 30.1, 22.8 ppm

**HR-MS** (*m/z*): $[M+H]^+$ calcd for $C_{13}H_{22}N_3O_4S^+$: 316.1326; found: 316.1328




***N*-(2-((4-(Hydroxyamino)-4-oxobutyl)(methyl)amino)ethyl)-*N*-methylbenzamide** (**31**): Ester **S20** (179 mg, 0.613 mmol, 1.0 equiv) was converted to hydroxamic acid **31** according to General Procedure A. The product was purified by RP-MPLC (15.5 g C18Aq Teledyne Isco #69-2203-559, gradient: 0% B for 2 CV, 0 → 14% B over 14 CV, then 14% B for 10 CV) to provide **31** as an amorphous, colorless solid (131 mg, 0.447 mmol, 73% yield).

**TLC** $R_f$ 0.26 (10% MeOH and 0.5% $NH_4OH$ in $CH_2Cl_2$)

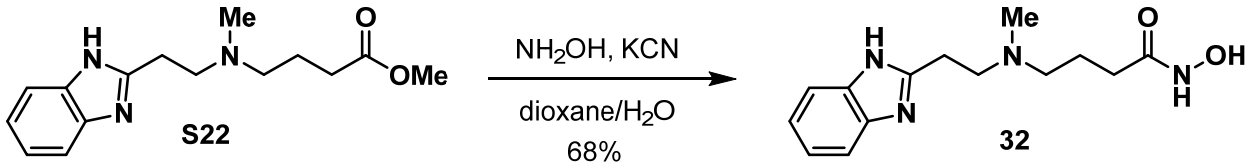
**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 9.49 (br s, 2H*, –NH–OH), 7.52 – 7.39 (m, 3H, Ar H), 7.39 – 7.25 (m, 2H, Ar H), 3.52 (t, *J* = 6.8 Hz, 1H, –CONMeC$\underline{H_2}$– rotamer A), 3.24 (t, *J* = 6.8 Hz, 1H, CONMeC$\underline{H_2}$ rotamer B), 2.96 (s, 1.5H, –CONC$\underline{H_3}$ rotamer A), 2.89 (s, 1.5H, –CONC$\underline{H_3}$ rotamer B), 2.59 – 2.51 (m, 1H, N–$CH_2$–C$\underline{H_2}$–N rotamer A), 2.43 – 2.27 (m, 2H, N–$CH_2$–C$\underline{H_2}$–N rotamer B and N–C$\underline{H_2}$–$CH_2$–$CH_2$– rotamer A), 2.26 – 2.05 (m, 2.5H, –$CH_2$–NC$\underline{H_3}$–$CH_2$– rotamer A and N–C$\underline{H_2}$–$CH_2$–$CH_2$– rotamer B), 2.05 – 1.74 (m, 3.5H, –C$\underline{H_2}$CONHOH and –$CH_2$–NC$\underline{H_3}$–$CH_2$– rotamer B), 1.73 – 1.58 (m, 1H, –$CH_2$–C$\underline{H_2}$–$CH_2$– rotamer A), 1.58 – 1.40 (m, 1H, –$CH_2$–C$\underline{H_2}$–$CH_2$– rotamer B) ppm; *partially exchanged with adventitious water.

**$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 170.9 (Ar–CO rotamer A), 169.9 (Ar–CO rotamer B), 169.0 (CONHOH), 136.8 (Ar$\underline{C}$–CO), 129.1 (ArCH rotamer A), 129.1 (ArCH rotamer B), 128.3 (ArCH), 126.6 (ArCH), 56.8 (N–$\underline{C}H_2$–$CH_2$–$CH_2$–), 54.8 (N–$CH_2$–$\underline{C}H_2$–N rotamer A), 54.2 (N–$CH_2$–$\underline{C}H_2$–N rotamer B), 48.4 (N–$\underline{C}H_2$–$CH_2$–N rotamer A), 44.6 (N–$\underline{C}H_2$–$CH_2$–N rotamer B), 41.9 (–$CH_2$–N$\underline{C}H_3$–$CH_2$– rotamer A), 41.7 (–$CH_2$–N$\underline{C}H_3$–$CH_2$– rotamer B), 37.6 (–CON$\underline{C}H_3$ rotamer A), 32.7 (–CON$\underline{C}H_3$ rotamer B), 30.1 (–

$\underline{C}H_2CONHOH$ rotamer A), 30.0 ($-\underline{C}H_2CONHOH$ rotamer B), 23.1 ($-CH_2-\underline{C}H_2-CH_2-$ rotamer A), 22.9 ($-CH_2-\underline{C}H_2-CH_2-$ rotamer B) ppm

Note: The $^1H$ and $^{13}C$ NMR spectra each show two rotamers with overlapping signals. An EXSY experiment confirmed that each set of two signals originates from the same nucleus**.** Overlapping rotamer signals were differentiated by the attached HSQC edit experiment. The ratio of rotamer A to B is 1:1.

**HR-MS** (*m/z*): $[2M+Na]^+$ calcd for $C_{30}H_{46}N_6NaO_6^+$: 609.3371; found: 609.3371



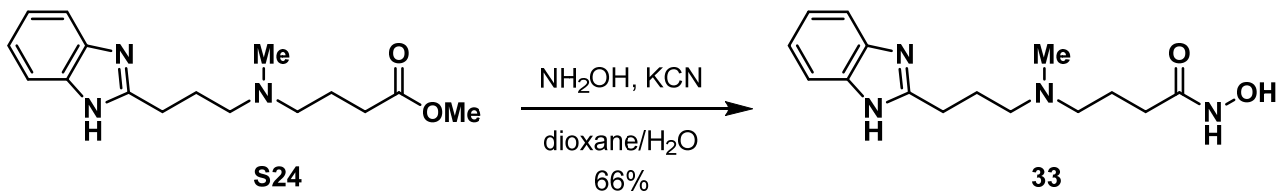

**4-((2-(Benzimidazol-2-yl)ethyl)(methyl)amino)-*N*-hydroxybutanamide (32):** Ester **S22** (178 mg, 0.646 mmol, 1.0 equiv) was converted to hydroxamic acid **32** according to General Procedure A. The product was purified by RP-MPLC (15.5 g C18Aq Teledyne Isco #69-2203-559, gradient: 0% B for 6 CV, 0 → 18% B over 25 CV, then 18% B over 4 CV) to provide **32** as white solid (122 mg, 0.442 mmol, 68% yield).

**TLC** $R_f$ 0.10 (20% MeOH in $CH_2Cl_2$)

**$^1$H NMR** (600 MHz, DMSO-$d_6$) $\delta$ 13.37 – 9.78 (br, 2H*), 9.71 – 7.75 (br, 1H*), 7.50 – 7.41 (m, 2H, Ar H), 7.13 – 7.07 (m, 2H, Ar H), 2.93 (t, $J$ = 7.5 Hz, 2H, $NCH_2$), 2.76 (t, $J$ = 7.5 Hz, 2H, $Ar-CH_2$), 2.33 (t, $J$ = 7.1 Hz, 2H, $NCH_2$), 2.18 (s, 3H, Me), 1.95 (t, $J$ = 7.4 Hz, 2H, $COCH_2$), 1.63 (app p, $J$ = 7.3 Hz, 2H, $CH_2-CH_2-CH_2$) ppm; *partially exchanged with adventitious water.

**$^{13}$C NMR** (151 MHz, DMSO-$d_6$) $\delta$ 169.2 (CONHOH), 153.9 (N=CNH), 121.1 (Ar CH), 55.9 ($CH_2$), 55.3 ($Ar-CH_2$), 41.6 (Me), 30.1 ($CH_2$), 26.6 ($CH_2$), 22.8 ($CH_2$) ppm; Due to tautomerism of the benzimidazole NH, two benzimidazole carbon signals were too broad to be identified in $^{13}C$ spectra. See the spectrum for **33**, where very broad signals could be observed.

**HR-MS** (*m/z*): $[M+H]^+$ calcd for $C_{14}H_{21}N_4O_2^+$: 277.1659; found: 277.1661




**4-((3-(Benzimidazol-2-yl)propyl)(methyl)amino)-*N*-hydroxybutanamide** (**33**): Ester **S24** (118 mg, 0.408 mmol, 1.0 equiv) was converted to hydroxamic acid **33** according to General Procedure A. The

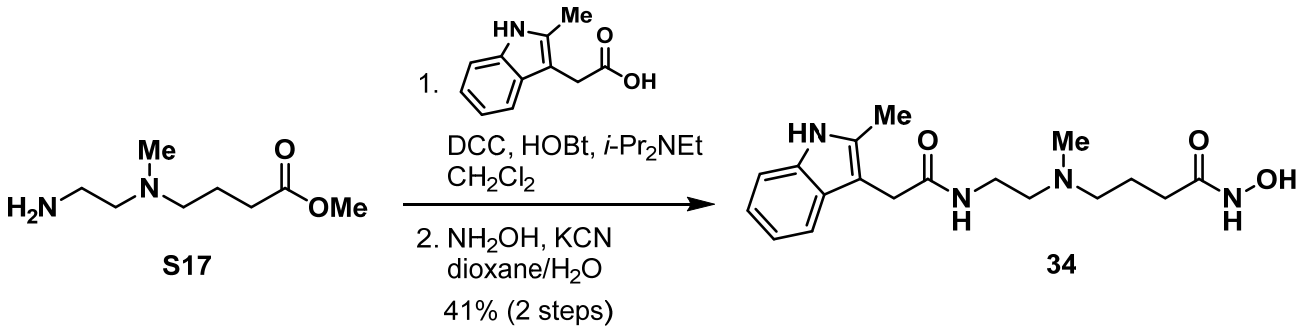
product was purified by RP-MPLC (15.5 g C18Aq Teledyne Isco #69-2203-559, gradient: 0% B for 8 CV, 0 → 19% B over 22 CV, then 19% B over 5 CV) to provide **33** as a colorless solid (78.6 mg, 0.271 mmol, 66% yield).

**TLC** $R_f$ 0.07 (20% MeOH in $CH_2Cl_2$)

**$^1$H NMR** (600 MHz, DMSO-$d_6$) δ 12.17 (br s, 1H*), 10.47 (br s, 1H*), 9.43 – 8.42 (br, 1H*), 7.50 – 7.41 (m, 2H), 7.12 – 7.08 (m, 2H), 2.81 (t, *J* = 7.7 Hz, 2H), 2.33 (t, *J* = 7.0 Hz, 2H), 2.25 (t, *J* = 7.1 Hz, 2H), 2.12 (s, 3H), 1.98 (t, *J* = 7.4 Hz, 2H), 1.87 (app p, *J* = 7.3 Hz, 2H), 1.62 (app p, *J* = 7.3 Hz, 2H) ppm; *partially exchanged with adventitious water.

**$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 169.2, 155.2, 142.2 (br), 134.9 (br), 121.1, 117.3 (br), 111.7 (br), 56.6, 56.4, 41.7, 30.2, 26.4, 25.4, 22.9 ppm; the broad signals are due to tautomerism of the benzimidazole NH.

**HR-MS** (*m/z*): $[M+H]^+$ calcd for $C_{15}H_{23}N_4O_2^+$: 291.1816; found: 291.1816




***N*-Hydroxy-4-(methyl(2-(2-(2-methyl-1*H*-indol-3-yl)acetamido)ethyl)amino)butanamide** (**34**): To a suspension of diamine **S17**·2HCl (84.6 mg, 0.342 mmol, 1.0 equiv), DCC (106 mg, 0.513 mmol, 1.5 equiv), HOBt (5 mg, 0.034 mmol, 0.1 equiv) and 2-methyl-3-indoleacetic acid (71.2 mg, 0.377 mmol, 1.1 equiv) in $CH_2Cl_2$ (8 mL) was added *i*-$Pr_2$NEt (0.146 mL, 0.856 mmol, 2.5 equiv). The reaction mixture was stirred at rt for 16 h, then cooled to –20 °C and filtered through a fritted syringe into EtOAc (50 mL). The solution was washed with saturated aqueous $NaHCO_3$ (3 × 40 mL), then dried ($MgSO_4$), filtered, and concentrated. The residue still contained DCU crystals and was therefore suspended in $Et_2O$/MeCN (1:1, 3 mL) and filtered through a cotton plug. The concentrated filtrate was directly converted to hydroxamic acid **34** according to General Procedure A. The product was purified by HPLC Basic Method (gradient: 1 → 50% B in 12 min) to provide **34** as an off-white solid (48.1 mg, 0.139 mmol, 41% yield (2 steps)).

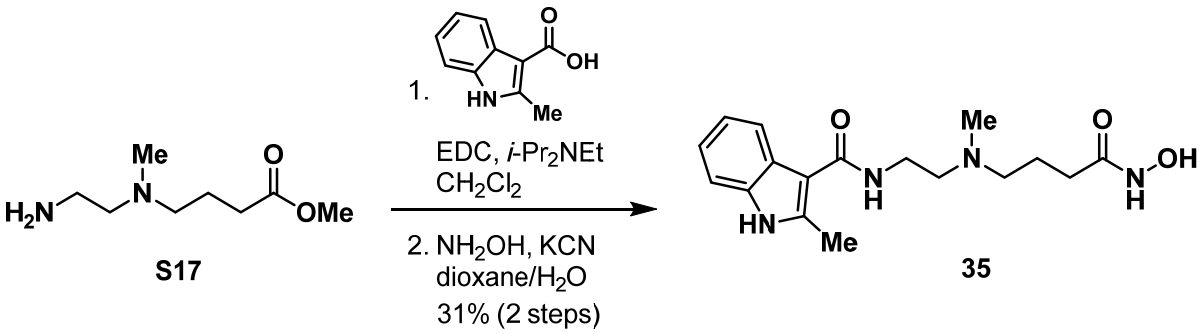
**TLC** $R_f$ 0.33 (20% MeOH in $CH_2Cl_2$)

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 10.78 (br s, 1H*), 9.45 (br s, 2H*), 7.60 (t, *J* = 5.6 Hz, 1H), 7.43 (dt, *J* = 7.6, 1.0 Hz, 1H), 7.22 (dt, *J* = 8.0, 1.0 Hz, 1H), 6.96 (ddd, *J* = 8.0, 7.0, 1.3 Hz, 1H), 6.90 (ddd, *J* = 8.0,

7.0, 1.2 Hz, 1H), 3.43 (s, 2H), 3.09 (app q, *J* = 6.4 Hz, 2H), 2.33 (s, 3H), 2.29 (t, *J* = 6.8 Hz, 2H), 2.22 (t, *J* = 7.2 Hz, 2H), 2.08 (s, 3H), 1.91 (t, *J* = 7.4 Hz, 2H), 1.55 (app p, *J* = 7.3 Hz, 2H) ppm; *partially exchanged with adventitious water.

**$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 170.6, 169.0, 135.1, 133.0, 128.4, 119.9, 118.1, 117.8, 110.2, 104.9, 56.5, 56.3, 41.7, 36.7, 31.6, 30.2, 22.9, 11.4 ppm

**HR-MS** (*m/z*): $[M+H]^+$ calcd for $C_{18}H_{27}N_4O_3^+$: 347.2078; found: 347.2077



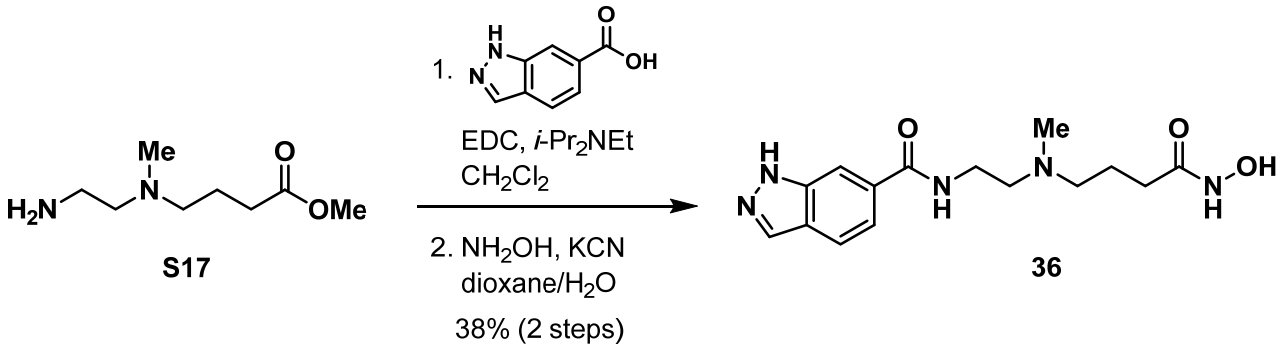

***N*-(2-((4-(Hydroxyamino)-4-oxobutyl)(methyl)amino)ethyl)-2-methyl-1*H*-indole-3-carboxamide** (**35**): The corresponding methyl ester of the title compound was prepared from ester **S17**·2HCl (70.4 mg, 0.285 mmol, 1.0 equiv) and 2-methyl-1*H*-indole-3-carboxylic acid (54.9 mg, 0.313 mmol, 1.1 equiv) according to General Procedure B. The crude product was directly converted to hydroxamic acid **35** according to General Procedure A. The product was purified by RP-MPLC (15.5 g C18 Teledyne Isco #69-2203-334, gradient: 0% B for 3 CV, then 0 → 15% over 12 CV) to provide **35** as white, fluffy solid (29.7 mg, 0.089 mmol, 31% yield (2 steps)).

**TLC** $R_f$ 0.17 (20% MeOH in $CH_2Cl_2$)

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 11.44 (s, 1H*), 10.80 – 8.18 (br, 2H*), 7.78 – 7.71 (m, 1H), 7.35 – 7.28 (m, 1H), 7.20 (t, *J* = 5.5 Hz, 1H), 7.11 – 7.01 (m, 2H), 3.41 – 3.33 (m, overlapped with water peak), 2.57 (s, 3H), 2.54 – 2.46 (m, overlapped with DMSO signal), 2.34 (t, *J* = 7.2 Hz, 2H), 2.20 (s, 3H), 1.98 (t, *J* = 7.5 Hz, 2H), 1.66 (app p, *J* = 7.3 Hz, 2H) ppm; *partially exchanged with adventitious water.

**$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 169.0, 165.2, 139.3, 134.6, 125.9, 120.9, 119.9, 119.2, 110.9, 107.7, 56.7, 56.5, 41.7, 36.6, 30.2, 23.1, 13.2 ppm

**HR-MS** (*m/z*): $[M+H]^+$ calcd for $C_{17}H_{25}N_4O_3^+$: 333.1921; found: 333.1921

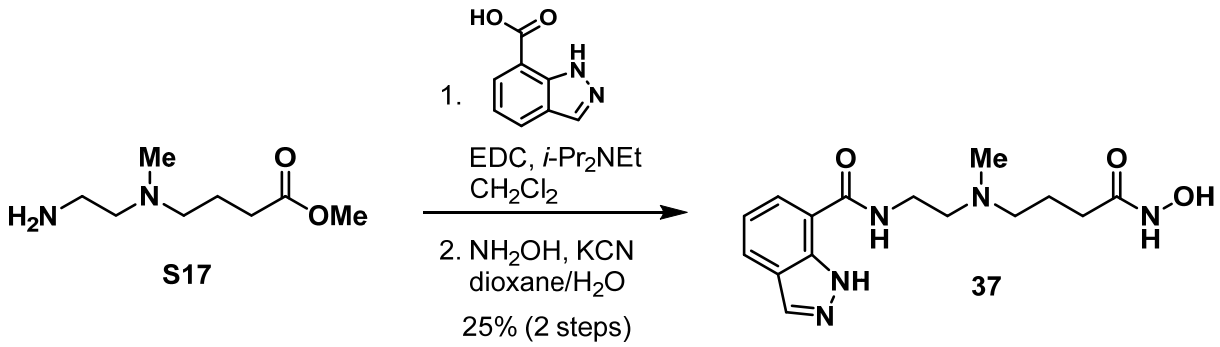



***N*-(2-((4-(Hydroxyamino)-4-oxobutyl)(methyl)amino)ethyl)-1H-indazole-6-carboxamide** (**36**): The corresponding methyl ester of the title compound was prepared from ester **S17**·2HCl (65.1 mg, 0.263 mmol, 1.0 equiv) and 1*H*-indazole-6-carboxylic acid (44.8 mg, 0.277 mmol, 1.05 equiv) according to General Procedure B. The crude product was directly converted to hydroxamic acid **36** according to General Procedure A. The product was purified by HPLC Basic Method (gradient: 1 → 25% B in 12 min) to provide **36** as a pale yellow solid (32.3 mg, 0.101 mmol, 38% yield (2 steps)).

**$^{1}$H NMR** (400 MHz, DMSO-$d_6$) $\delta$ 12.54 – 9.09 (br, 3H*), 8.50 (t, $J$ = 5.7 Hz, 1H), 8.13 (d, $J$ = 0.9 Hz, 1H), 8.04 (s, 1H), 7.80 (dd, $J$ = 8.5, 0.9 Hz, 1H), 7.57 (dd, $J$ = 8.5, 1.4 Hz, 1H), 3.38 (app q, $J$ = 6.5 Hz, 2H), 2.56 – 2.45 (m, overlapped with DMSO signal), 2.33 (t, $J$ = 7.3 Hz, 2H), 2.20 (s, 3H), 1.97 (t, $J$ = 7.3 Hz, 2H), 1.64 (app p, $J$ = 7.3 Hz, 2H) ppm; *partially exchanged with adventitious water.

**$^{13}$C NMR** (101 MHz, DMSO-$d_6$) $\delta$ 169.1, 166.5, 139.5, 133.4, 132.4, 124.2, 120.2, 119.2, 109.6, 56.5, 56.2, 42.0, 37.4, 30.2, 22.9 ppm

**HR-MS** (*m/z*): $[M+Na]^+$ calcd for $C_{15}H_{21}N_5NaO_3^+$: 342.1537; found: 342.1541




***N*-(2-((4-(Hydroxyamino)-4-oxobutyl)(methyl)amino)ethyl)-1*H*-indazole-7-carboxamide** (**37**): The corresponding methyl ester of the title compound was prepared from ester **S17**·2HCl (196.0 mg, 0.793 mmol, 1.0 equiv) and 1*H*-indazole-6-carboxylic acid (141 mg, 0.872 mmol, 1.1 equiv) according to General Procedure B. The crude product was directly converted to hydroxamic acid **37** according to

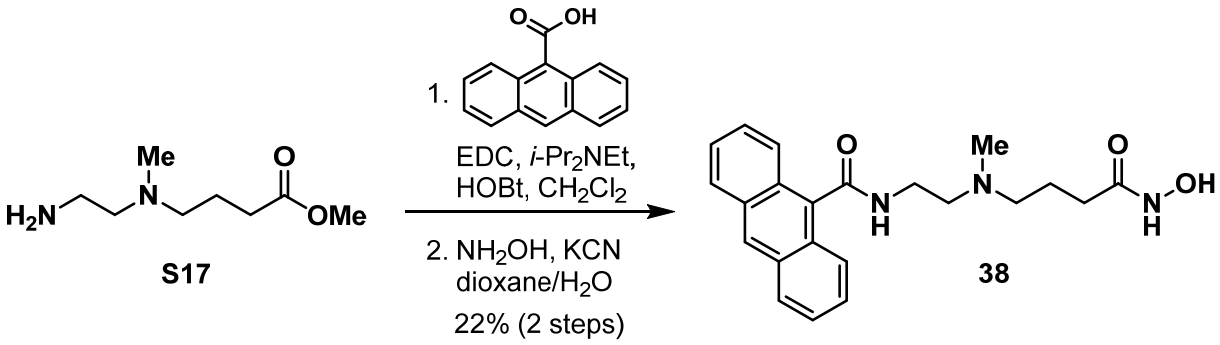
General Procedure A. The product was purified by HPLC Basic Method (gradient: 1 → 40% B in 12 min) to provide **37** as white solid (67.0 mg, 0.202 mmol, 25% yield (2 steps)).

**TLC** $R_f$ 0.08 (20% MeOH in $CH_2Cl_2$)

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 8.73 (br s, 1H*), 8.15 (s, 1H), 7.96 – 7.90 (m, 1H), 7.90 – 7.81 (m, 1H), 7.20 – 7.12 (m, 1H), 3.44 (app q, $J$ = 6.5 Hz, 2H), 2.52 (t, $J$ = 7.3 Hz, 2H), 2.34 (t, $J$ = 7.1 Hz, 2H), 2.21 (s, 3H), 2.00 (t, $J$ = 7.4 Hz, 2H), 1.65 (app p, $J$ = 7.4 Hz, 2H) ppm; *partially exchanged with adventitious water.

**$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 169.2, 165.7, 138.5, 133.0, 124.5, 124.2, 124.1, 119.4, 117.5, 56.6, 56.2, 41.9, 37.1, 30.1, 22.9 ppm

**HR-MS** (*m/z*): $[M+H]^+$ calcd for $C_{15}H_{22}N_5O_3^+$: 320.1717; found: 320.1719



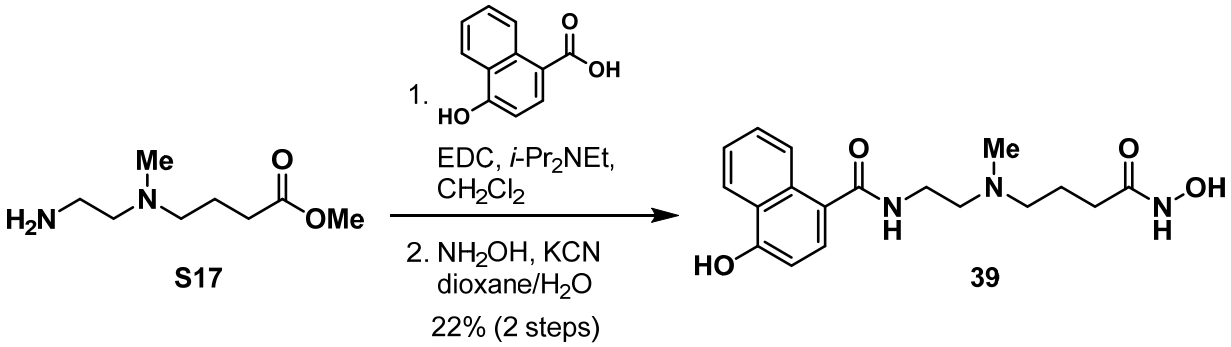

***N*-(2-((4-(Hydroxyamino)-4-oxobutyl)(methyl)amino)ethyl)anthracene-9-carboxamide** (**38**): The corresponding methyl ester of the title compound was prepared from ester **S17**·2HCl (92.7 mg, 0.375 mmol, 1.0 equiv) and anthracene-9-carboxylic acid (91.7 mg, 0.413 mmol, 1.1 equiv) according to General Procedure B. The crude product was purified by MPLC (12 g silica, gradient: 0 → 4% MeOH in $CH_2Cl_2$ over 13 CV, then 4% MeOH for 20 CV, then 4 → 8% MeOH over 10 CV) to provide the corresponding methyl ester of **38** (62.3 mg, TLC $R_f$ 0.46 (20% MeOH in $CH_2Cl_2$), *m/z*: $[M+H]^+$: 379.2), which was directly converted to hydroxamic acid **38** according to General Procedure A. The product was purified by RP-MPLC (15.5 g C18Aq Teledyne Isco #69-2203-559, gradient: 0% B for 2 CV, 0 → 22% B over 12 CV, 22% B for 9 CV, 22 → 36% B over 5 CV, then 36% B over 5 CV) to provide **38** as off-white fluffy powder (31.2 mg, 0.082 mmol, 22% yield (2 steps)).

**TLC** $R_f$ 0.16 (20% MeOH in $CH_2Cl_2$)

**$^1$H NMR** (600 MHz, DMSO-$d_6$) δ 8.74 (t, $J$ = 5.8 Hz, 1H), 8.64 (s, 1H), 8.14 – 8.10 (m, 2H), 8.09 – 8.04 (m, 2H), 7.60 – 7.51 (m, 4H), 3.58 (app q, $J$ = 6.3 Hz, 2H), 2.62 (t, $J$ = 6.6 Hz, 2H), 2.40 (t, $J$ = 7.2 Hz, 2H), 2.29 (s, 3H), 2.01 (t, $J$ = 7.5 Hz, 2H), 1.73 (app p, $J$ = 7.5 Hz, 2H) ppm; *partially exchanged with adventitious water.

**$^{13}$C NMR** (151 MHz, DMSO-$d_6$) δ 169.1, 168.1, 133.5, 130.7, 128.3, 127.3, 127.0, 126.3, 125.5 (2 Ar CH), 56.8, 56.6, 41.7, 37.2, 30.3, 23.2 ppm

**HR-MS** (*m/z*): $[M+H]^+$ calcd for $C_{22}H_{26}N_3O_3^+$: 380.1969; found: 380.1974



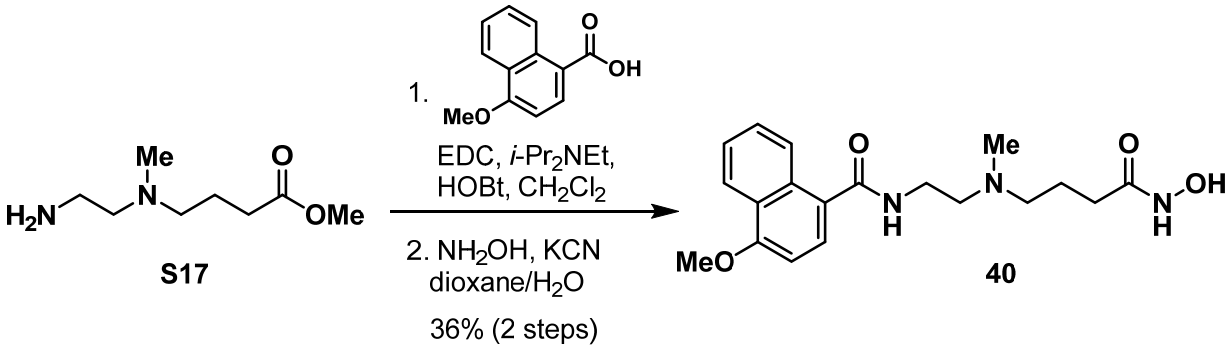

**4-Hydroxy-*N*-(2-((4-(hydroxyamino)-4-oxobutyl)(methyl)amino)ethyl)-1-naphthamide** (**39**): The corresponding methyl ester of the title compound was prepared from ester **S17**·2HCl (71.3 mg, 0.288 mmol, 1.0 equiv) and 4-hydroxy-1-naphthoic acid[10] (100 mg, 0.531 mmol, 1.0 equiv) according to General Procedure B. The product was purified by MPLC (4 g silica, gradient: 0 → 5% MeOH in $CH_2Cl_2$ over 20 CV, 5% MeOH for 30 CV, then 5 → 20% MeOH over 20 CV) to provide the corresponding methyl ester of **39** (78.4 mg, *m/z*: $[M+H]^+$: 345.2, $[M-H]^-$: 343.2), which was directly converted to hydroxamic acid **39** according to General Procedure A. The product was purified by RP-MPLC (15.5 g C18Aq Teledyne Isco #69-2203-559, gradient: 0% B for 6 CV, 0 → 14% over 20 CV, 14 → 17% B over 10 CV, then 17% B over 4 CV) to provide **39** as off-white solid (22.4 mg, 0.065 mmol, 22% yield (2 steps)).

**TLC** $R_f$ 0.03 (20% MeOH in $CH_2Cl_2$)

**$^1$H NMR** (600 MHz, DMSO-$d_6$) δ 10.98 – 9.72 (br, 2H*), 8.31 (d, *J* = 8.5 Hz, 1H), 8.21 – 8.14 (m, 2H), 7.55 – 7.49 (m, 1H), 7.49 – 7.43 (m, 2H), 6.84 (d, *J* = 7.8 Hz, 1H), 3.37 (app q, *J* = 6.5 Hz, 2H), 2.54 – 2.48 (m, overlapped with DMSO signal), 2.34 (t, *J* = 7.2 Hz, 2H), 2.21 (s, 3H), 1.99 (t, *J* = 7.4 Hz, 2H), 1.66 (app p, *J* = 7.4 Hz, 2H) ppm; *partially exchanged with adventitious water.

**$^{13}$C NMR** (151 MHz, DMSO-$d_6$) δ 169.2, 168.6, 155.0, 131.6, 126.7, 126.7, 125.5, 125.2, 124.7, 124.5, 122.2, 106.6, 56.6, 56.3, 41.9, 37.2, 30.2, 23.0 ppm

**HR-MS** (*m/z*): $[M+H]^+$ calcd for $C_{18}H_{24}N_3O_4^+$: 346.1761; found: 346.1762



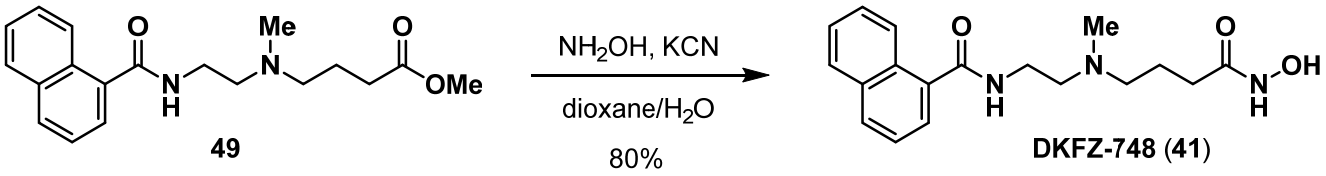

***N*-(2-((4-(Hydroxyamino)-4-oxobutyl)(methyl)amino)ethyl)-4-methoxy-1-naphthamide** (**40**): The corresponding methyl ester of the title compound was prepared from ester **S17**·2HCl (113.7 mg, 0.460 mmol, 1.0 equiv) and 4-methoxy-1-naphthoic acid (102 mg, 0.506 mmol, 1.1 equiv) according to General Procedure B. The crude product was directly converted to hydroxamic acid **40** according to General Procedure A. The product was purified by RP-MPLC (15.5 g C18Aq Teledyne Isco #69-2203-559, gradient: 0 → 4% B over 12 CV, 4 → 20% B over 16 CV, then 20% B for 2 CV) to provide **40** as white fluffy powder (59.7 mg, 0.166 mmol, 36% yield (2 steps)).

**TLC** $R_f$ 0.11 (20% MeOH in $CH_2Cl_2$)

**$^1$H NMR** (600 MHz, DMSO-$d_6$) δ 10.26 – 8.91 (br, 2H*), 8.32 (d, *J* = 8.4 Hz, 1H), 8.29 (t, *J* = 5.7 Hz, 1H), 8.20 (d, *J* = 8.4 Hz, 1H), 7.59 – 7.55 (m, 2H), 7.55 – 7.50 (m, 1H), 6.98 (d, *J* = 8.0 Hz, 1H), 4.00 (s, 3H), 3.39 (app q, *J* = 6.5 Hz, 2H), 2.52 (t, *J* = 6.9 Hz, 2H), 2.34 (t, *J* = 7.2 Hz, 2H), 2.22 (s, 3H), 1.98 (t, *J* = 7.4 Hz, 2H), 1.66 (app p, *J* = 7.3 Hz, 2H) ppm; *partially exchanged with adventitious water.

**$^{13}$C NMR** (151 MHz, DMSO-$d_6$) δ 169.1, 168.4, 155.9, 131.1, 127.1, 127.0, 126.3, 125.6, 125.5, 124.7, 121.6, 103.1, 56.6, 56.3, 55.8, 41.9, 37.2, 30.2, 23.1 ppm

**HR-MS** (*m/z*): $[M+H]^+$ calcd for $C_{19}H_{26}N_3O_4^+$: 360.1918; found: 360.1919



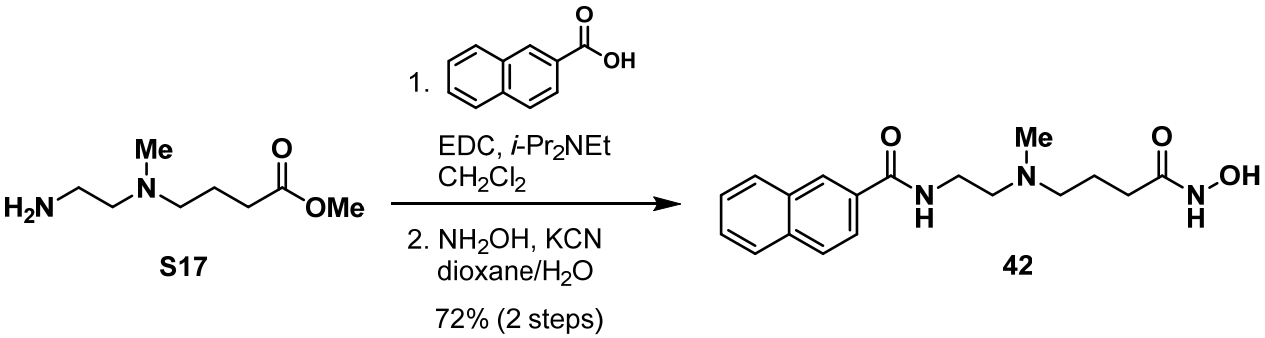

***N*-(2-((4-(Hydroxyamino)-4-oxobutyl)(methyl)amino)ethyl)-1-naphthamide** (**DKFZ-748**; **41**): Ester **49** (206 mg, 0.628 mmol, 1.0 equiv) was converted to hydroxamic acid **41** according to General Procedure A. The product was purified by RP-MPLC (15.5 g C18Aq Teledyne Isco #69-2203-559, gradient: 0% B for 3.5 CV, 0 → 25% over 22.5 CV, then 25% B for 9 CV) to provide **DKFZ-748** (**41**) as a white solid (166 mg, 0.504 mmol, 80% yield).

**TLC** $R_f$ 0.28 (20% MeOH in $CH_2Cl_2$)

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 10.37 (br s, 1H), 8.81 (br s, 1H*), 8.43 (t, *J* = 5.7 Hz, 1H), 8.27 – 8.20 (m, 1H*), 8.05 – 7.92 (m, 2H), 7.62 – 7.49 (m, 4H), 3.42 (app q, *J* = 6.5 Hz, 2H), 2.54 (t, *J* = 6.8 Hz, 2H), 2.35 (t, *J* = 7.2 Hz, 2H), 2.23 (s, 3H), 1.99 (t, *J* = 7.4 Hz, 2H), 1.67 (app p, *J* = 7.3 Hz, 2H) ppm; *partially exchanged with adventitious water.

**$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 169.1, 168.5, 135.2, 133.1, 129.7, 129.6, 128.2, 126.6, 126.2, 125.5, 125.0, 125.0, 56.6, 56.3, 41.9, 37.2, 30.2, 23.1 ppm

**HR-MS** (*m/z*): $[M+H]^+$ calcd for $C_{18}H_{24}N_3O_3^+$: 330.1812; found: 330.1814



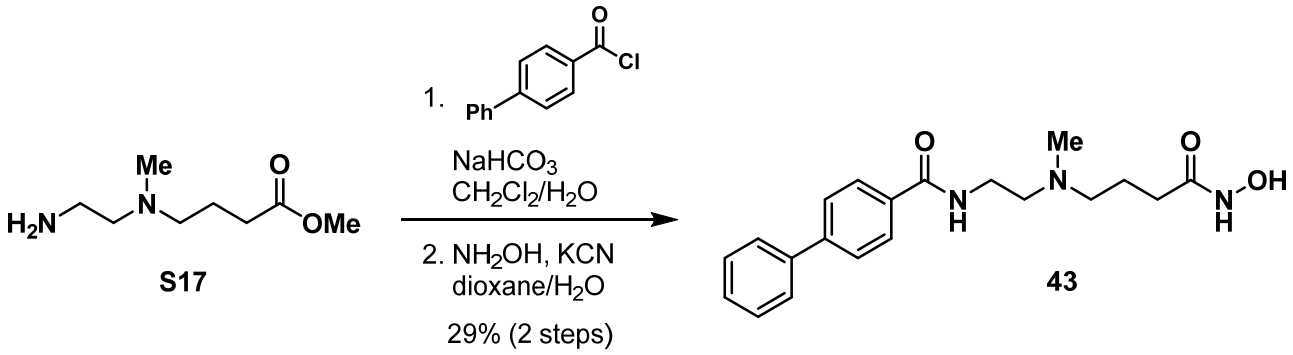

***N*-(2-((4-(Hydroxyamino)-4-oxobutyl)(methyl)amino)ethyl)-2-naphthamide** (**42**): The corresponding methyl ester of the title compound was prepared from ester **S17**·2HCl (86.1 mg, 0.348 mmol, 1.0 equiv) and 2-naphthoic acid (66.0 mg, 0.383 mmol, 1.1 equiv) according to General Procedure B. The crude product was directly converted to hydroxamic acid **42** according to General Procedure A. The product was purified by RP-MPLC (15.5 g C18Aq Teledyne Isco #69-2203-559, gradient: 0% B for 3 CV, then 0 → 20% over 22 CV, then 50% B for 4 CV) to provide **42** as white solid (82.8 mg, 0.251 mmol, 72% yield (2 steps)).

**TLC** $R_f$ 0.13 (20% MeOH in $CH_2Cl_2$)

**$^1$H NMR** (600 MHz, DMSO-$d_6$) δ 9.53 (br s, 2H*), 8.58 (t, *J* = 5.7 Hz, 1H), 8.44 (s, 1H), 8.05 – 7.89 (m, 4H), 7.64 – 7.56 (m, 2H), 3.41 (app q, *J* = 6.6 Hz, 2H), 2.52 (t, *J* = 7.1 Hz, 2H), 2.34 (t, *J* = 7.2 Hz, 2H), 2.21 (s, 3H), 1.99 (t, *J* = 7.4 Hz, 2H), 1.65 (app p, *J* = 7.3 Hz, 2H) ppm; *partially exchanged with adventitious water.

**$^{13}$C NMR** (151 MHz, DMSO-$d_6$) δ 169.1, 166.2, 134.1, 132.2, 132.0, 128.8, 127.8, 127.6, 127.5, 127.3, 126.7, 124.1, 56.5, 56.2, 42.0, 37.4, 30.2, 22.9 ppm

**HR-MS** (*m/z*): $[M+H]^+$ calcd for $C_{18}H_{24}N_3O_3^+$: 330.1812; found: 330.1814

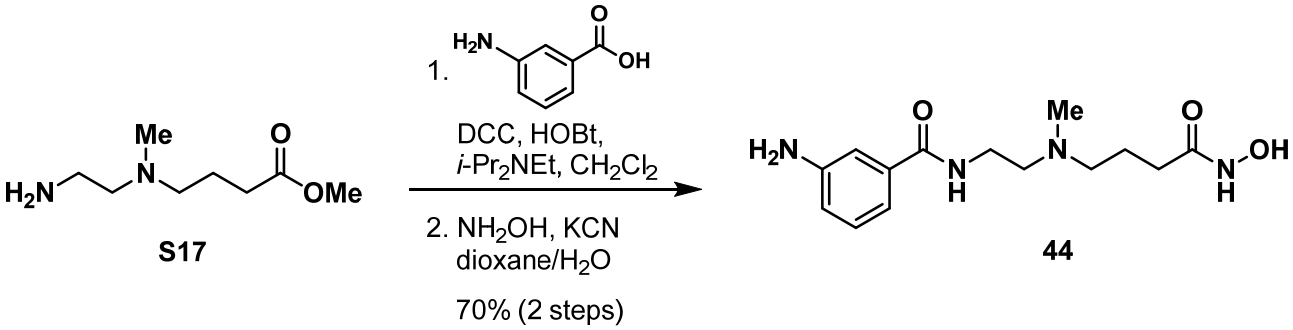



***N*-(2-((4-(Hydroxyamino)-4-oxobutyl)(methyl)amino)ethyl)-[1,1'-biphenyl]-4-carboxamide** (**43**): To a vigorously stirred solution of **S17**·2HCl (82 mg, 0.330 mmol, 1.0 equiv) in saturated aqueous $NaHCO_3$ (6 mL) and $CH_2Cl_2$ (10 mL) was added 4-phenylbenzoyl chloride (125 mg, 0.577 mmol, 1.8 equiv). After 135 min, the reaction mixture was diluted with water (100 mL) and extracted with $CH_2Cl_2$ (3 × 30 mL). The combined organic layers were washed with half saturated aqueous $K_2CO_3$ (twice) and brine (50 mL), dried ($MgSO_4$), filtered, and concentrated. The residue was directly converted to hydroxamic acid **43** according to General Procedure A. The product was purified by HPLC Basic Method (gradient: 1 → 75% B in 12 min) to provide **43** as a white fluffy solid (34.3 mg, 0.097 mmol, 29% yield (2 steps)).

**$^1$H NMR** (400 MHz, MeOD-$d_4$) δ 7.94 – 7.87 (m, 2H), 7.73 – 7.68 (m, 2H), 7.68 – 7.62 (m, 2H), 7.49 – 7.42 (m, 2H), 7.40 – 7.33 (m, 1H), 3.53 (t, *J* = 6.8 Hz, 2H), 2.63 (t, *J* = 6.8 Hz, 2H), 2.50 – 2.43 (m, 2H), 2.32 (s, 3H), 2.12 (t, *J* = 7.3 Hz, 2H), 1.81 (app p, *J* = 7.3 Hz, 2H) ppm

**$^{13}$C NMR** (101 MHz, MeOD-$d_4$) δ 172.1, 169.9, 145.7, 141.3, 134.3, 130.0, 129.1, 128.9, 128.1, 128.0, 57.9, 57.3, 42.4, 38.5, 31.6, 24.1 ppm

**HR-MS** (*m/z*): $[M+H]^+$ calcd for $C_{20}H_{26}N_3O_3^+$: 356.1969; found: 356.1972



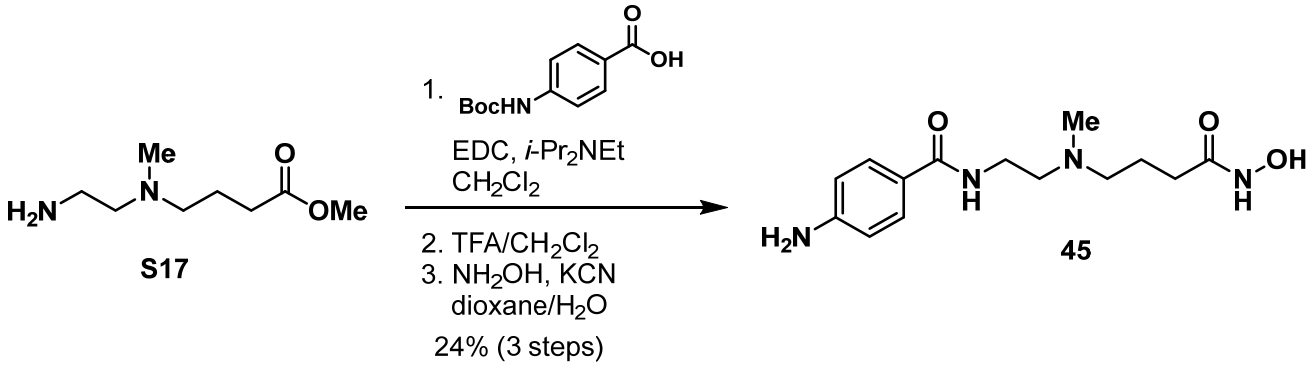

**3-Amino-*N*-(2-((4-(hydroxyamino)-4-oxobutyl)(methyl)amino)ethyl)benzamide** (**44**): To a suspension of diamine **S17**·2HCl (80.0 mg, 0.324 mmol, 1.0 equiv), DCC (100 mg, 0.486 mmol, 1.5 equiv), HOBt (5 mg, 0.032 mmol, 0.1 equiv) and 3-aminobenzoic acid (48.8 mg, 0.356 mmol, 1.1 equiv) in $CH_2Cl_2$ (8 mL) was added *i*-$Pr_2NEt$ (0.138 mL, 0.809 mmol, 2.5 equiv). The reaction mixture was stirred at rt for 16 h, then cooled to –20 °C and filtered through a fritted syringe. The filter cake was washed with ice-cold $CH_2Cl_2$ (4 mL), and the combined filtrates were concentrated in vacuo. The crude

product was directly converted to hydroxamic acid **44** according to General Procedure A. The product was purified by HPLC Basic Method (gradient: 1 → 30% B in 12 min) to provide **44** as an off-white powder (66.8 mg, 0.227 mmol, 70% yield (2 steps)).

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 10.82 – 8.38 (br, 2H), 8.08 (t, *J* = 5.7 Hz, 1H), 7.05 (t, *J* = 7.8 Hz, 1H), 7.00 (t, *J* = 2.0 Hz, 1H), 6.91 (dt, *J* = 7.6, 1.4 Hz, 1H), 6.70 – 6.63 (m, 1H), 5.20 (s, 2H*), 3.32 – 3.24 (m, overlapped with water peak), 2.43 (t, *J* = 7.0 Hz, 2H), 2.30 (t, *J* = 7.2 Hz, 2H), 2.17 (s, 3H), 1.96 (t, *J* = 7.4 Hz, 2H), 1.61 (app p, *J* = 7.4 Hz, 2H) ppm; *partially exchanged with adventitious water.

**$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 169.1, 166.9, 148.6, 135.6, 128.6, 116.3, 114.2, 112.7, 56.5, 56.2, 41.9, 37.1, 30.1, 22.9 ppm

**HR-MS** (*m/z*): $[M+H]^+$ calcd for $C_{14}H_{23}N_4O_3^+$: 295.1765; found: 295.1768

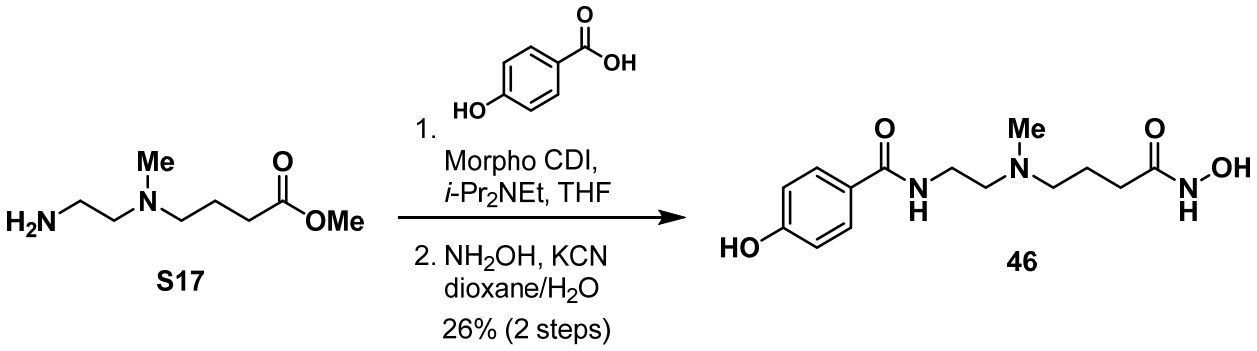



**4-Amino-*N*-(2-((4-(hydroxyamino)-4-oxobutyl)(methyl)amino)ethyl)benzamide** (**45**): The corresponding methyl ester of the title compound was prepared from ester **S17**·2HCl (103.8 mg, 0.420 mmol, 1.0 equiv) and Boc-protected 4-aminobenzoic acid (107 mg, 0.462 mmol, 1.1 equiv) according to General Procedure B. The crude product was dissolved in TFA/$CH_2Cl_2$ (5 mL, 20% TFA in $CH_2Cl_2$), stirred at rt for 1 h, then concentrated and residual TFA co-evaporated with MeOH (2 x 20 mL). The crude TFA salt was directly converted to hydroxamic acid **45** according to General Procedure A. The product was purified by HPLC Basic Method (gradient: 1 → 30% B in 12 min) to provide **45** as off-white solid (30.2 mg, 0.103 mmol, 24% yield (3 steps)).

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 8.42 (s, 1H), 10.66 – 8.26 (m, 2H), 7.87 (t, *J* = 5.6 Hz, 1H), 7.58 – 7.49 (m, 2H), 6.56 – 6.48 (m, 2H), 5.57 (s, 2H), 3.27 (app q, *J* = 6.4 Hz, 2H), 2.41 (t, *J* = 7.1 Hz, 2H), 2.29 (t, *J* = 7.2 Hz, 2H), 2.16 (s, 3H), 1.95 (t, *J* = 7.4 Hz, 2H), 1.61 (app p, *J* = 7.2 Hz, 2H) ppm

**$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 169.1, 166.1, 151.5, 128.6, 121.3, 112.5, 56.5, 56.4, 42.0, 37.0, 30.1, 22.9 ppm

**HR-MS** (*m/z*): $[M+H]^+$ calcd for $C_{14}H_{23}N_4O_3^+$: 295.1765; found: 295.1768

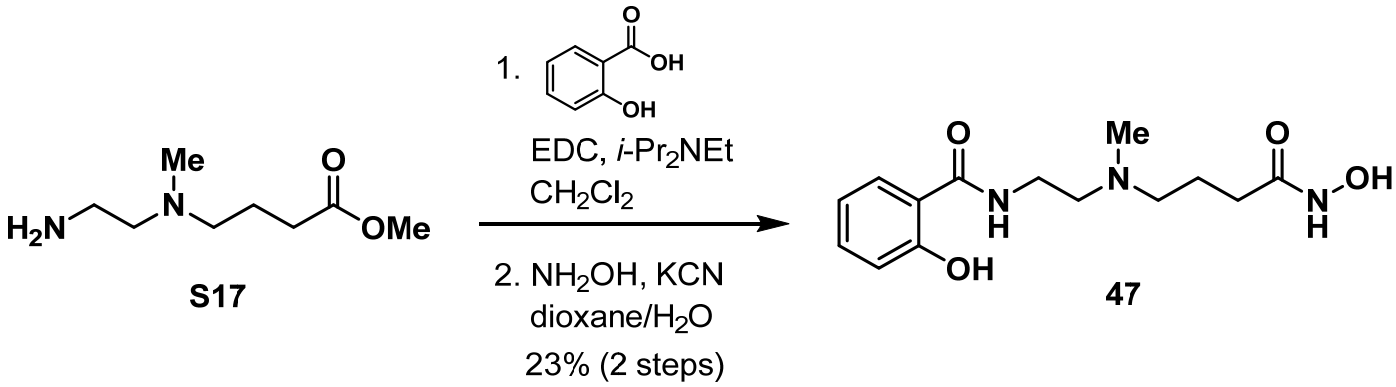



**4-Hydroxy-*N*-(2-((4-(hydroxyamino)-4-oxobutyl)(methyl)amino)ethyl)benzamide** (**46**): The corresponding methyl ester of the title compound was prepared from ester **S17**·2HCl (103.8 mg, 0.420 mmol, 1.0 equiv) and 4-hydroxybenzoic acid (63.8 mg, 0.462 mmol, 1.1 equiv) according to General Procedure B, but in THF with Morpho CDI (N-Cyclohexyl-N′-(2-morpholinoethyl)carbodiimide methyl-p-toluenesulfonate) as coupling reagent. The crude product was directly converted to hydroxamic acid **46** according to General Procedure A. The product was purified by HPLC Acidic Method but with 0.05% formic acid as pH modifier (gradient: 1 → 8% B in 12 min) to provide the formate salt of **46** as an orange-red amorphous solid (37.1 mg, 0.109 mmol, 26% yield (2 steps)).

**$^1$H NMR** (400 MHz, $D_2O$) δ 8.42 (s, 1H), 7.66 (d, *J* = 7.5 Hz, 2H), 6.91 (d, *J* = 7.5 Hz, 2H), 3.72 (t, *J* = 5.6 Hz, 2H), 3.37 (t, *J* = 5.6 Hz, 2H), 3.29 – 3.14 (m, 2H), 2.91 (s, 3H), 2.26 (t, *J* = 6.9 Hz, 2H), 2.01 (app p, *J* = 7.5 Hz, 2H) ppm

**$^{13}$C NMR** (101 MHz, $D_2O$) δ 173.9, 173.8 (br, $HCO_2^-$), 173.6, 162.5, 132.3, 127.2, 118.3, 58.2, 58.1, 43.1, 37.7, 31.9, 22.4 ppm

**HR-MS** (*m/z*): $[M+H]^+$ calcd for $C_{14}H_{22}N_3O_4^+$: 296.1605; found: 296.1607




**2-Hydroxy-*N*-(2-((4-(hydroxyamino)-4-oxobutyl)(methyl)amino)ethyl)benzamide** (**47**): The corresponding methyl ester of the title compound was prepared from ester **S17**·2HCl (88.7 mg, 0.359 mmol, 1.0 equiv) and salicylic acid (54.5 mg, 0.395 mmol, 1.1 equiv) according to General Procedure B. The crude product was directly converted to hydroxamic acid **47** according to General Procedure A.

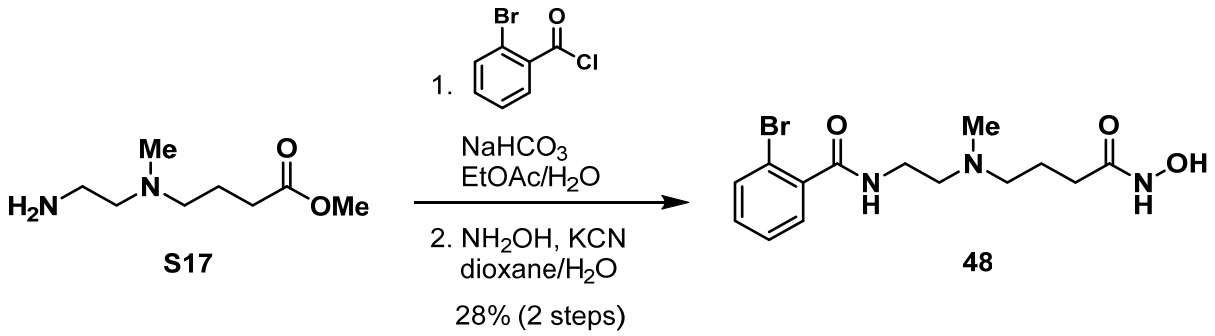
The product was purified by HPLC Basic Method (gradient: 1 → 5% B in 9 min) to provide **47** as an off-white solid (24.9 mg, 0.084 mmol, 23% yield (2 steps)).

**TLC** $R_f$ 0.24 (20% MeOH in $CH_2Cl_2$)

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 10.34 (s, 1H*), 8.97 – 8.42 (m, 2H*), 8.77 (t, $J$ = 5.5 Hz, 1H), 7.82 (dd, $J$ = 7.9, 1.7 Hz, 1H), 7.43 – 7.34 (m, 1H), 6.93 – 6.83 (m, 2H), 3.42 – 3.36 (m, overlapped with water peak), 2.57 – 2.51 (m, 2H), 2.36 (t, $J$ = 7.3 Hz, 2H), 2.23 (s, 3H), 1.96 (t, $J$ = 7.4 Hz, 2H), 1.70 – 1.58 (m, 2H) ppm; The four Ar H show rotamer signals with a ratio of 1:0.16. Only the major rotamer is reported. *partially exchanged with adventitious water.

**$^{13}$C NMR** (151 MHz, DMSO-$d_6$) δ 169.1, 168.5, 159.7, 133.5, 127.9, 118.6, 117.3, 115.8, 56.4, 55.6, 41.7, 36.8, 30.0, 22.7 ppm; Only signals of the major rotamer are reported.

**HR-MS** (*m/z*): $[M+H]^+$ calcd for $C_{14}H_{22}N_3O_4^+$: 296.1605; found: 296.1609



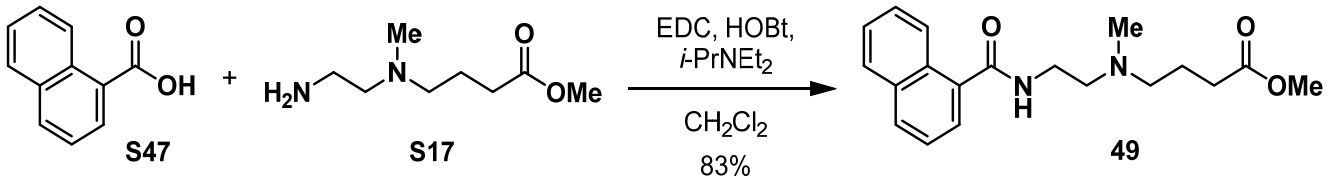

**2-Bromo-*N*-(2-((4-(hydroxyamino)-4-oxobutyl)(methyl)amino)ethyl)benzamide** (**48**): To a solution of **S17**·2HCl (103.8 mg, 0.420 mmol, 1.0 equiv) partitioned in saturated aqueous $NaHCO_3$ (25 mL) and EtOAc (25 mL) in a separatory funnel was added 2-bromo benzoyl chloride (120 µL, 0.924 mmol, 2.2 equiv) in two portions, followed by vigorous shaking. The phases were separated and the organic layer was washed with half saturated aqueous $K_2CO_3$ (2 × 25 mL) and brine (25 mL), then dried ($MgSO_4$), filtered, and concentrated. The residue was directly converted to hydroxamic acid **48** according to General Procedure A. The product was purified by HPLC Basic Method (gradient: 1 → 30% B in 15 min) to provide **48** as an off-white solid (41.5 mg, 0.116 mmol, 28% yield (2 steps)).

**TLC** $R_f$ 0.13 (10% MeOH in $CH_2Cl_2$)

**$^1$H NMR** (400 MHz, DMSO-$d_6$) δ 10.64 – 8.57 (br, 2H) 8.31 (t, $J$ = 5.7 Hz, 1H), 7.68 – 7.60 (m, 1H), 7.45 – 7.39 (m, 1H), 7.39 – 7.30 (m, 2H), 3.32 – 3.24 (m, overlapped with water peak), 2.46 (t, $J$ = 7.0 Hz, 2H), 2.30 (t, $J$ = 7.2 Hz, 2H), 2.18 (s, 3H), 1.97 (t, $J$ = 7.4 Hz, 2H), 1.62 (app p, $J$ = 7.3 Hz, 2H) ppm

**$^{13}$C NMR** (101 MHz, DMSO-$d_6$) δ 169.1, 167.1, 139.3, 132.7, 130.7, 128.7, 127.5, 118.9, 56.5, 56.0, 41.9, 37.2, 30.1, 23.0 ppm

**HR-MS** (*m/z*): $[M+H]^+$ calcd for $C_{14}H_{21}BrN_3O_3^+$: 358.0761; found: 358.0764




S47 S17 49

**Methyl 4-((2-(1-naphthamido)ethyl)(methyl)amino)butanoate** (**49**): The title compound was prepared from ester **S17**·2HCl (418 mg, 1.692 mmol, 1.0 equiv) and recrystallized 1-naphthoic acid (321 mg, 1.861 mmol, 1.1 equiv) according to General Procedure B. The product was purified by MPLC (12 g silica, gradient: 0 → 7% MeOH with 0.5% NH4OH in $CH_2Cl_2$ over 10 CV) to provide **49** as a pale yellow oil (459 mg, 1.398 mmol, 83% yield).

**TLC** $R_f$ 0.58 (10% MeOH and 0.5% $NH_4OH$ in $CH_2Cl_2$)

**$^1$H NMR** (400 MHz, $CDCl_3$) δ 8.38 – 8.30 (m, 1H), 7.93 – 7.82 (m, 2H), 7.63 (dd, *J* = 7.0, 1.3 Hz, 1H), 7.58 – 7.41 (m, 3H), 6.71 (br s, 1H), 3.64 – 3.55 (m, 2H), 3.48 (s, 3H), 2.64 – 2.57 (m, 2H), 2.41 (t, *J* = 6.9 Hz, 2H), 2.28 (t, *J* = 7.1 Hz, 2H), 2.22 (s, 3H), 1.78 (app p, *J* = 7.0 Hz, 2H) ppm

**$^{13}$C NMR** δ (101 MHz, $CDCl_3$) δ 174.2, 169.7, 134.8, 133.8, 130.5, 130.3, 128.4, 127.1, 126.4, 125.6, 125.2, 124.9, 56.8, 56.4, 51.5, 41.6, 37.4, 32.0, 22.7 ppm

**HR-MS** (*m/z*): $[M+H]^+$ calcd for $C_{19}H_{25}N_2O_3^+$: 329.1860; found: 329.1861

# Supplementary methods biology

## Thermal shift assay with HDAC10 – Differential scanning fluorimetry

Protein thermal stability was measured using TwinStrep-HDAC10 protein, which was prepared as previously described.[11] The TwinStrep-HDAC10 protein concentration for each sample here was 0.27 mg/mL (~4 μM), each test compound was added from a DMSO stock to a final concentration of 500 μM and a final DMSO content of 1%. Two control experiments, one without DMSO and a 1% DMSO vehicle control was conducted. A temperature gradient of 1 ºC $min^{-1}$ from 20 to 95 ºC was used and fluorescence emission of tryptophan at 330 nm and 350 nm was recorded. Analysis was done using the fluorescence recorded at 350 nm and is presented as the normalized 1$^{st}$ derivative. Melting temperatures are determined as the inflection point of the 1$^{st}$ derivative. The experiment was conducted two times independently and melting curves are presented superimposed.

## HDAC-Glo assay for HDAC 1, 2, 3, 6 and 8[12]

HDAC6 and class I HDAC inhibition was tested using the HDAC-Glo™ I/II Assay and Screening System (G6421, Promega) with recombinant human HDACs (BPS Bioscience; HDAC1 cat. #50051; HDAC2 cat. #50002; HDAC3/NcoR2 complex cat. #50003; HDAC6 cat. #50006; HDAC8 cat. #50008). The assay was carried out in a 384-well plate (Corning 4512) format according to the manufacturer's description. Inhibitors were tested at eight serial dilutions in triplicates ranging from 50 μM – 86.7 pM or 100 μM – 8.67 nM. Drug dosing was performed from 10 mM and 0.1 mM DMSO stock solutions with a D300e Digital Dispenser (Tecan). HDACs (7 ng/mL for HDAC1, 25 ng/mL for HDAC2, 200 ng/mL for HDAC3/Ncor2 complex, 100 ng/mL for HDAC6, 200 ng/mL for HDAC8) and inhibitors were incubated together at rt for 30 min. After addition of the HDAC-Glo™ I/II reagent, plates were shaken (800 rpm orbital shaker, 30 s), centrifuged (300 g, 1 min) and incubated at rt for 30 min. Luminescence was detected with a CLARIOstar (BMG Labtech) plate reader. Luminescence signal was normalized with 100 μM SAHA treated negative controls and uninhibited positive controls. $pIC_{50}$-values were calculated from log(inhibitor) vs normalized luminescence by nonlinear regression four parameters least squares fit of Y=Bottom + (Top-Bottom)/(1+10^((LogIC50-X)*HillSlope)) in GraphPad Prism version 7.05 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com.

## HDAC10 TR-FRET assay[12]

TR-FRET assays were performed in white 384-well plates (Corning 4512) using 50 mM HEPES pH 8.0, 150 mM NaCl, 10 mM $MgCl_2$, 1 mM EGTA and 0.01% Brij-35 as buffer. The concentrations of reagent

in 15 µL final assay volume were 5 nM TwinStrep-GST-HDAC10 (preparation described previously[12]), 25 nM "Tubastatin-AF647-Tracer" (synthesis described previously[12]) and 0.1 nM DTBTA-$Eu^{3+}$-labelled Streptactin (synthesis described previously[12]). Inhibitors were tested at eight serial dilutions in triplicates ranging from 50 µM – 86.7 pM or 100 µM – 8.67 nM and dosed from 10 mM and 0.1 mM DMSO stock solutions with a D300e Digital Dispenser (Tecan). After drug dosing to the premixed assay reagents in buffer, plates were shaken (800 rpm orbital shaker, 30 s), centrifuged (300 g, 1 min) and incubated at rt in the dark for 90 min. TR-FRET was measured with a CLARIOstar (BMG Labtech) plate reader, equipped with TR-FRET filters. Sample wells were excited with 100 flashes and fluorescence emission detected at 665 nm and 620 nm. FRET ratios were calculated from 665 nm/620 nm ratio and normalized for each plate using 50 µM SAHA treated negative controls and uninhibited positive controls. $pIC_{50}$-values were calculated as described in the HDAC-Glo assay.

## Crystal structure determination of HDAC10–inhibitor complexes

The "humanized" version of HDAC10 was expressed and purified as previously described.[13] A solution of inhibitor in DMSO (final concentration of 2 mM for **14** or **DKFZ-728**, or 4 mM for SAHA or **DKFZ-711**) was added to protein solutions containing 10 mg/mL HDAC10, 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (pH 7.5), 300 mM KCl, 5% glycerol, and 1 mM tris(2-carboxyethyl)phosphine (TCEP) and allowed to incubate on ice for 1 h. To the HDAC10–inhibitor solutions, trypsin was added at a 1:1000 molar ratio and allowed to digest at room temperature for 1 h. Each HDAC10–inhibitor solution was filtered using a 0.22 µm centrifuge filter prior to crystallization.

Each HDAC10–inhibitor solution was crystallized by the sitting drop vapor diffusion method at 4 °C. A 100 nL drop of the HDAC10–inhibitor solution was dispensed onto a 100 nL drop of precipitant buffer on a 96-well crystallization plate using a Mosquito crystallization robot (TTP Labtech) with the exception of the HDAC10–DKFZ-728 solution where 200 nL of protein solution was dispensed onto 130 nL of precipitant buffer. To each combined drop, 25 nL of microseed crystals of the HDAC10–Tubastatin A complex were added prior to sealing the 96-well tray (70 nL for HDAC10–**DKFZ-728**). Each drop was equilibrated against 80 µL of precipitant buffer in the well reservoir. Crystals formed in approximately 1 day.

Crystallization of the HDAC10–SAHA complex was achieved using 0.125 M $NaH_2PO_4$, 0.075 M $Na_2HPO_4$, and 20% (w/v) PEG3350 as the precipitant buffer. Crystallization of the HDAC10–**14** complex was achieved using 0.131 M $NaH_2PO_4$, 0.044 M $Na_2HPO_4$, 3% (v/v) glycerol, and 20% (w/v) PEG3350 as the precipitant buffer. Crystallization of the HDAC10–**DKFZ-728** complex was achieved using 0.192 M $NaH_2PO_4$, 0.008 M $Na_2HPO_4$, and 20% (w/v) PEG3350 as the precipitant buffer.

Crystallization of the HDAC10–**DKFZ-711** complex was achieved using 0.100 M $KH_2PO_4$, 0.100 M $K_2HPO_4$, and 20% (w/v) PEG3350 as the precipitant buffer.

All X-ray diffraction data reported for the HDAC10 complexes were collected on NE-CAT beamline 24-ID-C at the Advanced Photon Source of Argonne National Laboratory (Argonne, Il). Diffraction data was integrated using iMosflm[14] and scaled using Aimless in the CCP4 program suite.[15,16] Initial electron density maps were phased by molecular replacement using the Y307F HDAC10–trifluoroketone inhibitor complex [Protein Data Bank (PDB) entry 5TD7][17] using Phaser.[18,19] The model was built into the electron density using COOT[20] and refinement was performed using PHENIX.[21] Simulated annealing refinement was performed to reduce phase bias. The inhibitor was built into the model at the final stages of refinement. MolProbity[22] was utilized to determine the quality of the final model. All data collection and refinement statistics are listed in the table below.

**Collection and Refinement Statistics Data**

| HDAC10 Complex | HDAC10-SAHA | HDAC10-inhibitor 14 | HDAC10-DKFZ-728 | HDAC10-DKFZ-711 |
|---|---|---|---|---|
| **Space group** | $P3_121$ | $P3_121$ | $P3_121$ | $P3_121$ |
| **a,b,c (Å)** | 80.3, 80.3, 249.2 | 80.5, 80.5, 245.6 | 80.5, 80.6, 243.4 | 80.4, 80.4, 248.3 |
| **α, β, γ (°)** | 90, 90, 120 | 90, 90, 120 | 90, 90, 120 | 90, 90, 120 |
| **$R_{merge}$[b]** | 0.134 (1.152) | 0.142 (1.246) | 0.148 (1.391) | 0.103 (1.179) |
| **$R_{pim}$[c]** | 0.046 (0.430) | 0.049 (0.457) | 0.052 (0.470) | 0.048 (0.569) |
| **$CC_{1/2}$[d]** | 0.996 (0.616) | 0.994 (0.574) | 0.996 (0.667) | 0.999 (0.639) |
| **Redundancy** | 9.6 (9.7) | 9.3 (8.3) | 9.1 (9.4) | 9.5 (9.3) |
| **Completeness (%)** | 99.8 (99.9) | 100 (99.9) | 99.8 (100) | 100 (100) |
| **I/σ** | 9.7 (2.0) | 10.8 (2.1) | 9.4 (1.8) | 12.6 (2.1) |
| **Refinement** | | | | |
| **Resolution (Å)** | 69.58–2.10 (2.16–2.10) | 69.71–2.15 (2.21–2.15) | 69.50–2.20 (2.27–2.20) | 69.64–2.15 (2.21–2.15) |
| **No. reflections** | 55122 (5454) | 51508 (5072) | 47446 (4673) | 51726 (5083) |
| **$R_{work}/R_{free}$[e]** | 0.183/0.205 (0.291/0.345) | 0.190/0.222 (0.277/0.318) | 0.196/0.245 (0.307/0.353) | 0.182/0.216 (0.254/0.266) |
| **Number of Atoms** | | | | |
| **Protein** | 4911 | 4848 | 4873 | 4889 |
| **Ligand** | 50 | 39 | 39 | 50 |
| **Solvent** | 236 | 239 | 244 | 248 |
| **Average B factor (Å$^2$)** | | | | |
| **Protein** | 46 | 40 | 49 | 48 |
| **Ligand** | 57 | 49 | 54 | 56 |
| **Solvent** | 46 | 40 | 47 | 48 |
| **Root-Mean-Square Deviation** | | | | |
| **Bond lengths (Å)** | 0.007 | 0.007 | 0.007 | 0.007 |

| **Bond angles (°)** | 0.9 | 0.9 | 0.9 | 0.9 |
|---|---|---|---|---|
| **Ramachandran Plot (%)** | | | | |
| **Favored** | 96.70 | 96.47 | 96.50 | 96.52 |
| **Allowed** | 2.99 | 3.37 | 3.50 | 3.32 |
| **Outliers** | 0.31 | 0.16 | 0.00 | 0.16 |
| **PDB Entry** | 7SGG | 7SGI | 7SGK | 7SGJ |

[a] Values in parentheses refer to the highest-resolution shell of data.

[b] $R_{merge} = \sum_h\sum_i|I_{i,h} - \langle I\rangle_h|/\sum_h\sum_i I_{i,h}$, where $\langle I\rangle_h$ is the average intensity calculated for reflection *h* from *i* replicate measurements.

[c] $R_{p.i.m.} = (\sum_h(1/(N-1))^{1/2}\sum_i|I_{i,h} - \langle I\rangle_h|)/\sum_h\sum_i I_{i,h}$, where N is the number of reflections and $\langle I\rangle_h$ is the average intensity calculated for reflection *h* from replicate measurements.

[d] Pearson correlation coefficient between random half-datasets.

[e] $R_{work} = \sum||F_o| - |F_c||/\sum|F_o|$ for reflections contained in the working set. $|F_o|$ and $|F_c|$ are the observed and calculated structure factor amplitudes, respectively. $R_{free}$ is calculated using the same expression for reflections contained in the test set held aside during refinement.

[f] Per asymmetric unit.

[g] Calculated with MolProbity.

## Docking procedures

For molecular docking to HDAC6, the crystal structure of human HDAC6 domain with the accession code 5edu was downloaded from the Protein Database and prepared. The co-crystallized ligand Trichostatin A was extracted and its binding region was used to define the active site. Further, from this molecule the coordinates of the hydroxamic acid substructure were used as a constraint in the docking process of DKFZ-711 and SAHA. For the docking of DKFZ-748 into HDAC10, the crystal structure of the HDAC10–DKFZ-728 complex was prepared, the ligand DKFZ-728 extracted, active site defined and the position of the hydroxamic acid substructure of DKFZ-728 served as constraint.

Minimized 3D-structures of the ligands were generated and served as input for the docking software SurflexDock v.2.51 from BioPharmics IT as implemented in the software package SYBYL-X 1.3, Tripos, 1699 South Hanley Rd., St. Louis, Missouri, 63144, USA.

The ligands were flexibly docked into the respective active sites with the constraint to place the hydroxamic acid substructure at the same region as it was found in the co-crystallised Trichostatin A and the co-crystallised HDAC10-DKFZ-728 complex, respectively. The resulting top docking poses were visually inspected and showed little variability. One representative pose each is displayed in the figures.

## Cell culture procedure

All cell lines were cultivated under sterile conditions in polystyrene cell culture flasks (Greiner 658170) at 37 °C and 5% $CO_2$ in a humidified atmosphere using D-MEM growth medium (Sigma D5796), supplemented with 10% FCS (Capricorn Scientific FBS-12A) and 1% penicillin–streptomycin (Sigma P4333) unless stated otherwise. Medium for BE(2)-C cells was additionally supplemented with 1% non-essential amino acids (Sigma M7145). Cells were passaged near confluency by removing old medium, washing with DPBS (Gibco 14190-094), trypsinization (Sigma T4049) and seeding in fresh growth medium. Cell lines were routinely screened for mycoplasma in-house and verified using DNA fingerprinting authentication by Multiplexion (Heidelberg, Germany).

## BRET assay for HDAC6 and HDAC10[12]

Production and cultivation of transfected HeLa mono-clones stably expressing HDAC-nanoBRET fusion proteins for HDAC10 and HDAC6-catalytic domain 2 (HDAC6CD2) was described previously.[12] The intracellular target engagement assay on HDAC10 and HDAC6CD2 was performed using the NanoBRET™ Target Engagement Intracellular HDAC Assay[23] (Promega N2081 and N2090) as described by the kit manufacturer in a 96-well plate (Corning 3600) format with $2\times10^4$ cells per well and a tracer concentration of 0.3 µM. Inhibitors were tested at ten serial dilutions in triplicates ranging from 10 µM – 12.9 pM or 40 µM – 139 pM and dosed from 10 mM and 0.1 mM DMSO stock solutions with a D300e Digital Dispenser (Tecan). DMSO concentrations were normalized to 0.5 % for all wells. For the cellular compound stability experiment, **DKFZ-728** was diluted to 10 µM and tubastatin A to 1 µM in OPTI-MEM (Gibco #11058021), which was used to cultivate BE(2)-C cells for 72 h. After 0, 24, 48 and 72 h, some cell culture medium was removed from the live culture and used to perform the NanoBRET assay with HeLa HDAC10 cells as described above, but by manual serial dilution to eight dose levels in triplicates ranging from 10 µM – 61 nM or 1 µM – 6.1 nM. After dosing, assay plates were shaken at 800 rpm and incubated at 37 °C for 2 h followed by measurement of 450 nm and 650 nm luminescence (80 nm bandwidth) at room temperature with a CLARIOstar (BMG Labtech) plate reader 2 min after NanoLuc substrate addition.

BRET ratios were calculated from 650 nm/450 nm luminescence and normalized for each plate using 50 µM SAHA treated negative controls and uninhibited positive controls. $pIC_{50}$-values were calculated as described in the HDAC-Glo assay.

## Pharmacokinetic profiling

PK profiling was performed by WuXi AppTec (Shanghai, China) from 10 mM DMSO stocks of inhibitor **17**, **DKFZ-728** and **DKFZ-748** with standardized in-house assays.

**MDR1-MDCKII Bi-Directional Permeability Assay.** MDR1-MDCKII cells (obtained from Piet Borst at the Netherlands Cancer Institute) were seeded onto polyethylene membranes in 96-well insert systems (acceptor plate: Corning #359205, insert plate: Corning # 351131) at 0.25 x $10^6$ cells/mL for 4-7 days for confluent cell monolayer formation. Test compounds were diluted with the transport buffer (HBSS with 10 mM HEPES, pH 7.4) from DMSO stock solution to a concentration of 2 µM (DMSO<1%) and applied to the apical (A) or basolateral (B) side of the cell monolayer. Permeation of the test compounds from A to B direction or B to A direction was determined in duplicates. Reference compounds were digoxin tested at 10 µM from A to B direction and B to A direction, and nadolol and metoprolol tested at 2 µM in A to B direction. The plate was incubated at 37 °C with 5% $CO_2$ at saturated humidity without shaking for 2.5 hours. Test and reference compounds were quantified by LC-MS/MS analysis based on the peak area ratio of analyte/internal standard.

After transport assay, the Lucifer yellow rejection assay was applied to determine the cell monolayer integrity. Buffers were removed from both apical and basolateral chambers, followed by the addition of 75 µL 100 µM Lucifer yellow in transport buffer and 250 µL transport buffer in apical and basolateral chambers, respectively. The plate was incubated at 37 °C with 5% CO2 at saturated humidity without shaking for 30 min, then 20 µL of Lucifer yellow samples were taken from the apical sides and replaced with 60 µL transport buffer, then 80 µL of Lucifer yellow samples are taken from the basolateral sides. The relative fluorescence unit (RFU) of Lucifer yellow was measured at 425/528 nm (excitation/emission) with an Envision plate reader.

Data Analysis:

The apparent permeability coefficient Papp (cm/s) was calculated using the equation:

$$P_{app} = (dC_r/dt) \times V_r / (A \times C_0)$$

[$dC_r/dt$] is the cumulative concentration of compound in the receiver chamber as a function of time (µM/s).

[$V_r$] is the solution volume in the receiver chamber (0.075 mL on the apical side, 0.25 mL on the basolateral side).

[A] is the surface area for the transport, i.e. 0.0804 $cm^2$ for the area of the monolayer.

[$C_0$] is the initial concentration in the donor chamber in µM.

**Mouse liver microsomal stability assay.** Microsomal stability in CD-1 mouse liver microsomes (Xenotech #M1000) was determined by mixing a pre-warmed liver microsome preparation (0.56 mg/mL in 100 mM potassium phosphate buffer) with test compound working solution (100 µM in 99% MeCN) and NADPH working solution (10 mM NADPH, 10 mM $MgCl_2$) to achieve a final concentration of 0.5 mg protein/mL microsomes, 1 µM test compound and 1 µM NADPH in the incubation wells. After the addition of NADPH, the plate was incubated at 37 °C for 60 min and a timer was started to remove 60 µL aliquots and mix them with 180 µL stop solution (200 ng/mL tolbutamide and 200 ng/mL labetalol in acetonitrile, cooled to 4°C) after 5, 15, 30, 45, and 60 min. A time zero (T0) sample was prepared by mixing NADPH work solution with stop solution first, then adding the microsome preparation and test compounds. A separate no co-factor plate (NCF60) was prepared with NAPDH working solution replaced by buffer and also incubated at 37 °C for 60 min, with one aliquot taken and quenched at 60 min. Quenched samples were shaken for 10 min, then centrifuged at 4000 rpm at 4 °C for 20 minutes, then 80 µL supernatant was transferred into 240 µL HPLC water. Plates were sealed, shaken and submitted to LC-MS/MS analysis.

Data Analysis:

The equation of first order kinetics was used to calculate $T_{1/2}$ and $CL_{int(mic)}$ in µL/min/mg:

$$C_t = C_0 \bullet e^{-k_e \bullet t}$$

$$\text{when } C_t = \frac{1}{2} C_0,$$

$$T_{1/2} = \frac{Ln2}{k_e} = \frac{0.693}{k_e}$$

$$CL_{int(mic)} = \frac{0.693}{\text{In vitro } T_{1/2}} \bullet \frac{1}{\text{mg / mL microsomal protein in reaction system}}$$

**Mouse plasma stability assay.** Plasma stability was determined in CD-1 mouse plasma (Beijing Vital River Laboratory Animal Technology), thawed in a water bath at 37 °C and cleared by centrifugation at 4000 rpm for 5 min. Using an Apricot automation workstation and 96-well reaction plates, 98 µL/well of blank plasma was combined with 2 µL test compound working solution (100 µM in DMSO). Duplicates for each test compound were incubated for 0, 10, 30, 60 or 120 min at 37 °C in a water bath, then 400 µL stop solution (200 ng/mL tolbutamide and 200 ng/mL labetalol in acetonitrile) was added, plates were sealed and shaken for 20 min, then centrifuged at 4000 rpm and 4 °C for 20 minutes and 50 µL supernatant was transferred into 100 µL HPLC water and submitted to LC-MS/MS analysis.

Data Analysis:

The % remaining of test compound after incubation in plasma was calculated using following equation:

% Remaining = 100 x (PAR at appointed incubation time / PAR at T0 time).

[PAR] is the peak area ratio of analyte versus internal standard (IS).

**Mouse plasma binding assay.** Plasma binding was determined by high-throughput equilibrium dialysis in 96-well format using micro-equilibrium dialysis devices (HTDialysis #1006) and HTD 96 a/b regenerated cellulose membrane strips with a molecular mass cutoff of 12–14 kDa (HTDialysis #1101). Briefly, the dialysis device was assembled following the manufacturer's instructions using separated and equilibrated dialysis membrane strips. The blank matrix consisted of CD-1 mouse plasma (Beijing Vital River Laboratory Animal Technology) and was thawed by running under cold water and cleared by centrifugation at 3220 rpm for 5 min. The pH of the plasma was confirmed to be within 7.0–8.0. Test and control compounds were used in DMSO working solutions at 400 μM. Loading matrix was prepared by adding 5 μL DMSO working solution in a 1:200 ratio to blank matrix (995 μL). Time zero (T0) samples were prepared in triplicates as a 1:1 mixture of loading matrix and dialysis buffer (PBS) and immediately diluted with stop solution (200 ng/mL tolbutamide, 200 ng/mL labetalol and 50 ng/mL metformin in acetonitrile). To load the dialysis device, aliquots of 150 μL loading matrix were placed on the donor side of each dialysis well in triplicate, and 150 μL dialysis buffer was placed on the receiver side of the wells. The dialysis plate was placed in a humidified incubator at 37 °C with 5% $CO_2$ on a shaking platform that rotated slowly (about 100 rpm) for 4 hours, then aliquots of 50 μL were taken from both the buffer side and the matrix side of the dialysis device and were transferred into new 96-well plates. Each sample was mixed with an equal volume of opposite blank matrix (buffer or blank plasma matrix) to reach a final volume of 100 μL of 1:1 matrix/dialysis buffer (v/v) in each well. All samples were further processed by adding 500 μL of stop solution containing internal standards. The mixture was vortexed and centrifuged at 4000 rpm for about 20 minutes. An aliquot of 100 μL supernatant each was then removed for LC-MS/MS analysis. Blank samples were prepared the same way from blank matrix and buffer without test compound.

Data Analysis:

The % Unbound, % Bound, and % Recovery values were calculated using the following equations:

$\%unbound = 100 \times F / T$; $\%Bound = 100 - \%Unbound$; $\%Recovery = 100 \times (F + T) / T0$

[F] is the analyte concentration or peak area ratio of analyte/internal standard on the buffer (receiver) side of the membrane.

[T] is the analyte concentration or peak area ratio of analyte/internal standard on the matrix (donor) side of the membrane.

[T0] is the analyte concentration or the peak area ratio of analyte/internal standard in the loading matrix sample at time zero.

## Chemoproteomic profiling

**Preparation of cell lysates for affinity pulldown assays.** BE(2)C cell lysates were prepared from 30–50 confluent T175 flasks, harvested by trypsination, following three PBS washes and flash freezing of the combined pellets in liquid nitrogen. Lysis was performed by re-suspending the cell pellets in 0.35 mL freshly prepared lysis buffer per confluent T175 flask harvested, incubation for 5 min on ice, then lysates were cleared by ultracentrifugation. Lysis buffer composition: 0.8% IGEPAL, 50 mM Tris-HCl pH 7.5, 5% glycerol, 1.5 mM $MgCl_2$, 150 mM NaCl, 1 mM $Na_3VO_4$, 25 mM NaF, 1 mM DTT, supplemented with protease inhibitors (Sigma S8820-20TAB).

**Competition pulldown assays.** Selectivity profiling of DKFZ-748 and SAHA was performed in triplicates, for CHDI00465983 in duplicates. A volume of 0.5 mL lysate (adjusted to 5 mg/mL protein concentration and 0.4% IGEPAL) was pre-incubated with 11 doses of inhibitor (DMSO vehicle, 3 nM, 10 nM, 30 nM, 100 nM, 300 nM, 1000 nM, 3000 nM, 10000 nM, 30000 nM, 100000 nM) for 1 h at 30 °C in an end-over-end shaker, followed by incubation with 18 µL affinity matrix (1:1:1 mixture of iC, iQ and iA)[24] for 30 min at 30 °C in an end-over-end shaker. To assess the degree of protein depletion from lysates by the affinity matrix, a second pulldown (PDPD) with fresh beads was performed in six replicates, using the unbound protein fraction from the vehicle control flow through. The beads were washed (1 x 1 mL of lysis buffer without inhibitors and only 0.4% IGEPAL, 2 x 2 mL of lysis buffer without inhibitors and only 0.2% IGEPAL, 3 x 1 mL of lysis buffer without inhibitors and without IGEPAL) and captured proteins were denatured with 8 M urea buffer (containing 10 mM dithiothreitol), alkylated with 55 mM chloroacetamide and digested with 30 ng trypsin per sample. Resulting peptides were desalted on a C18 filter plate (Waters 186002318), vacuum dried and stored at –20 °C until LC-MS/MS measurement.

**LC-MS/MS measurement of competition pulldown assays.** Peptides were analyzed via LC-MS/MS on a Dionex Ultimate3000 nano HPLC coupled to an Orbitrap Fusion Lumos mass spectrometer (Thermo Fisher Scientific), operated by the Thermo Scientific Xcalibur software. Peptides were loaded on a trap column (100 µm x 2 cm, packed in house with Reprosil-Gold C18 ODS-3 5 µm resin, Dr. Maisch, Ammerbuch, Germany) and washed with 5 µL/min solvent A (0.1 % formic acid in HPLC grade water) for 10 min. Peptides were then separated on an analytical column (75 µm x 40 cm, packed in house with Reprosil-Gold C18 3 µm resin, Dr. Maisch, Ammerbuch, Germany) using a 50 min gradient ranging from 4-32 % solvent B (0.1 % formic acid, 5 % DMSO in acetonitrile) in solvent A (0.1 % formic acid, 5 % DMSO in HPLC grade water) at a flow rate of 300 nL/min. The mass spectrometer was operated in data dependent mode, automatically switching between MS1 and MS2 spectra. MS1

spectra were acquired over a mass-to-charge (m/z) range of 360-1300 m/z at a resolution of 60,000 (at m/z 200) in the Orbitrap using a maximum injection time of 50 ms and an automatic gain control (AGC) target value of 4E5. Up to 12 peptide precursors were isolated (isolation width of 1.2 Th, maximum injection time of 75 ms, AGC value of 1E5), fragmented by HCD using 30 % normalized collision energy (NCE) and analyzed in the Orbitrap at a resolution of 30,000. The dynamic exclusion duration of fragmented precursor ions was set to 20 s.

**Competition pulldown assay protein identification and quantification.** Protein identification and quantification was performed using MaxQuant[1] (v1.6.1.0) by searching the LC-MS/MS data against all canonical protein sequences as annotated in the Swissprot reference database (v03.12.15, 20193 entries, downloaded 22.03.2016) using the embedded search engine Andromeda. Carbamidomethylated cysteine was set as fixed modification and oxidation of methionine and N-terminal protein acetylation as variable modifications. Trypsin/P was specified as the proteolytic enzyme and up to two missed cleavage sites were allowed. Precursor tolerance was set to 10 ppm and fragment ion tolerance to 20 ppm. The minimum length of amino acids was set to seven and all data were adjusted to 1% PSM and 1% protein FDR. Label-free quantification[25] was performed with LFQ min. ratio count of 1 (unique and razor) and match between runs was enabled.

**(Competition) Pulldown assay data analysis.** For the competition assays, relative binding was calculated based on the protein intensity ratio relative to the DMSO control for every single inhibitor concentration. $EC_{50}$ values were derived in GraphPad Prism (GraphPad Software, La Jolla California USA) by nonlinear regression four parameters least squares fit of Y=Bottom + (Top-Bottom)/(1+10^((LogEC50-X)*HillSlope)), with Top fixed to 1. Mean $EC_{50}$ values were calculated from the dose response replicates and the obtained mean $EC_{50}$ values were multiplied with a protein-dependent correction factor (cf), resulting in the apparent $K_D$ value ($K_D^{app}$). The correction factor is determined by calculating the ratio of the protein intensity of two consecutive pulldowns of the vehicle control sample.[26] Correction factors were set to the median of correction factors derived from 6 replicates ($cf^{HDAC1}$=1, $cf^{HDAC2}$=1, $cf^{HDAC8}$=0.75, $cf^{HDAC6}$=1, $cf^{HDAC10}$=1, $cf^{HDAC5}$=1, $cf^{ALDH11}$=0.89, $cf^{ALDH2}$=0.82, $cf^{ISOC1}$=0.85, $cf^{ISOC2}$=0.41, $cf^{GATD3A}$=1, $cf^{MBLAC2}$=0.78). Median correction factors equal to or larger than 1 indicate no relevant target depletion and were hence set to 1. Targets of the inhibitors were annotated manually. A protein was considered a target or interactor of a target if the resulting binding curve showed a sigmoidal curve shape with a dose dependent decrease of binding to the beads. Additionally, the number of unique peptides and MS/MS counts per condition were taken into account.

**Data Availability.** The mass spectrometry proteomics data including the used Swissprot reference database have been deposited to the ProteomeXchange Consortium via the PRIDE[27] partner repository with the dataset identifier PXD032671.

## SDS-PAGE and Western blot analysis

**Acetylation Western blots.** BE(2)-C cells were seeded in full medium at a density of 5 x $10^6$ cells per 15 cm dish the night before treatment, then treated by replacing the seeding medium with treatment medium containing the indicated drug concentration and 0.2% DMSO for all treatment conditions. Cells were incubated for 4 h, then harvested by PBS wash, trypsinization at 37 °C for 5 min, diluted with full medium at 4 °C and washed by centrifugation and subsequent re-suspending in fresh PBS at 4 °C. Then the cell suspension for each treatment was split into two aliquots for preparation of a whole cell lysate and an aliquot for histone isolation. The supernatant was removed and cell pellets were flash frozen. Whole cell lysates were prepared by adding 1x lysis buffer (Cell Signaling #9803) containing 1x protease inhibitor (Serva #39101.01) to cell pellets, then sonication 2x for 30 s and removal of cell debris by centrifugation with 17000 g at 4 °C for 10 min. Histones were isolated by cell lysis with hypotonic lysis buffer and precipitation with trichloroacetic acid as described previously.[28] Protein concentrations were determined by Bradford assay (Bio-Rad #5000006), then 15 µg protein from histone preparation or whole cell lysate were diluted 5:1 with 5x Lämmli buffer (30% (v/v) glycerin, 3% (wt/v) SDS), 62.5 mM Tris, 4.3 mM bromophenol blue, adjusted to pH 6.8, 0.15 M DTT added freshly) and denatured at 95 °C for 10 min. Samples were loaded onto a freshly prepared 15% polyacrylamide gel. SDS-PAGE and blotting was performed with a Mini PROTEAN Tetra Cell (Bio-Rad, Germany) electrophoresis system and a PowerPac Basic (Bio-Rad, Germany) power supply. SDS-PAGE was run at 80–120 V with running buffer containing 25 mM Tris, 0.19 M glycine and 0.1 % (wt/v) SDS. Proteins were blotted onto a 0.2 µm Amersham Protran nitrocellulose membrane (Cytiva 1060001) for 70 min at 90 V (blotting buffer: 20 mM boric acid, 1 mM EDTA, adjusted to pH 8.8 with NaOH). The membrane was blocked for 1 h at rt with 5% skimmed milk powder (Gerbu #1602) in TBST buffer (20 mM Tris, 136 mM NaCl, 0.1% Tween-20), then incubated with the respective primary antibody, diluted in 5% skimmed milk powder in TBST, overnight at 4 °C. The blot was washed 3 x 10 min with TBST buffer, then incubated with the respective secondary antibody at rt for 1 h, and washed 3 x 10 min prior to fluorescence readout using an Odyssey Sa Near-Infrared Imaging System. Quantification was performed using Image Studio Lite Version 5.2. Protein Ladder: PageRuler Prestained (Thermo Scientific #26616), primary antibodies: α-tubulin (mouse monoclonal, diluted 1:2000, abcam ab7291),

α-tubulin acetyl K40 (rabbit monoclonal, diluted 1:2000, abcam ab179484), histone H3 (rabbit polyclonal, diluted 1:1000, Cell Signaling #9715) and histone H3 acetyl K9 (rabbit monoclonal, diluted 1:1000, Cell Signaling #9649), secondary antibodies: IRDye 680RD donkey anti-mouse IgG (diluted 1:10000, LI-COR Biosciences #926-68072), IRDye 800CW donkey anti-rabbit IgG (diluted 1:10000, LI-COR Biosciences #926-32213).

**Western blots against autophagy markers p62 and LC3-1/II.** Western blots against autophagy markers was performed as described in Körholz et al.[29] Briefly, BE(2)-C cells were treated on 10 cm dishes for 24 h with inhibitors, following lysis in SDS lysis buffer (0.5 M Tris-HCl pH 6.8, 2% SDS, 8.7% glycerol, 1 mM DTT). Protein samples were denatured at 95 °C for 10 min and 30 µg protein per lane was loaded in bromophenol blue loading buffer. Proteins were separation by SDS-PAGE, followed by blotting to polyvinylidene fluoride membranes using a semidry electroblot chamber (blotting time according to the size of the protein of interest). Membranes were blocked in blocking solution (Tris-buffered saline, 20% nonfat milk from milk powder, 20% FCS, 3% BSA, 1% normal goat serum, 0.2% Tween20) for 1 h. Incubation with a primary antibody solution over night was performed at 4 °C followed by 1 h incubation with a peroxidase-conjugated secondary antibody at room temperature. The following antibodies were used for detection by the electrogenerated chemiluminescence (ECL) method: anti-p62 (Sigma P0067), anti-LC3B (Sigma L7543), anti-β-actin (Sigma A1978). Quantification of Western blot images was performed using ImageJ-software version 1.0.

## Lysotracker assay

Enlargement of the lysosomal compartment upon treatment with **DKFZ-728** or SAHA was determined using the LysoTracker DND-99 probe in BE(2)-C cells as described previously.[30] Briefly, cells were treated for 24 h with HDACi or lysosomal inhibitors, then stained 1 h with 50 nM LysoTracker Red DND-99 in medium under standard cell culture conditions. Cells were washed with ice-cold RPMI (without phenol-red) and detached by trypsinization (Gibco #25300-054) for 3 minutes at 37 °C. Cells were centrifuged, resuspended in RPMI (without phenol-red) and LysoTracker Red fluorescence was quantified on a BD FACSCanto II platform using the PE filter setting.

## Cytotoxicity assay

Cells were seeded the previous day in 96-well plates (Greiner 655 180) in full medium. Initial seeding densities were for BE(2)-C 5.5k, for HEK293T 2.5k and for HeLa 1.25k cells per well. Drugs were dosed in triplicates for each dose level from DMSO stock solutions with a D300e Digital Dispenser (Tecan) once and all wells were normalized to 0.5% DMSO. Cells were cultivated for 72 h after dosing, then per well 50 µL CellTiter-Blue (Promega G8081) solution, diluted 1:5 in medium, was added and plates incubated at 37 °C for at least 90 min. Fluorescence was recorded at 560/590 nm in a

CLARIOstar (BMG Labtech) plate reader. Full viability was defined by vehicle controls, 0% viability was defined by only medium containing wells. $pIC_{50}$-values were calculated using log(inhibitor) vs normalized fluorescence by nonlinear regression four parameters least squares fit in GraphPad Prism version 7.05 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com.

## Co-treatment with the Helmholtz Drug Repurposing Library

BE(2)-C cells, cultured as described in the cell culture procedure section, were detached by trypsinization (Gibco #25300-054) and pooled from multiple confluent cell culture dishes. Cell suspensions were filtered through a 40 µm cell strainer (Corning #352340) and diluted to 77.78 k cells/mL in full medium, then 45 µL (3.5 k cells) per well was added to 384-well culture plates (Perkin Elmer #6007689) using a FlexDrop IV Exi dispenser (Perkin Elmer). Additional 2.5 µL medium containing either HDAC10 inhibitor **37** or vehicle control was added to each well using an automated JANUS model G1 Integrator workstation equipped with an MDT 384 dispensing head (Perkin Elmer) to reach a final concentration of 5 µM. Plates were incubated at 37 °C, 95% humidity and 5% $CO_2$ for 20 h, then 2.5 µL of drug library stocks was added with the JANUS workstation to reach a final concentration of 5 µM or 0.1 µM, or the matching vehicle control concentration in two columns of each plate. Additionally, each plate was prepared with two columns of positive controls, treated with 5 µM mitoxantrone dihydrochloride (Sigma-Aldrich M6545). Plates were incubated at 37 °C, 95% humidity and 5% $CO_2$ for 48 h, then equilibrated to rt and 25 µL/well ATPLite 1step reagent (Perkin Elmer #6016731) was added using a FlexDrop dispenser IV Exi (Perkin Elmer). Plates were incubated in the dark for 10 minutes and luminescence was read 0.1 sec/well with an Envision plate reader model 2104 (Perkin Elmer) with an ultrasensitive detector. Assay signals were normalized to the vehicle controls and positive controls of each plate as percent inhibition in ActivityBase Version 9.4 (IDBS):

Inhibition [%] = 100 – ((RawData – MeanLowerBound)/(MeanUpperBound – MeanLowerBound) × 100).

## Targeted metabolomics of acetylated spermidines

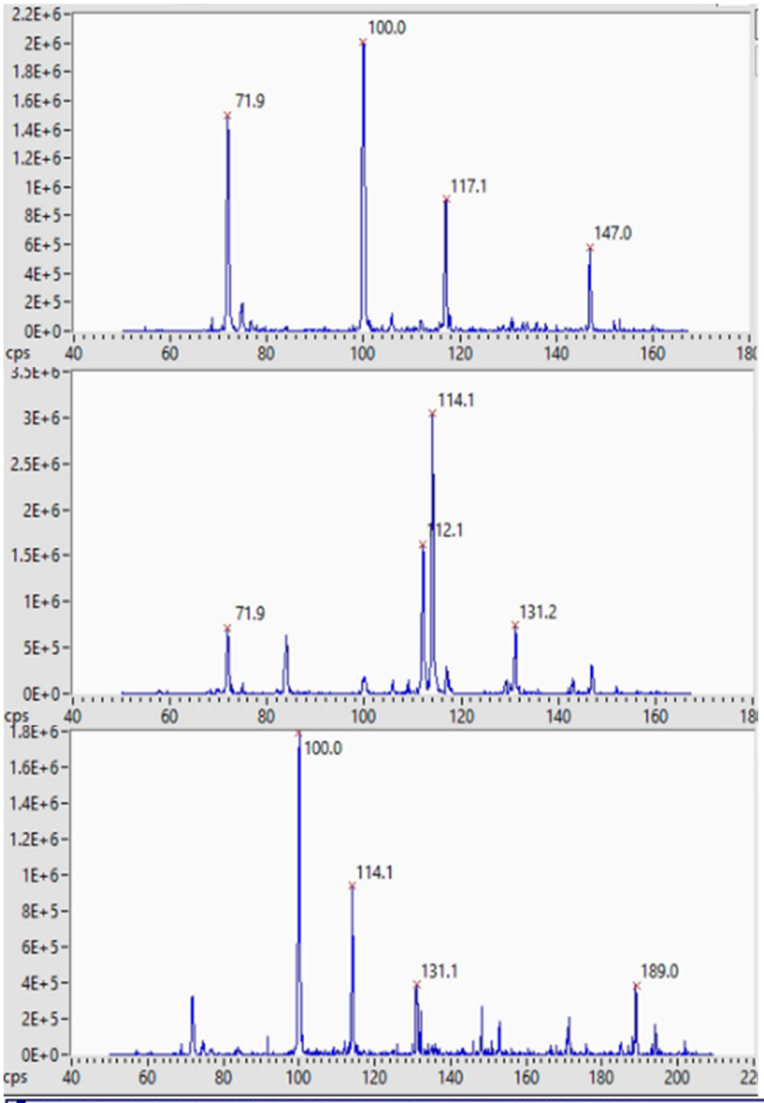
**Preparation of inhibitor-treated cell samples.** BE(2)-C cells were seeded in a volume of 19 mL full medium at a density of 2.9 x $10^6$ cells per 15 cm dish the night before treatment, then 1 mL of 20x drug treatment solution in full medium was added to reach the indicated drug concentration and 0.4% DMSO for all treatment conditions. Cells were cultivated for 24 h and checked for equal confluency (70–100%) in all treatment conditions before harvest. Then, the medium was removed, cells were washed with 0.9% NaCl (Braun #08609249), detached by trypsinization at 37 °C for 5 min, diluted with full medium at 4 °C and washed two times at 4 °C by centrifugation at 300 g and 4 °C for 5 min and subsequent re-suspending in fresh 0.9% NaCl. Then, 10 x $10^6$ cells from each replicate were taken, as confirmed by

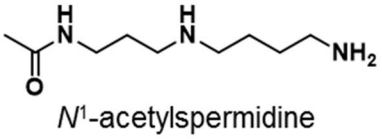
cell counting of small aliquots, and pelleted by centrifugation at 1000 g and 4 °C for 5 min. Supernatant was aspirated and cell pellets were snap-frozen in liquid nitrogen before storage at –80°C.

**Preparation of HDAC10 knockdown cell samples.** BE(2)-C cells were seeded at a density of 0.4 x $10^6$ cells per 10 cm dish on 17 dishes per replicate, allowing for three technical replicates per condition. Transfection with siRNAs against HDAC10 or with control siRNAs was performed as described previously.[29] A pool of two siRNAs was used against HDAC10 (Ambion/ThermoFisher Scientific siRNA IDs 33581, 120681) and for negative control transfection, respectively (Silencer Negative Control #1 and Silencer Negative Control #5, Ambion/ThermoFisher Scientific AM4611, AM4642). Medium was changed on the morning following transfection. For harvesting, cells were washed in ice-cold sterile 0.9% NaCl, then trypsinized and transferred to 50 mL Falcon tubes. A fraction of the cell suspension (750 µL) was used for cell counting (Vi-Cell XR automated cell counter, Beckmann Coulter). Cells were centrifuged at 300 g and 4 °C for 5 min and washed with 0.9% NaCl, then resuspended in 0.9% NaCl at a cell number of 10 x $10^6$ cells per mL. Of this cell suspension, 15 x $10^6$ cells for quantification of acetyled spermidines were transferred to pre-cooled 1.5 mL Eppendorf tubes. Cells were centrifuged in a table-top centrifuge at 2700 g for 3 min at 4 °C. Supernatant was aspirated and cell pellets were snap-frozen in liquid nitrogen before storage at –80°C.

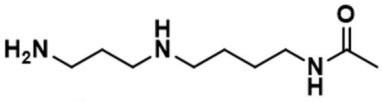
**Extraction and quantification of acetylated spermidines.**[31,32] For quantification of acetylated spermidines, pellets of at least 10 x $10^6$ cells were extracted two times on ice with 300 µL MeOH/MeCN (50:50) by vortexing, followed by 5 min centrifugation. The resulting supernatants were transferred to a new Eppendorf tube, dried without heat using the Eppendorf Concentrator Plus (Eppendorf, Hamburg), then re-suspended in 75 µL water. LC-MS/MS analysis was performed on a Waters Acquity I-class Plus UPLC system (Binary Solvent Manager, thermostatic Column Manager and FTN Sample Manager) (Waters, USA) coupled to an QTRAP 6500+ (Sciex, USA) mass spectrometer with electrospray ionization (ESI) source using the following settings: curtain gas: 30 psi; collision gas: low; ion spray: 4500 V; source temperature: 500°C; ion source gas 1:40 (GS1) and ion source gas 2:50 (GS2).
Acetylated spermidines were separated by chromatography on an Acquity HSS T3 column (150 mm x 2.1 mm, 1.7 µm, Waters) kept at 20 °C and a flow rate of 0.3 mL/min. Eluent A: water + 0.1% formic acid, eluent B: MeCN + 0.1% formic acid. Gradient: 0% B for 1 min, 0 → 20% B over 4 min, 20 → 100% B in 0.5 min, then 100% B for 1 min.
Identification and quantification of acetylation isomers was based on unique transitions extracted from the MS/MS fragmentation patterns of commercially available standards of $N^1$-acetylspermidine (Cayman Chemical #9001535), $N^8$-acetylspermidine (MedChemExpress #HY-113253A) and $N^{1,8}$-

diacetylspermidine (Cayman Chemical #21588). Fragmentation patterns and relative retention times shown below are in accordance with previously reported data.[31]

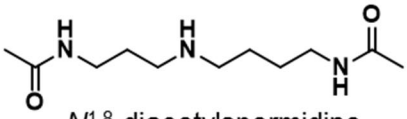


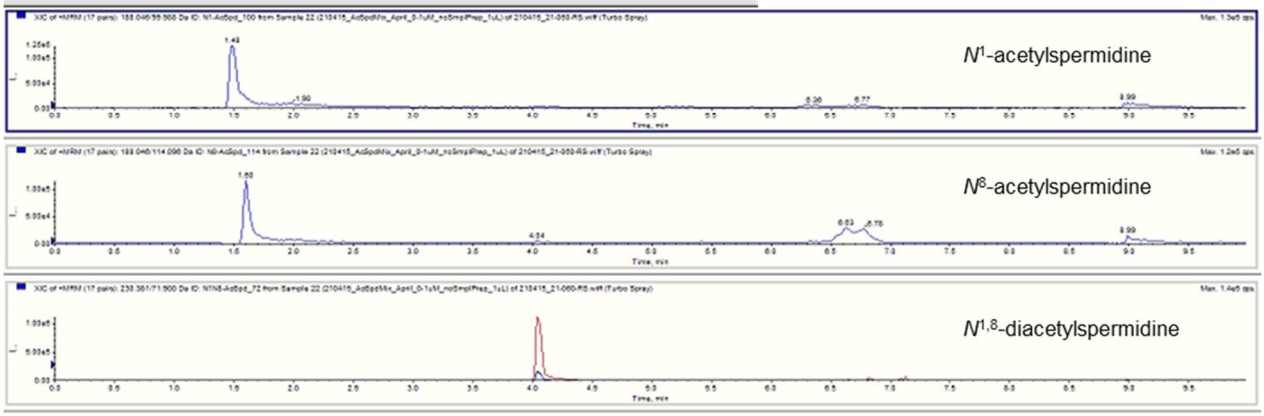
$N^1$-acetylspermidine



$N^8$-acetylspermidine



$N^{1,8}$-diacetylspermidine



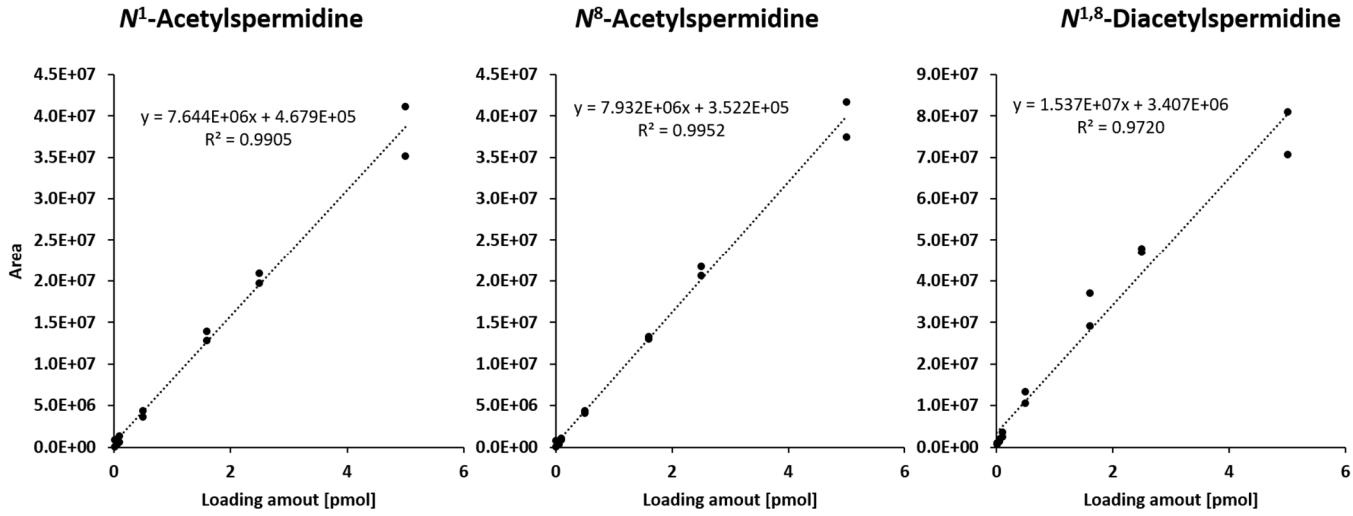
Fragmentation patterns of acetylated spermidines (top) and chromatographic traces (bottom) of acetylation isomer specific transitions. $N^1$-acetylspermidine: 188.046/99.998; $N^8$-acetylspermidine: 188.046/114.096; $N^{1,8}$-diacetylspermidine: 230.381/99.990 (red), 230.381/71.900 (blue).

Acetylated spermidines were quantified in positive electrospray ion mode with the settings and specific transitions described in the following table:

| Compound | Quantifier / Qualifier transition | Q1 mass (Da) | Q3 mass (Da) | Dwell time (msec) | DP (volt) | EP (volt) | CE (volt) | CXP (volt) |
|---|---|---|---|---|---|---|---|---|
| **$N^1$-Acetylspermidine** | Quantifier | 188.046 | 99.988 | 60 | 25 | 10 | 23 | 11 |
| | Qualifier 1 | 188.046 | 117.118 | 60 | 25 | 10 | 16 | 13 |
| **$N^{1,8}$-Diacetylspermidine** | Quantifier | 230.381 | 99.99 | 60 | 71 | 10 | 29 | 54 |
| | Qualifier 1 | 230.381 | 114.065 | 60 | 71 | 10 | 27 | 52 |
| | Qualifier 2 | 230.381 | 71.9 | 60 | 71 | 10 | 39 | 4 |
| **$N^8$-Acetylspermidine** | Quantifier | 188.046 | 114.096 | 60 | 25 | 10 | 23 | 12 |
| | Qualifier 1 | 188.046 | 112.149 | 60 | 25 | 10 | 27 | 7 |
| | Qualifier 2 | 188.046 | 131.192 | 60 | 25 | 10 | 20 | 8 |

Q1 mass: precursor ion (m/z), Q3 mass: product ion (m/z). EP: entrance potential, CE: collision energy, CXP: cell exist potential.

Calibration curves were generated from standard solutions prepared in 0.1 M HCl and are depicted below. Data acquisition was performed with Analyst (Sciex, USA), while data quantification was performed with the SciexOS software suite (Sciex, USA).



Calibration curves generated for $N^1$-acetylspermidine ($R_t$ = 1.49 min), $N^8$-acetylspermidine ($R_t$ = 1.62 min) and $N^{1,8}$-diacetylspermidine ($R_t$ = 4.05 min) from external standards using seven calibration points in duplicates.

## HeLa cell growth inhibition and polyamine rescue assay

**Cell culture and reagents.** HeLa S3 cells were obtained from ATCC (CCL-2.2; Manassas, VA) and grown in MEM-alpha (Corning #10-022-CV) with 10% fetal bovine serum, sodium pyruvate (ThermoFisher #11360-070), and penicillin/streptomycin solution (Corning #30-002-CI). Cells were maintained in a humidified atmosphere at 37 °C, 5% $CO_2$ and were routinely screened for mycoplasma infection using MycoAlert (Lonza #LT07-118, Walkersville, MD). Aminoguanidine (AG; 1 mM) was used in all studies including the addition of exogenous polyamines to limit extracellular polyamine oxidation by bovine serum amine oxidase.[33] DFMO was provided by Dr. Patrick Woster at the Medical University of South Carolina. Aminoguanidine (#396494) and $N^8$-AcSpd (A3658) were purchased from Sigma Chemical Co. (St. Louis, MO).

**CRISPR-Cas9-mediated HDAC10 knockout.** Oligonucleotides targeting the *HDAC10* gene were synthesized, annealed and inserted into the single guide RNA scaffold of the pLentiCRISPRv2-Blast vector (AddGene Watertown, MA, #83480) via the BsmBI sites according to methods previously described.[34] Three different oligo pairs were chosen for construct synthesis targeting *HDAC10*, and ligation of each was confirmed by Sanger sequencing of the sgRNA region. Transfections of HeLa S3 cells was performed using Lipofectamine 3000 (Invitrogen L3000-001) followed by single cell isolation through limiting dilution arrays and selection in growth media containing 10 μg/mL Blasticidin (Corning, #30-100-RB). Lysates of individual CRISPR-Cas9-edited clones were prepared in 4% SDS for HDAC10 knockout screening by Western blot. Proteins (30 μg/lane) were separated on 4-12% Bolt Bis-Tris polyacrylamide gels in MES running buffer (Invitrogen NP0002) and transferred onto Immunoblot PVDF membranes (BioRad, Hercules, CA). Primary antibodies to HDAC10 (1:1000; Sigma H3413) and PCNA (1:4000; Calbiochem NA03) were incubated overnight at 4 °C with rocking; IR-dye conjugated secondary antibodies (1:10000; Rockland, Limerick, PA, 611-132-122 and 1:20000; ThermoFisher Scientific #A-21057) allowed detection using a LI-COR Odyssey imager with ImageStudioLite analysis software (Lincoln, NE). Biallelic knockout through indel generation was subsequently confirmed in each cell line following PCR amplification of genomic DNA (extracted using Zymo Quick gDNA Micropreps #D3020; Irvine, CA) with primers flanking the guide RNA target of the *HDAC10* gene. PCR products were TOPO-TA cloned into the pCR4 vector for sequencing according to the manufacturer's protocol (#45-0071; Invitrogen). All primers were synthesized by IDT (Coralville, IA), and Sanger sequencing

was performed by the Genetic Resources Core Facility of the Johns Hopkins University School of Medicine. Stable cell lines with biallelic indels in exon 2 of *HDAC10* (created by sgRNA1 targeting AGTGCGAGATCGAGCGTCCTG) were used for all subsequent experiments.

**Cell proliferation studies.** Cells were seeded in triplicate at 1000 cells/well of 96-well plates and allowed to attach overnight. Medium was aspirated and replaced with 100 μL fresh medium containing 1 mM aminoguanidine, 5 mM DFMO, and increasing concentrations of $N^8$-AcSPD. After incubation for 96 h, 20 μL of CellTiter-Blue reagent (Promega G8080) was added per well and cells were incubated an additional 2 h. Fluorescence was measured ($560_{Ex}/590_{Em}$) on a SpectraMax M5 platereader (Molecular Dynamics, Sunnyvale, CA). In experiments using HDAC10 inhibitors, cells were pretreated 24 h with the inhibitor, after which the medium was replaced with that containing inhibitor, DFMO, and $N^8$-AcSPD for an additional 96 h incubation. Full viability was defined by cells treated only with aminoguanidine, 0% viability was defined by only medium containing wells.

## HPLC analysis of polyamine concentrations

Cells were seeded at 0.6 x $10^5$ cells per T-25 flask (Sarstedt #83.3910.002) and allowed to attach overnight. Media was aspirated and replaced with that containing 1 mM aminoguanidine, 5 mM DFMO, and $N^8$-AcSPD at final concentrations of 5, 10, or 50 μM. After incubation for 96 h, cell lysates were collected for protein quantitation and precipitation by adding 50 μL 1.2 N perchloric acid to a 50 μL aliquot of lysate. After centrifugation, acid-extracted supernatants were used for pre-column derivatization of polyamines with dansyl chloride (Sigma D2625) in the presence of diaminoheptane (Sigma D17408) as an internal standard. For experiments including HDAC10 inhibitors, cells were seeded as above and allowed to adhere to the flask approximately 4 h, at which time the inhibitor was added. After pre-treating the cells for 24 h, the media was aspirated and refreshed with that containing inhibitor, DFMO, aminoguanidine, and $N^8$-AcSPD, for an additional 96 h incubation. Dansyl-labeled polyamines were quantified by HPLC using the methods of Kabra et al.[35] and are presented relative to total cellular protein in the lysate, which was determined from centrifuged lysates using the method of Bradford (Bio-Rad #5000006), with interpolation on a bovine serum albumin standard curve. Absorbance of protein samples was measured in duplicates at 595 nm using a SpectroMax M5 platereader. For preparation of HPLC standards, $N^8$-AcSpd (#A3658), putrescine (#P77505), SPD (#S2501), and SPM (#S2876) were purchased from Sigma.

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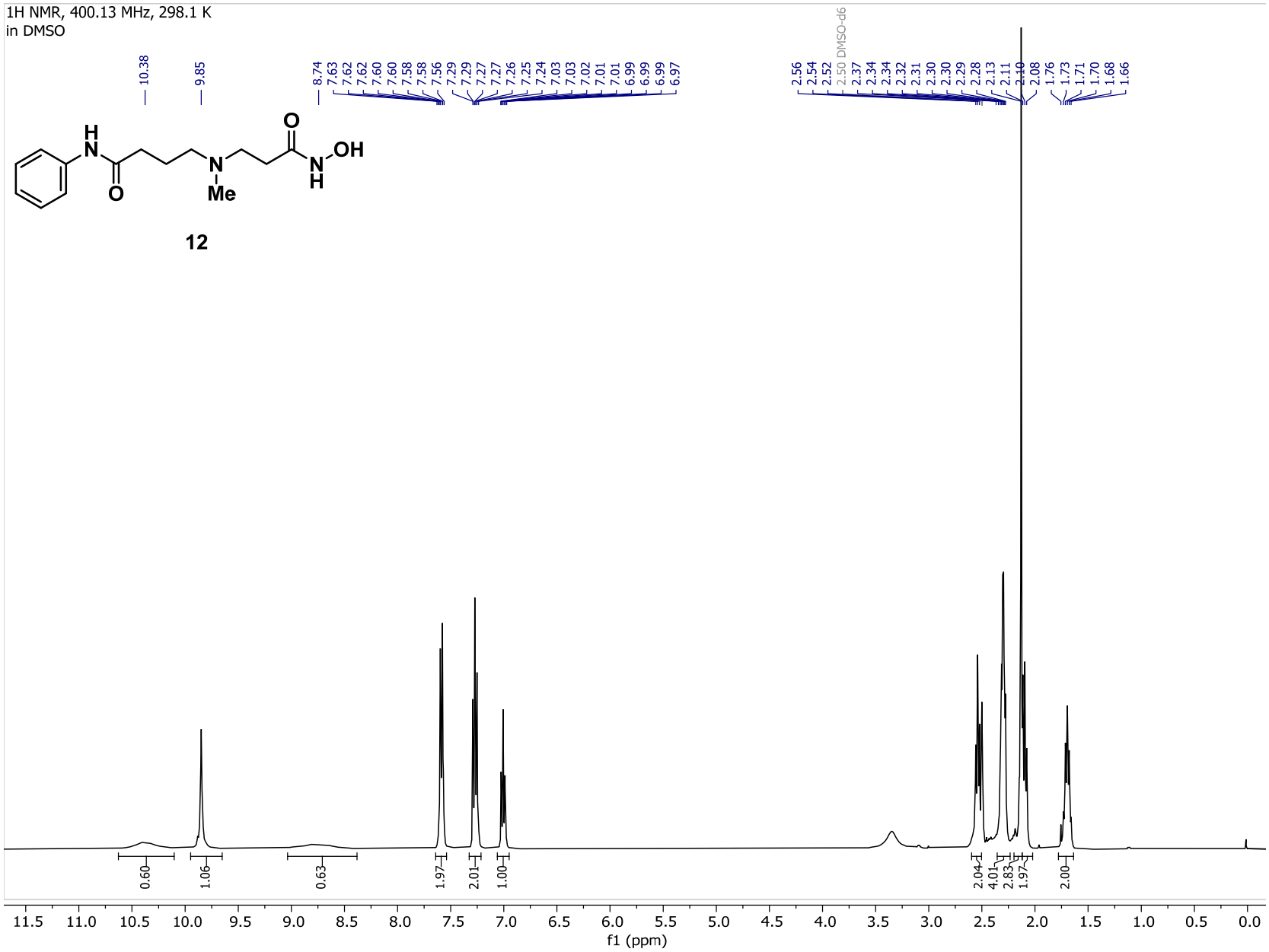
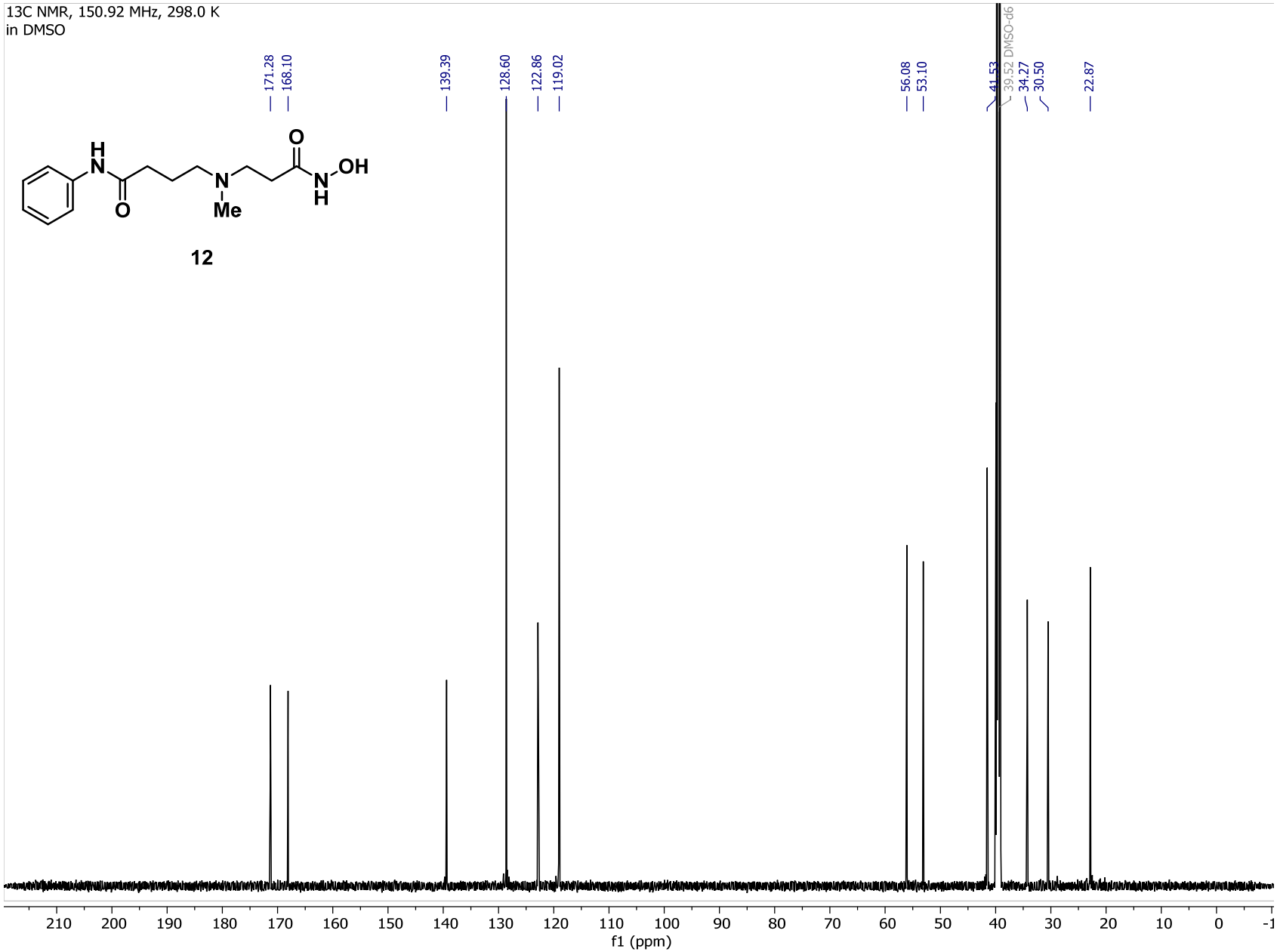
## NMR spectra and HPLC chromatograms

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in DMSO
171.28
168.10
139.39
128.60
122.86
119.02
56.08
53.10
41.53
39.52 DMSO-d6
34.27
30.50
22.87
H
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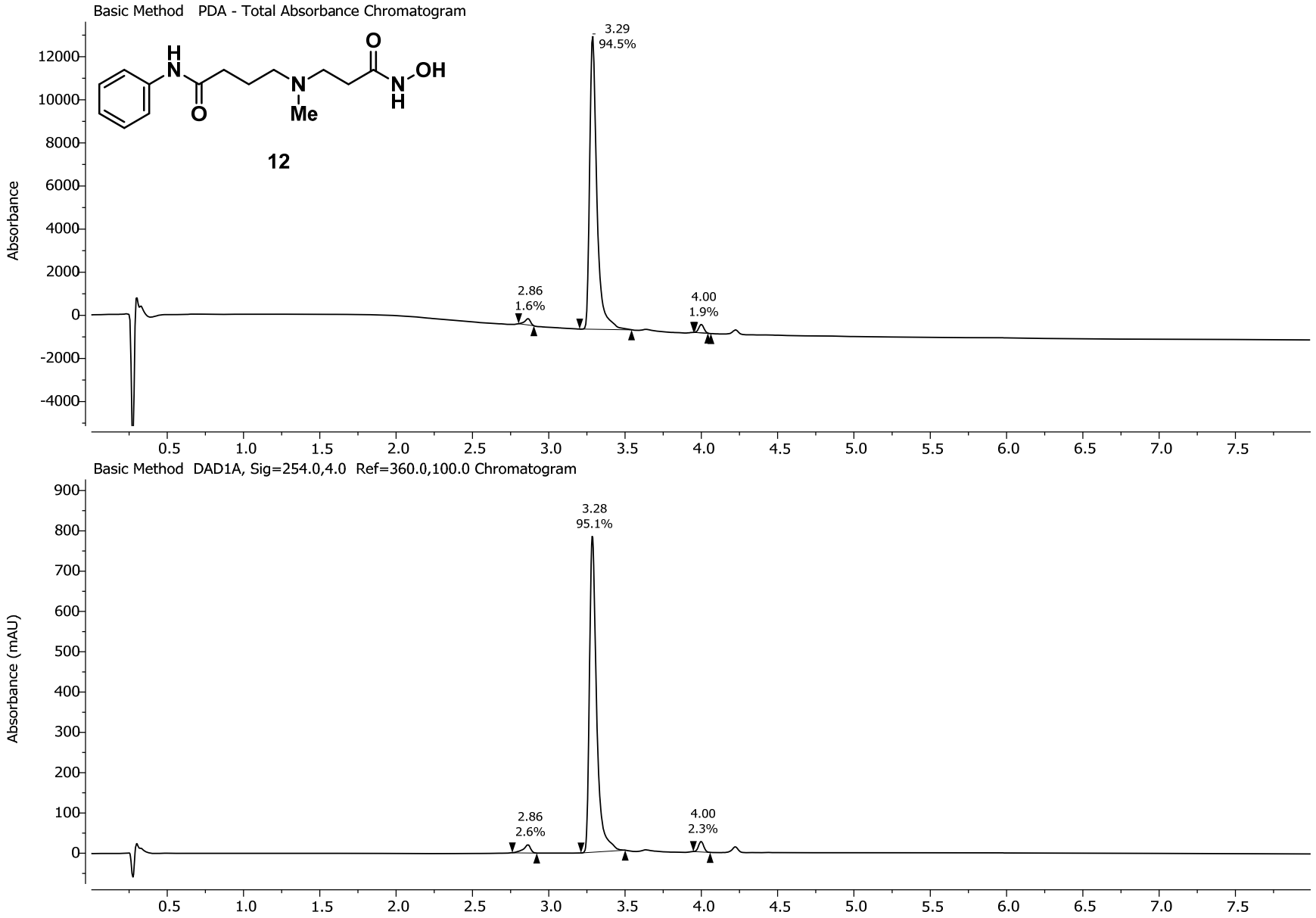
f1 (ppm)


Basic Method PDA - Total Absorbance Chromatogram
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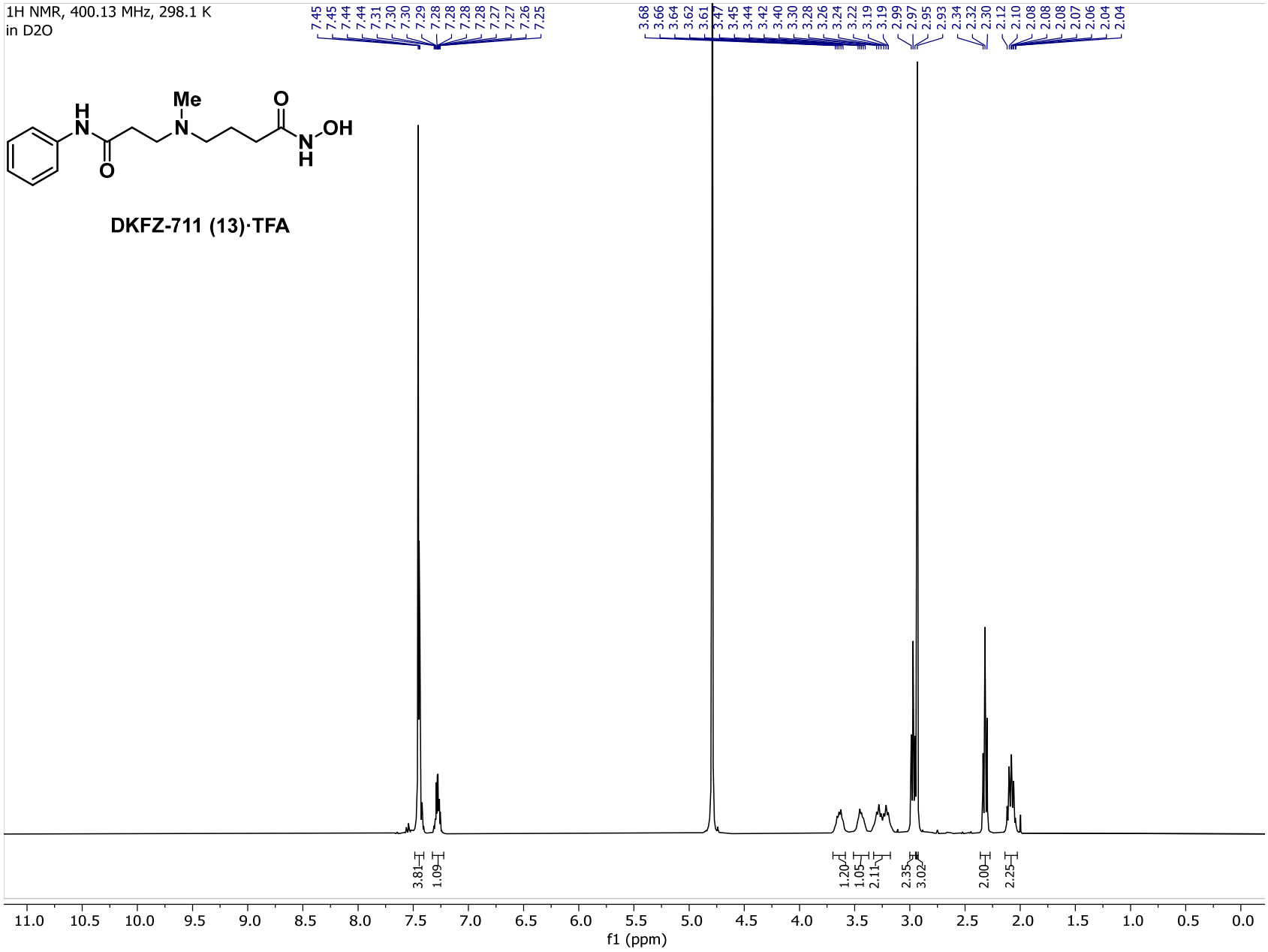
3.29
94.5%
2.86
1.6%
4.00
1.9%
Absorbance
Basic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
3.28
95.1%
2.86
2.6%
4.00
2.3%
Absorbance (mAU)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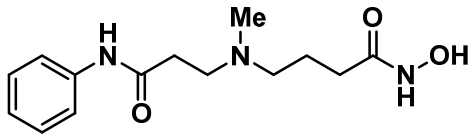
DKFZ-711 (13)·TFA
f1 (ppm)

19F NMR, 376.46 MHz, 298.1 K
in D2O

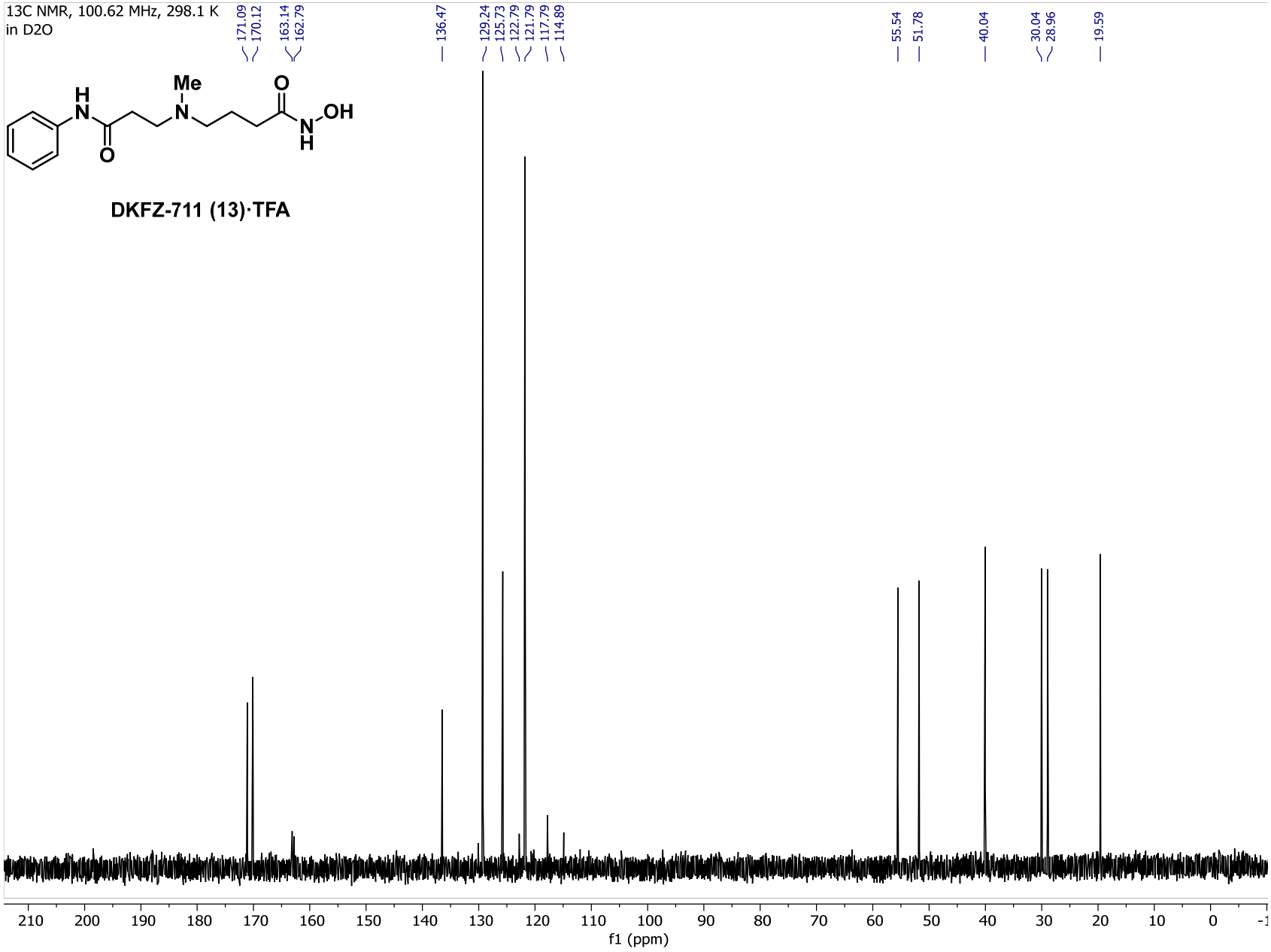
-78.39




**DKFZ-711 (13)·TFA**

20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210 -220 -230 -24

f1 (ppm)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in D2O
171.09
170.12
163.14
162.79
136.47
129.24
125.73
122.79
121.79
117.79
114.89
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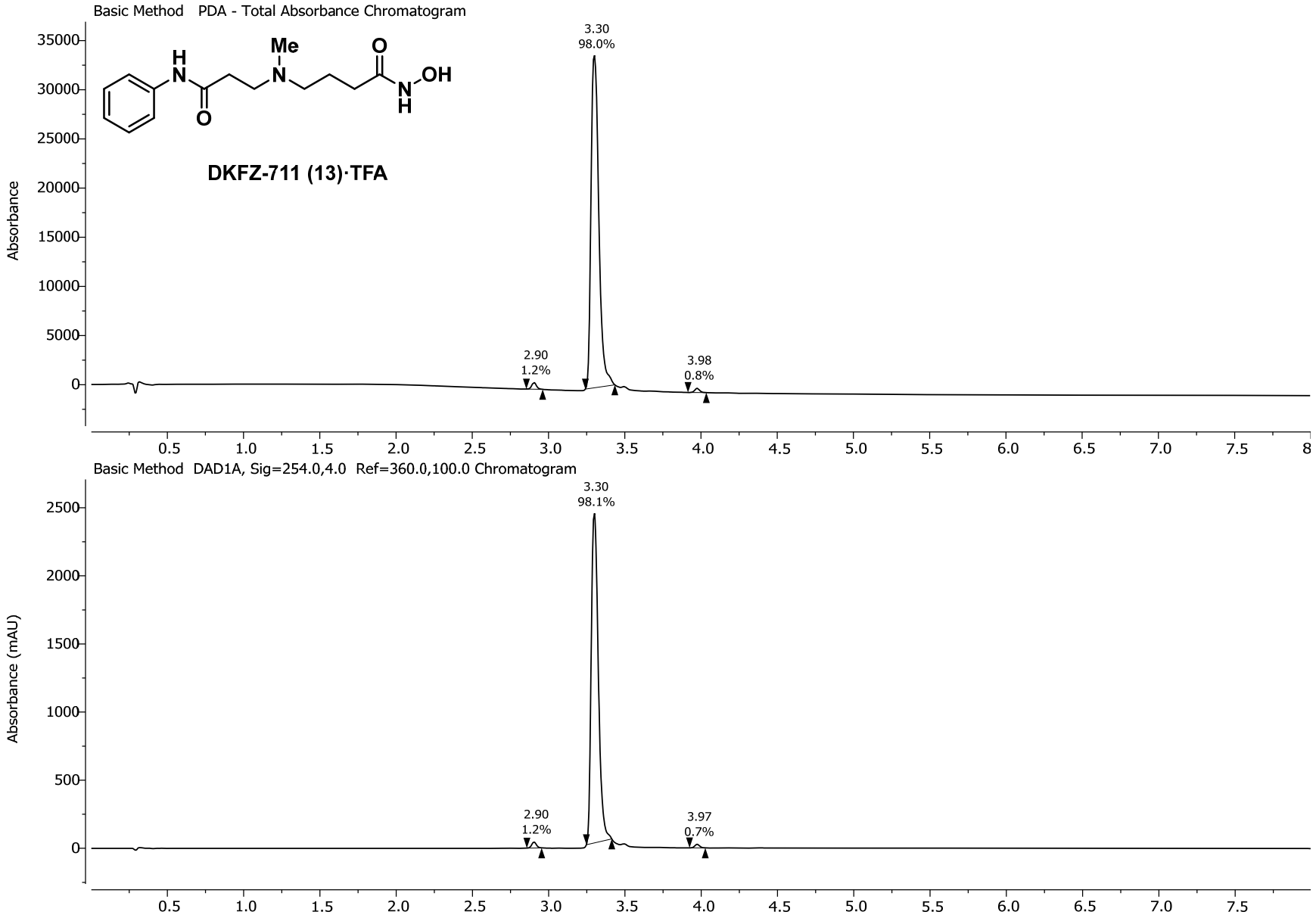
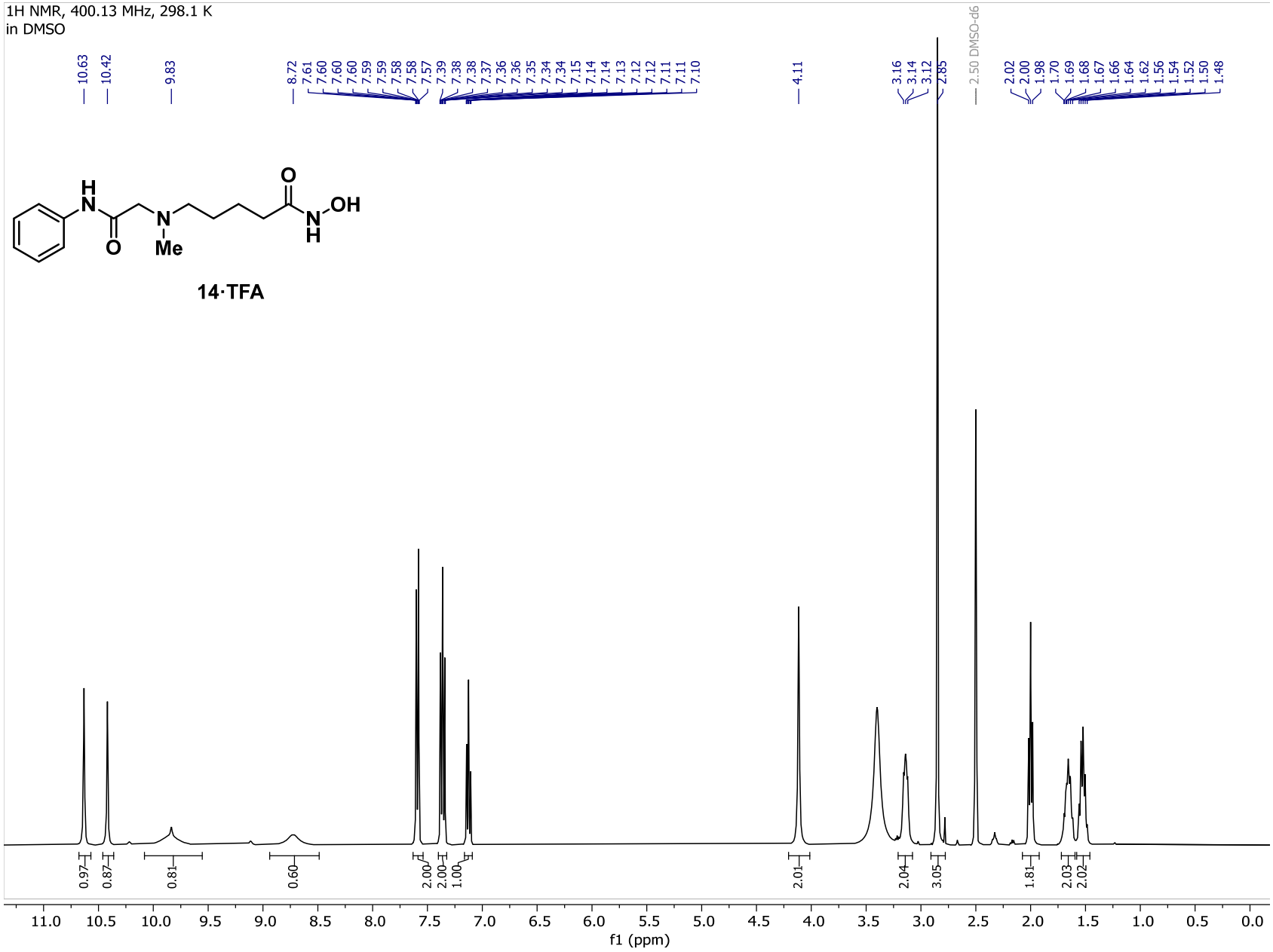
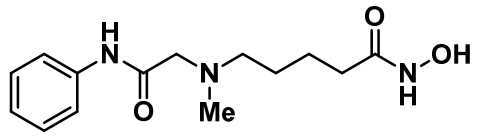
51.78
40.04
30.04
28.96
19.59
DKFZ-711 (13)·TFA
f1 (ppm)

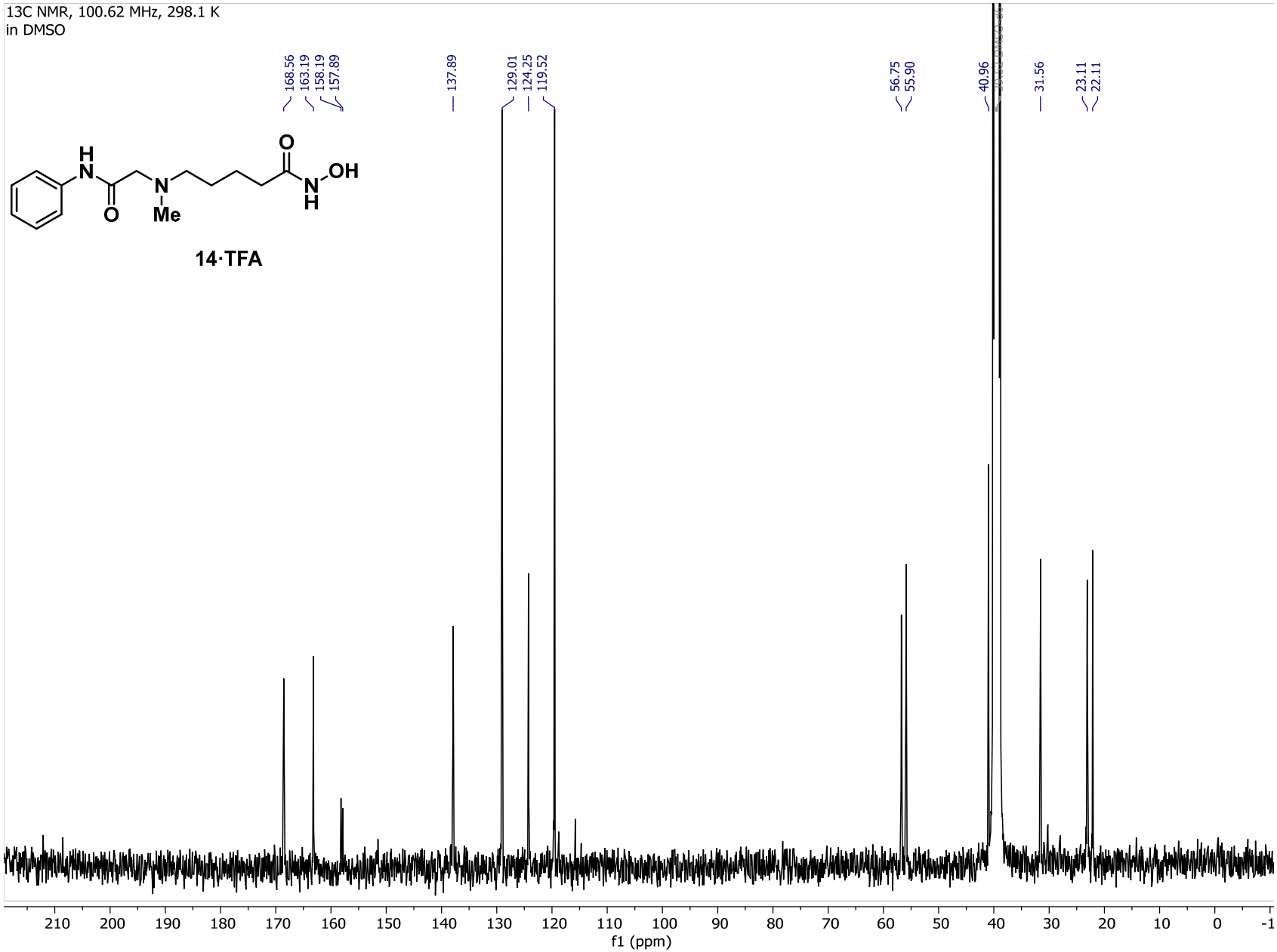

Basic Method PDA - Total Absorbance Chromatogram
DKFZ-711 (13)·TFA
Absorbance
3.30
98.0%
2.90
1.2%
3.98
0.8%
Basic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
Absorbance (mAU)
3.30
98.1%
2.90
1.2%
3.97
0.7%

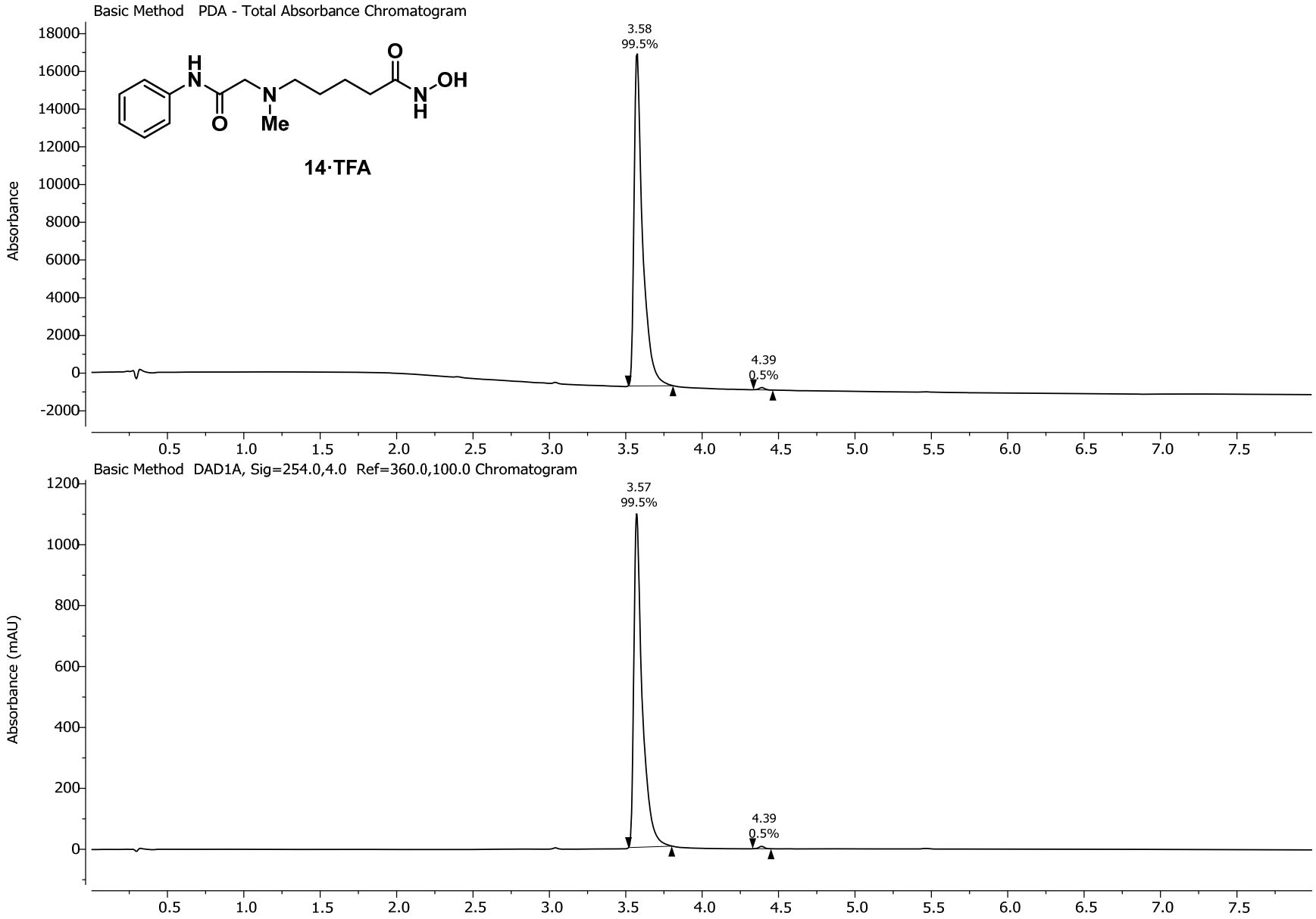

1H NMR, 400.13 MHz, 298.1 K
in DMSO
10.63
10.42
9.83
8.72
7.61
7.60
7.60
7.60
7.59
7.59
7.58
7.58
7.57
7.39
7.38
7.38
7.37
7.36
7.36
7.35
7.34
7.34
7.15
7.14
7.14
7.13
7.12
7.12
7.11
7.11
7.10
4.11
3.16
3.14
3.12
2.85
2.50 DMSO-d6
2.02
2.00
1.98
1.70
1.69
1.68
1.67
1.66
1.64
1.62
1.56
1.54
1.52
1.50
1.48
H
N
O
N
Me
O
N
H
OH
14·TFA
0.97
0.87
0.81
0.60
2.00
2.00
1.00
2.01
2.04
3.05
1.81
2.03
2.02
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

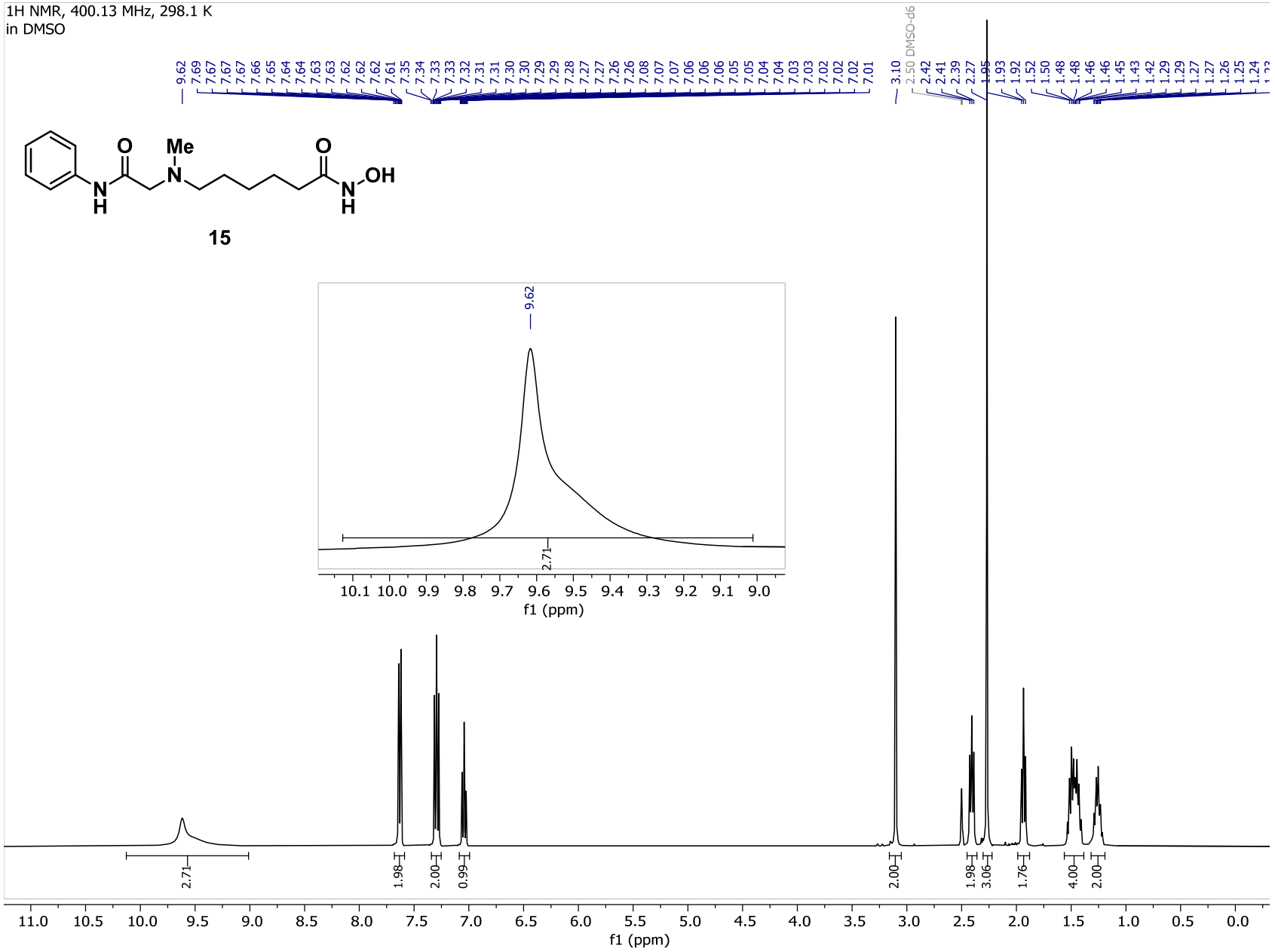
19F NMR, 376.46 MHz, 298.1 K
in DMSO

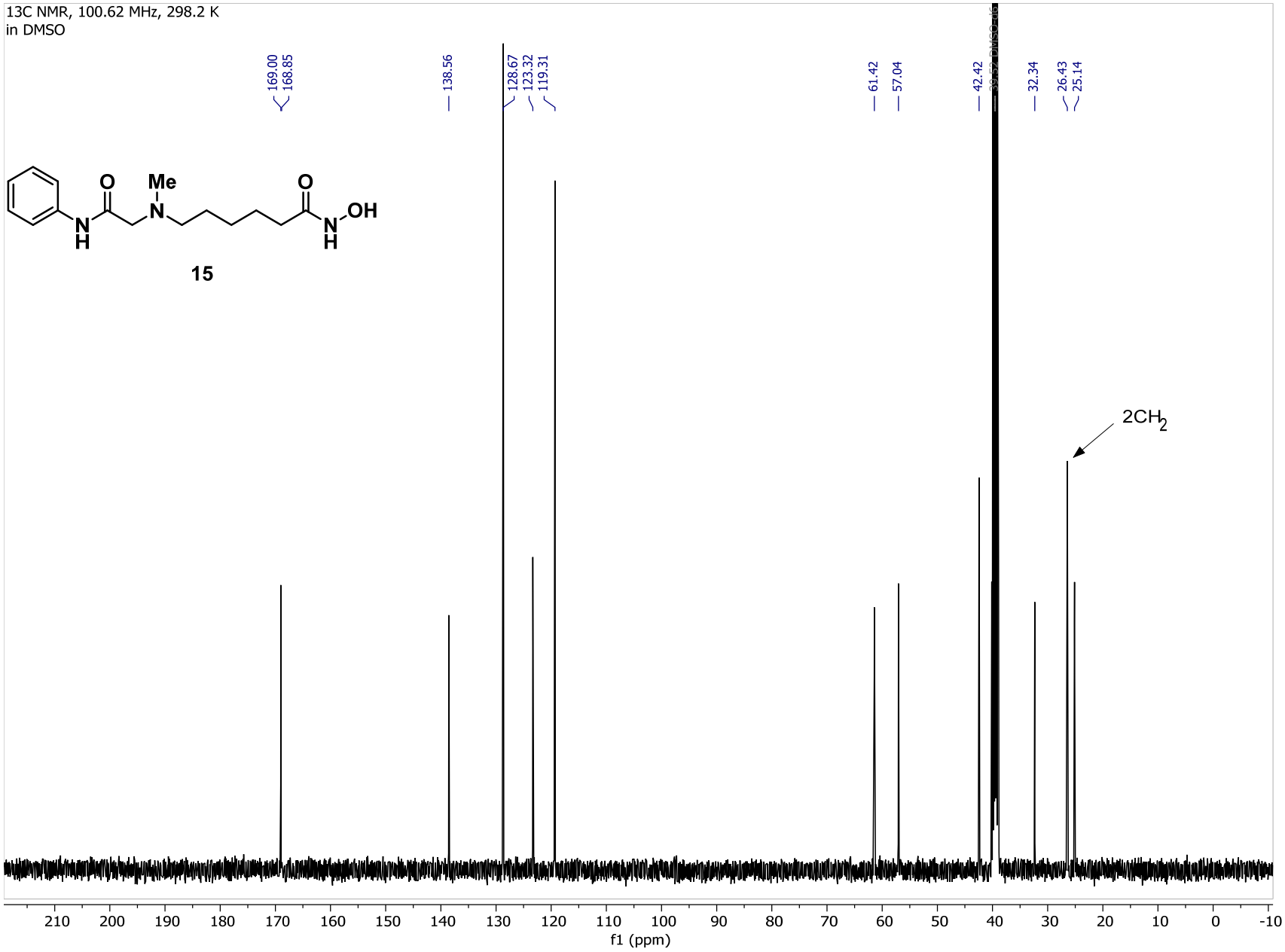


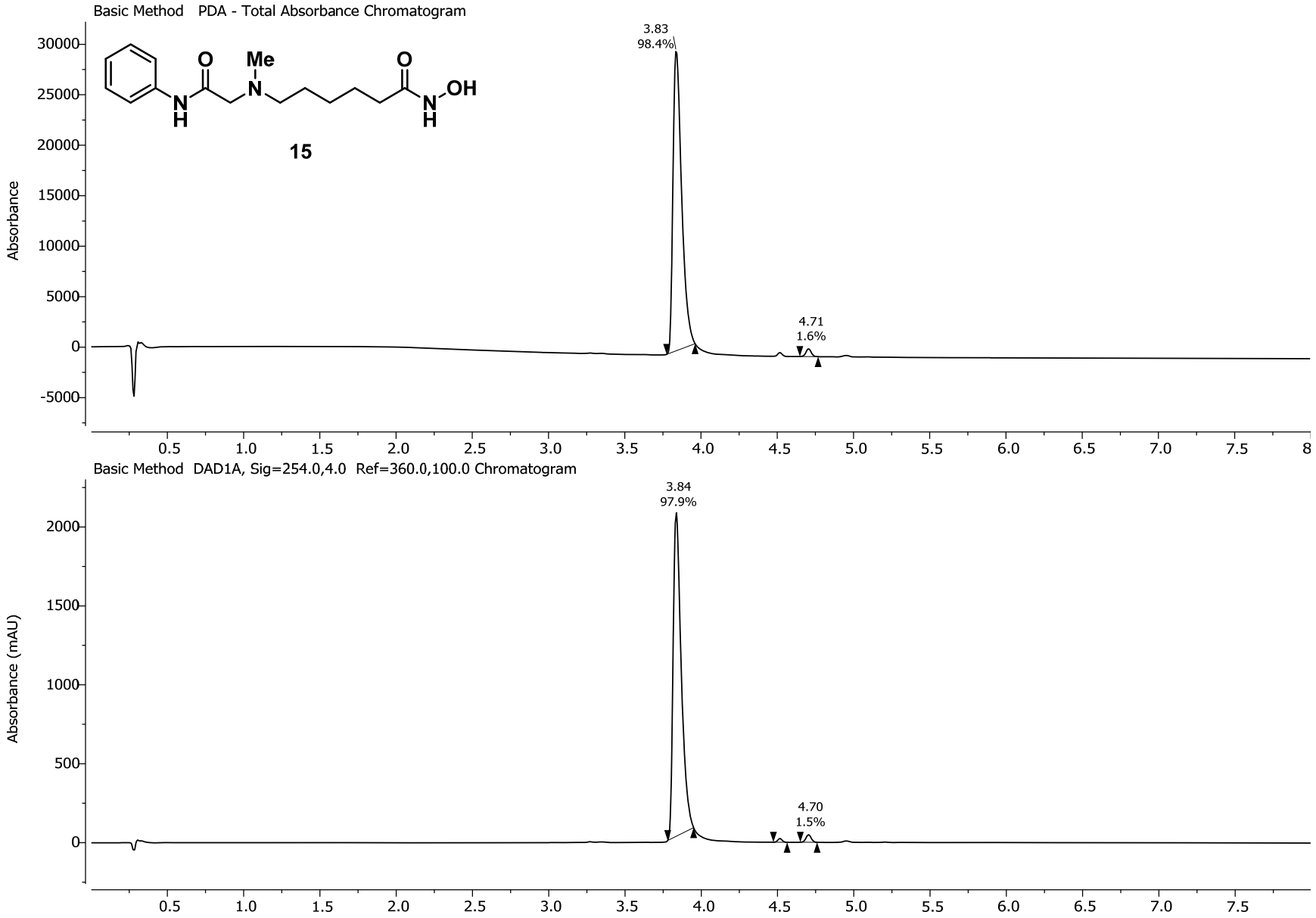

**14·TFA**

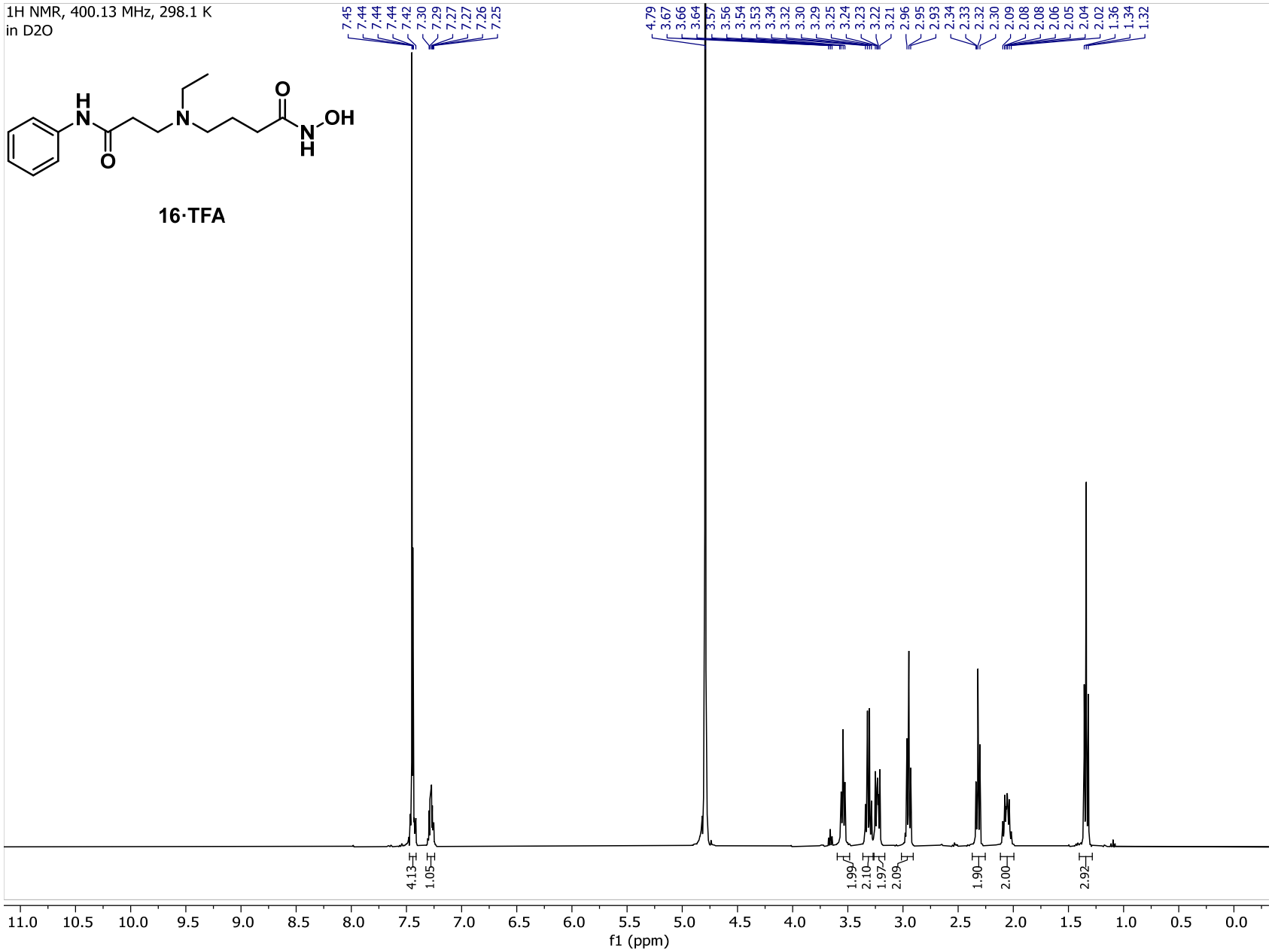

13C NMR, 100.62 MHz, 298.1 K
in DMSO
168.56
163.19
158.19
157.89
137.89
129.01
124.25
119.52
56.75
55.90
40.96
31.56
23.11
22.11
H
N
O
N
Me
O
N
H
OH
14·TFA
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -1
f1 (ppm)


Basic Method PDA - Total Absorbance Chromatogram
Absorbance
3.58
99.5%
4.39
0.5%
14·TFA
Basic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
Absorbance (mAU)
3.57
99.5%
4.39
0.5%

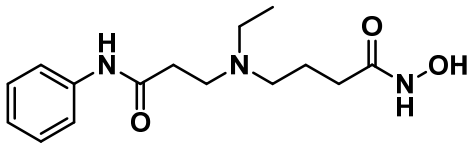

1H NMR, 400.13 MHz, 298.1 K
in DMSO
9.62
7.69
7.67
7.67
7.67
7.66
7.65
7.64
7.64
7.63
7.63
7.62
7.62
7.62
7.61
7.35
7.34
7.33
7.33
7.32
7.31
7.31
7.30
7.30
7.29
7.29
7.28
7.27
7.27
7.26
7.26
7.08
7.07
7.07
7.06
7.06
7.06
7.05
7.05
7.04
7.04
7.03
7.03
7.02
7.02
7.02
7.01
3.10
2.50 DMSO-d6
2.42
2.41
2.39
2.27
1.95
1.93
1.92
1.52
1.50
1.48
1.48
1.46
1.46
1.45
1.43
1.42
1.29
1.29
1.27
1.27
1.26
1.25
1.24
O
Me
O
N
H
N
N
OH
H
15
9.62
2.71
10.1 10.0 9.9 9.8 9.7 9.6 9.5 9.4 9.3 9.2 9.1 9.0
f1 (ppm)
2.71
1.98
2.00
0.99
2.00
1.98
3.06
1.76
4.00
2.00
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


13C NMR, 100.62 MHz, 298.2 K
in DMSO
169.00
168.85
138.56
128.67
123.32
119.31
61.42
57.04
42.42
39.52 DMSO-d6
32.34
26.43
25.14
2CH2
O
Me
O
N
N
OH
H
H
15
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

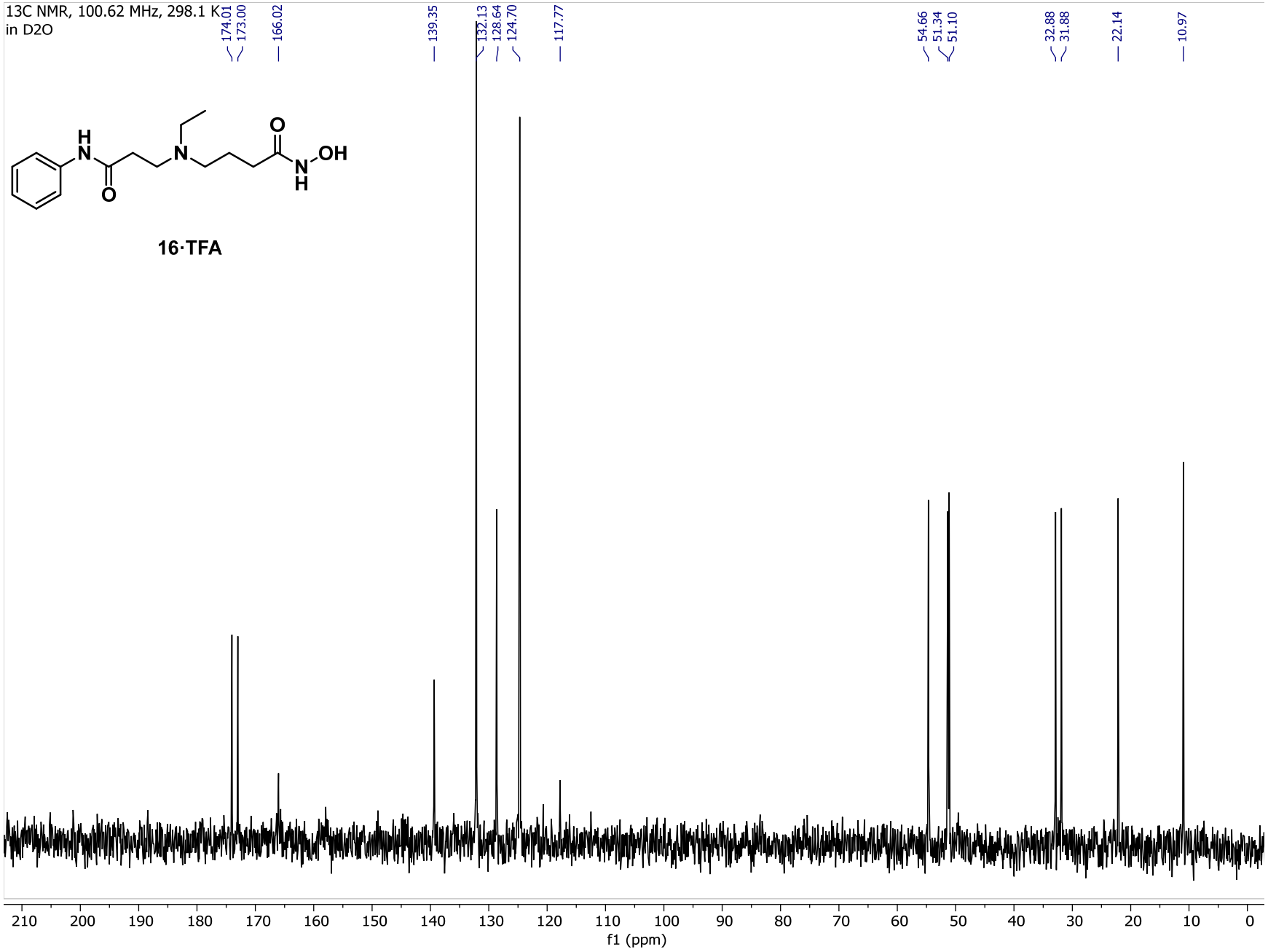

Basic Method PDA - Total Absorbance Chromatogram
Absorbance
3.83
98.4%
4.71
1.6%
15
Basic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
Absorbance (mAU)
3.84
97.9%
4.70
1.5%

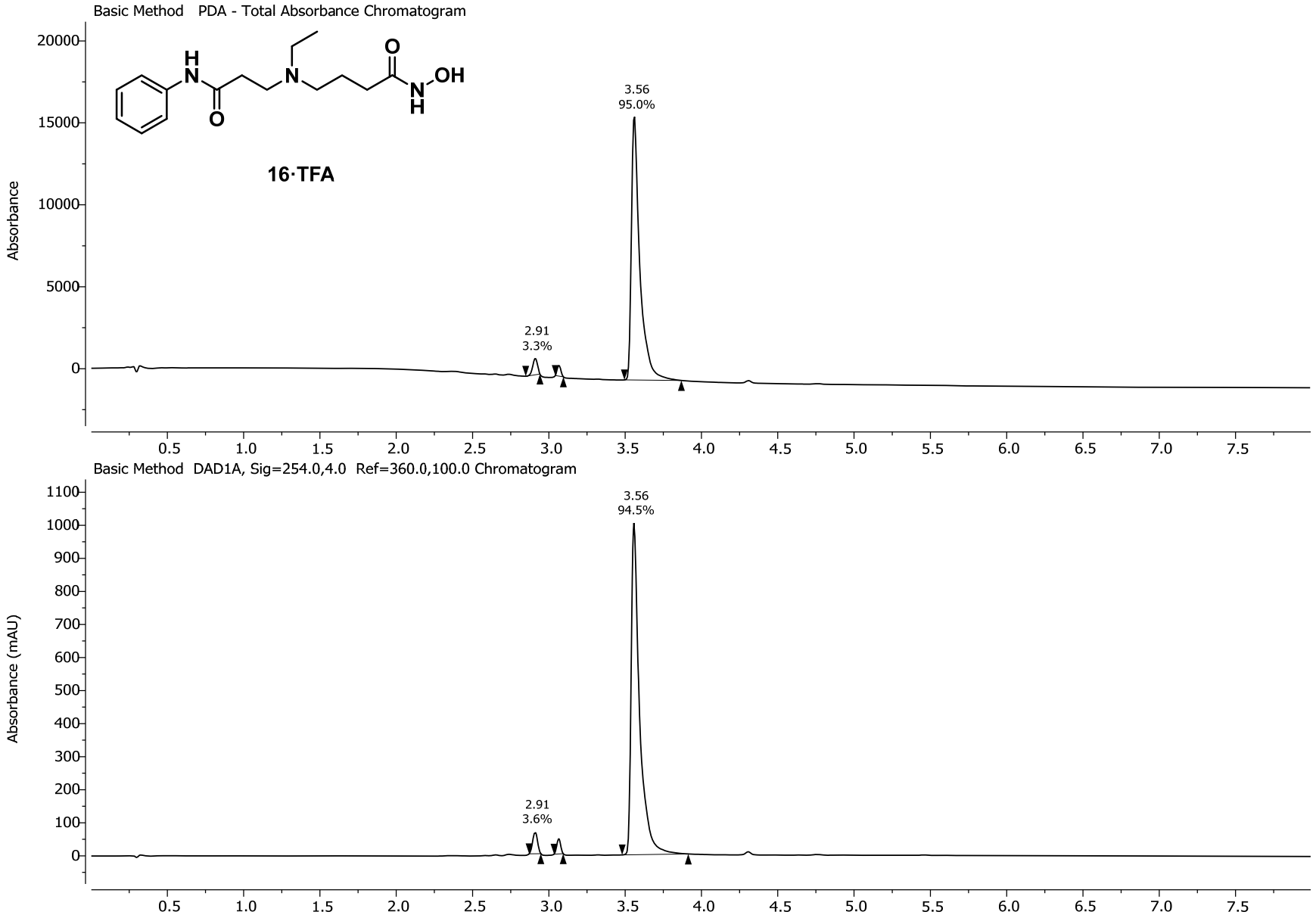

1H NMR, 400.13 MHz, 298.1 K
in D2O
16·TFA
f1 (ppm)

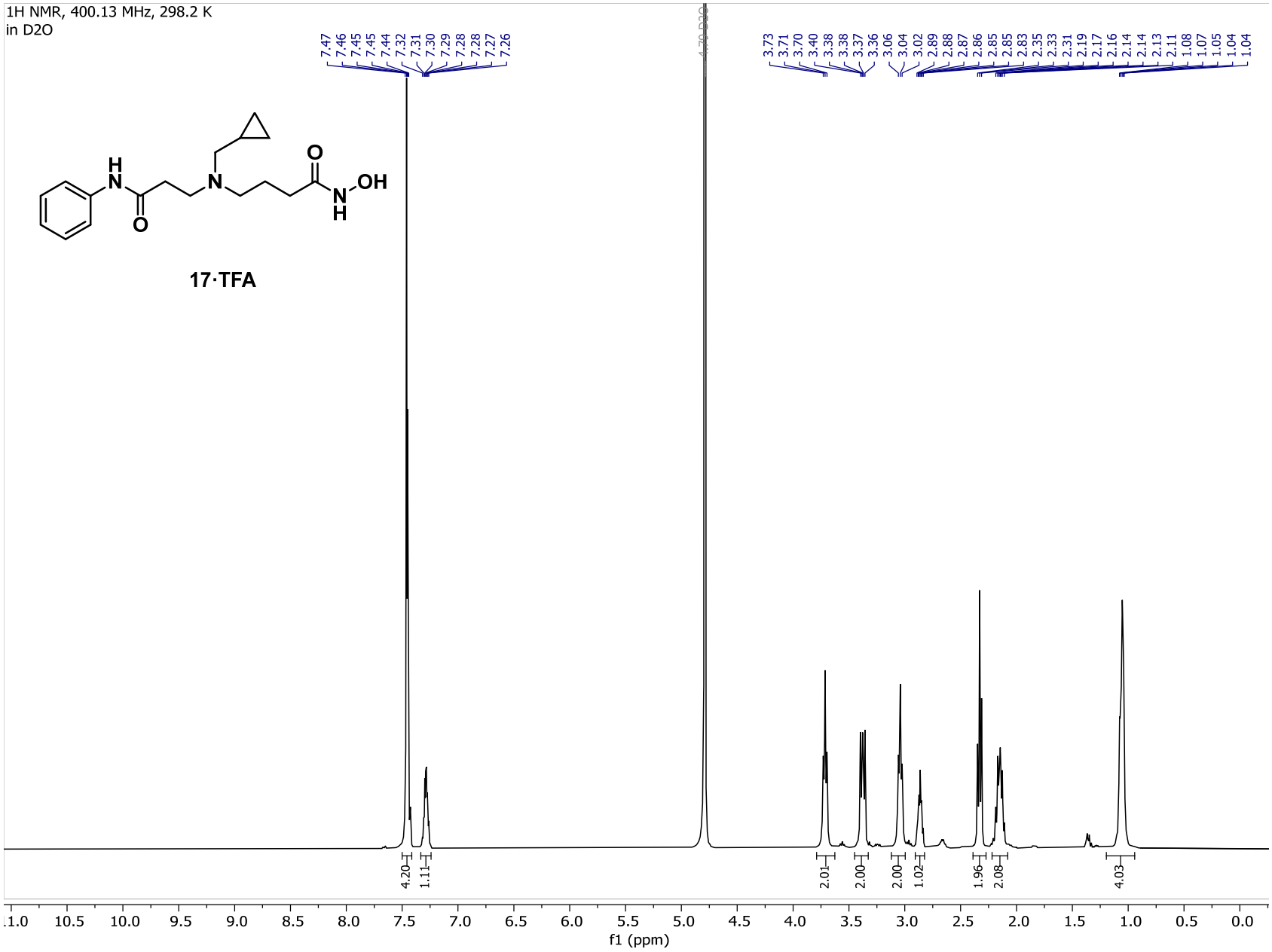
19F NMR, 376.23 MHz, 295.7 K
in D2O



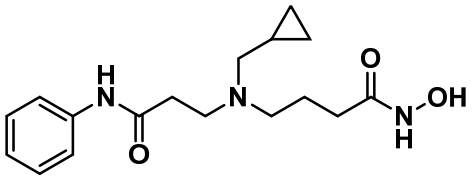
**16·TFA**


13C NMR, 100.62 MHz, 298.1 K
in D2O
174.01
173.00
166.02
139.35
132.13
128.64
124.70
117.77
54.66
51.34
51.10
32.88
31.88
22.14
10.97
16·TFA
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

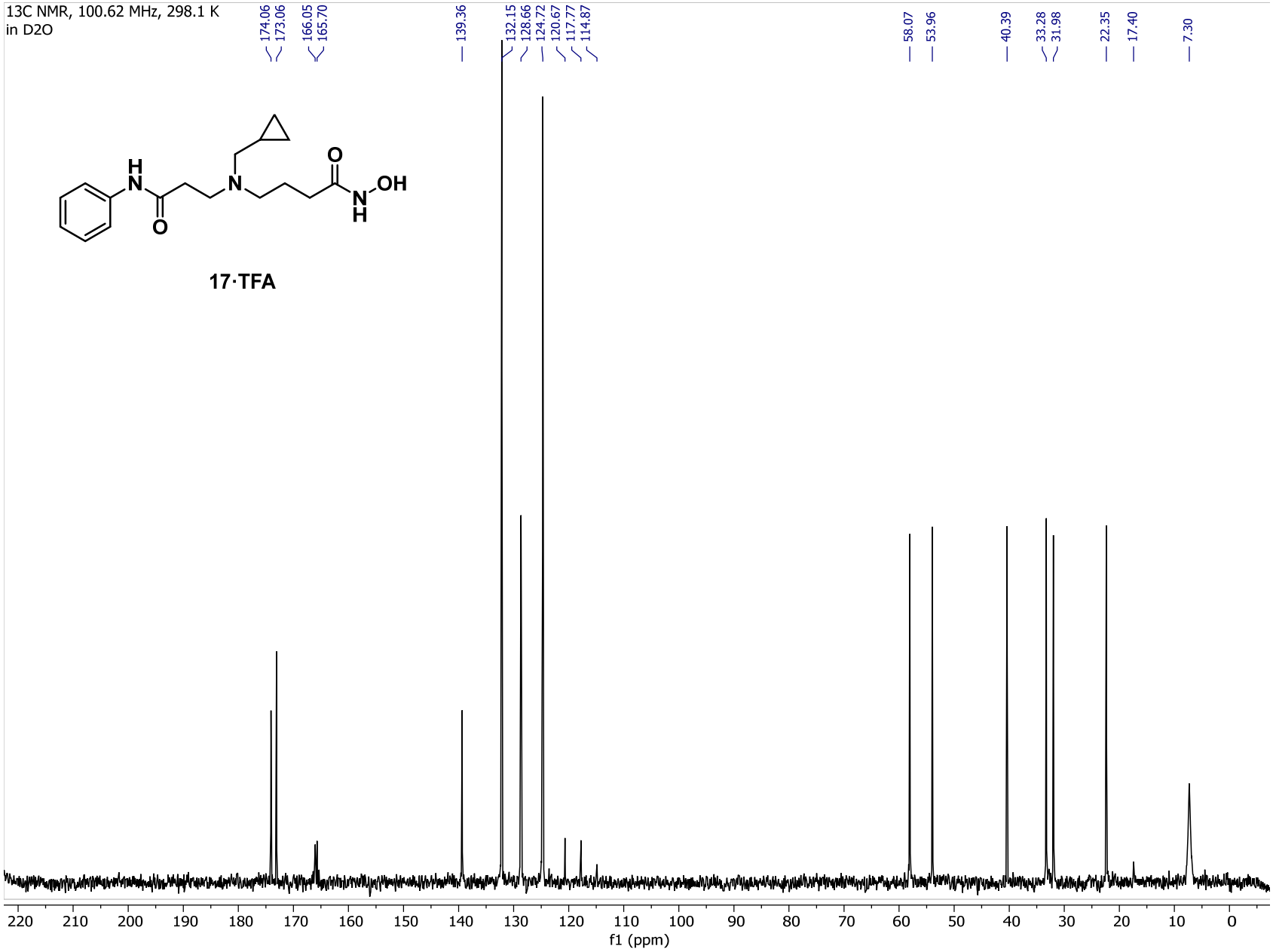

Basic Method PDA - Total Absorbance Chromatogram
Absorbance
16·TFA
3.56
95.0%
2.91
3.3%
Basic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
Absorbance (mAU)
3.56
94.5%
2.91
3.6%

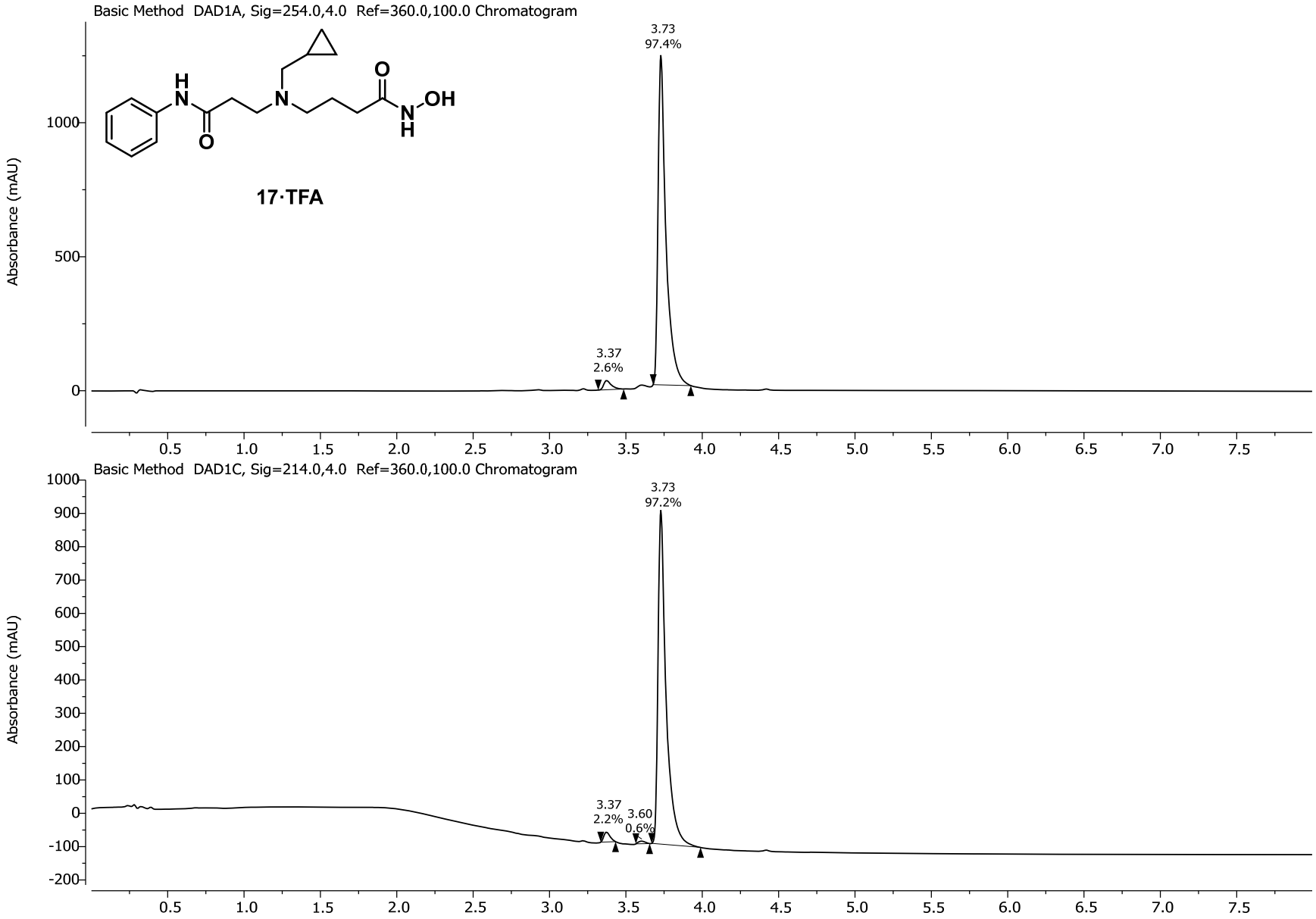

1H NMR, 400.13 MHz, 298.2 K
in D2O
7.47
7.46
7.45
7.45
7.44
7.32
7.31
7.30
7.29
7.28
7.28
7.27
7.26
3.73
3.71
3.70
3.40
3.38
3.38
3.37
3.36
3.06
3.04
3.02
2.89
2.88
2.87
2.86
2.85
2.85
2.83
2.35
2.33
2.31
2.19
2.17
2.16
2.14
2.14
2.13
2.11
1.08
1.07
1.05
1.04
1.04
H
N
O
N
O
N
H
OH
17·TFA
4.20
1.11
2.01
2.00
2.00
1.02
1.96
2.08
4.03
f1 (ppm)

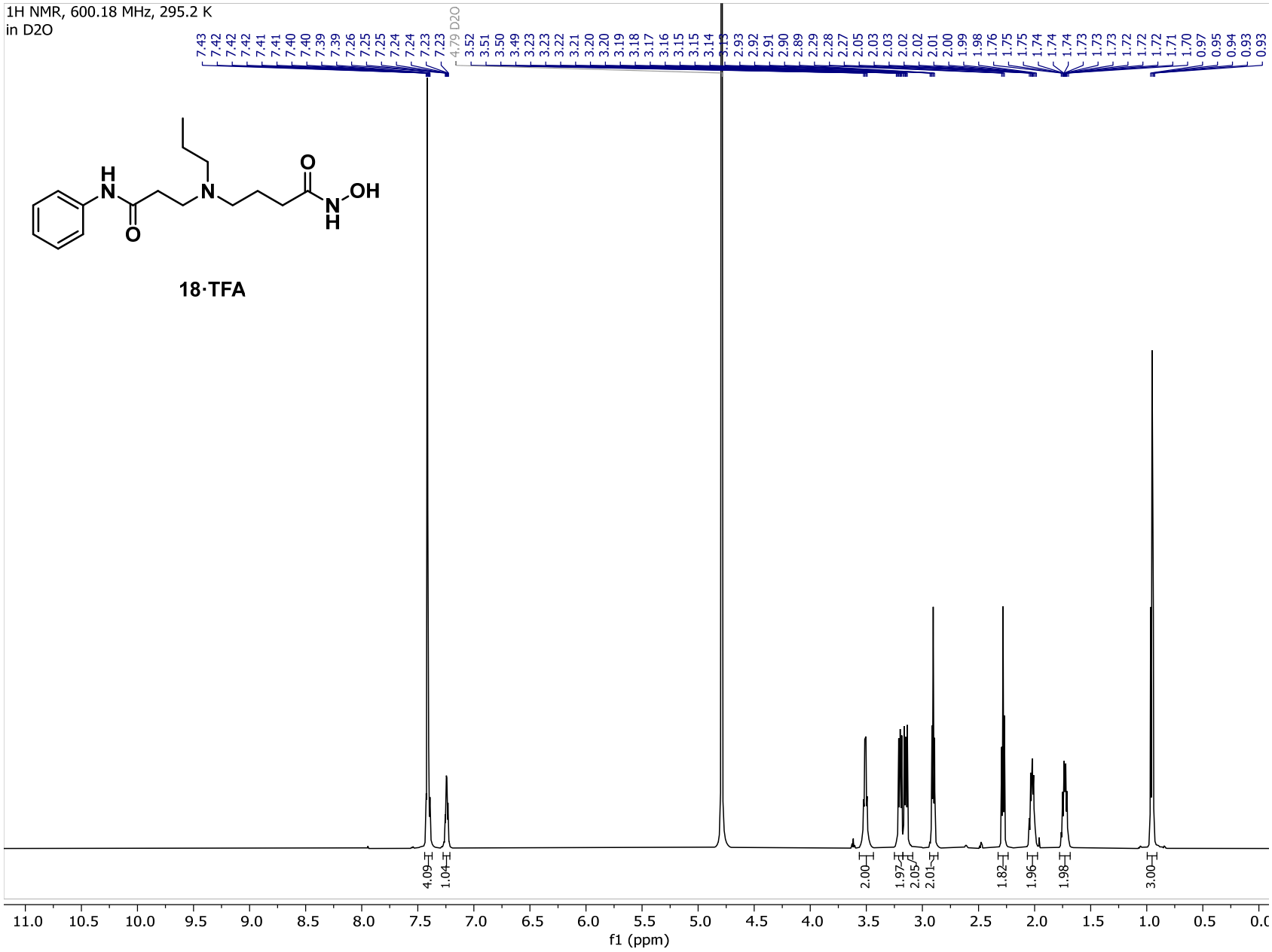
19F NMR, 376.46 MHz, 298.1 K
in D2O

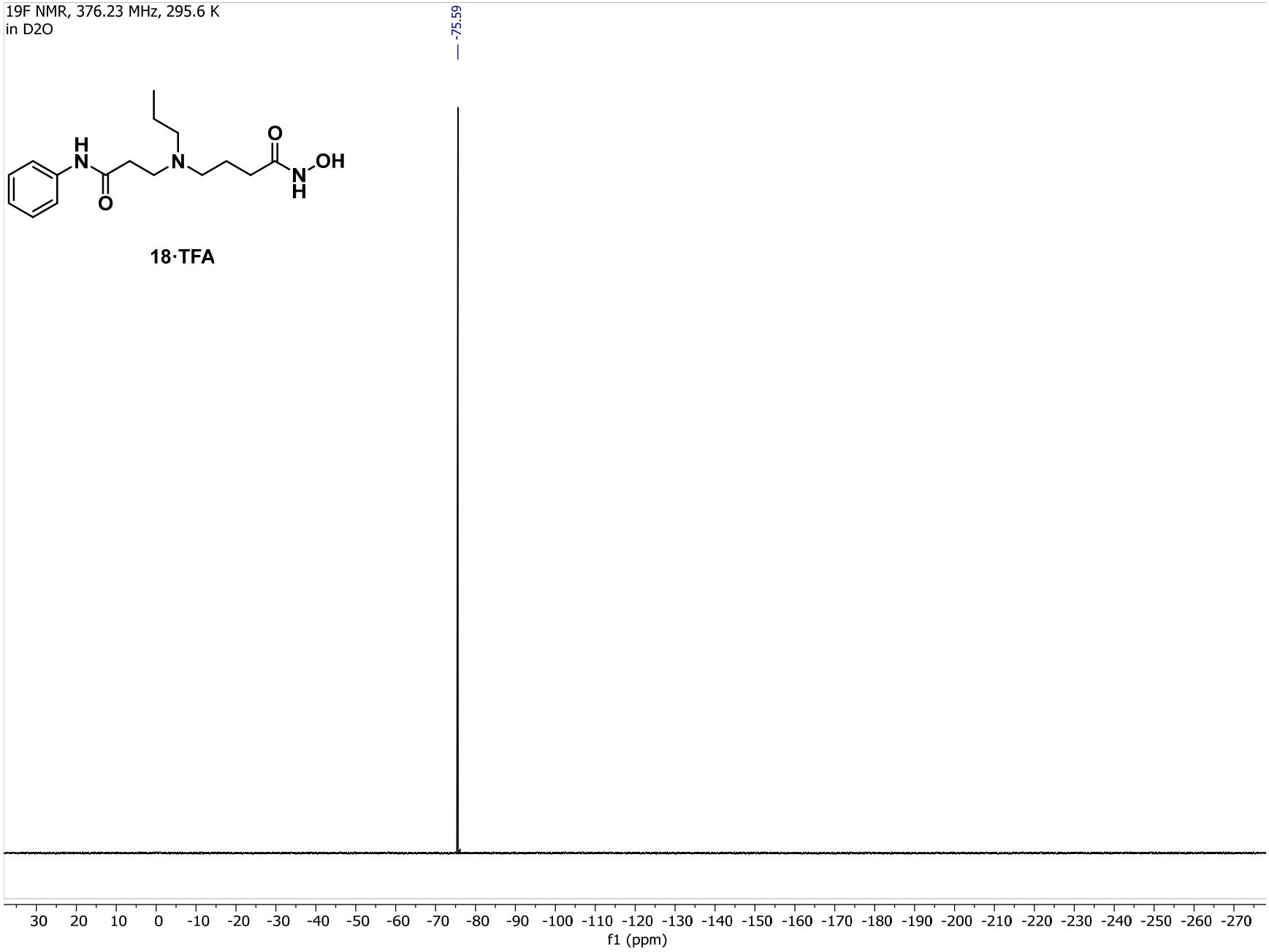


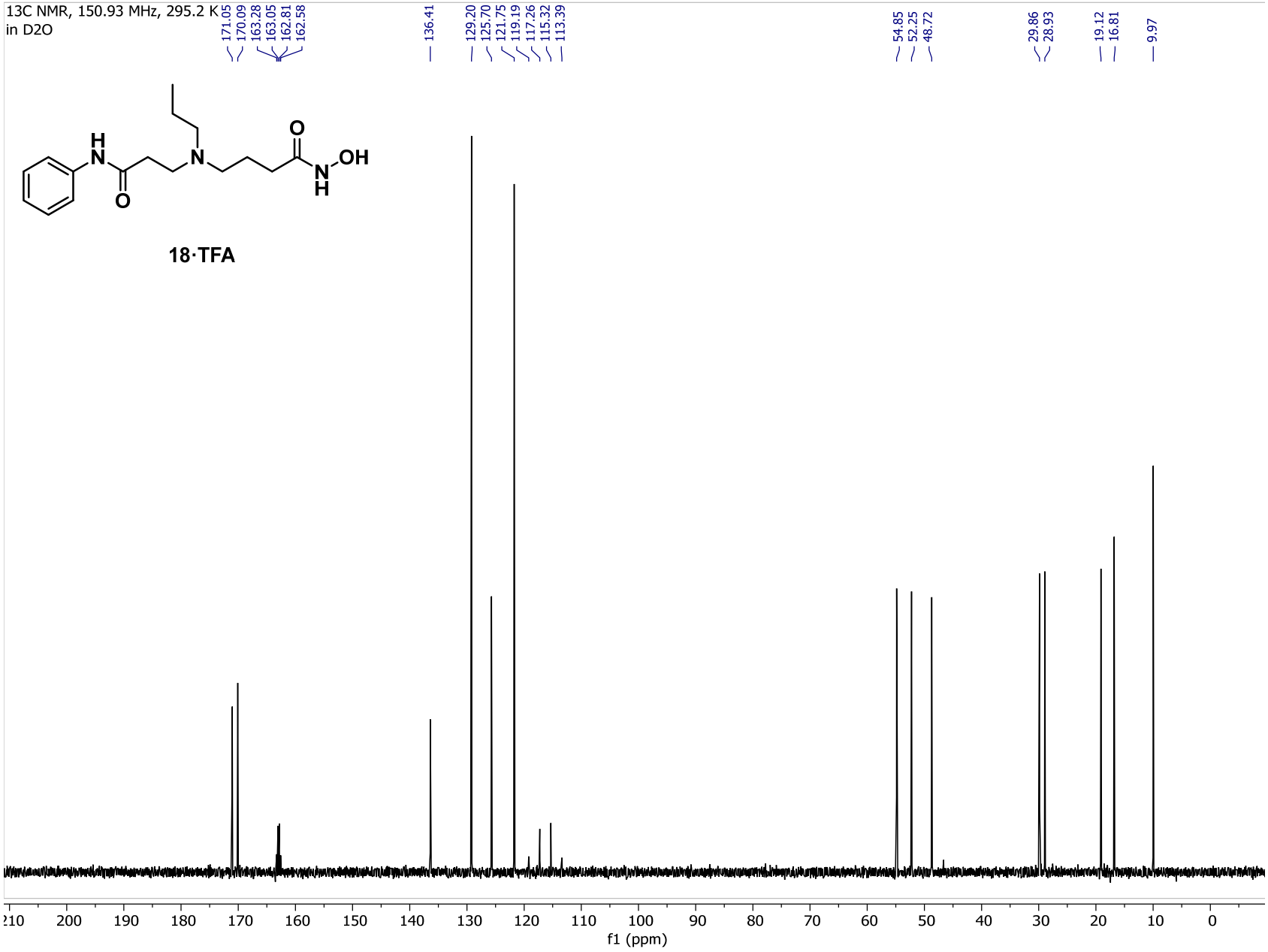

**17·TFA**

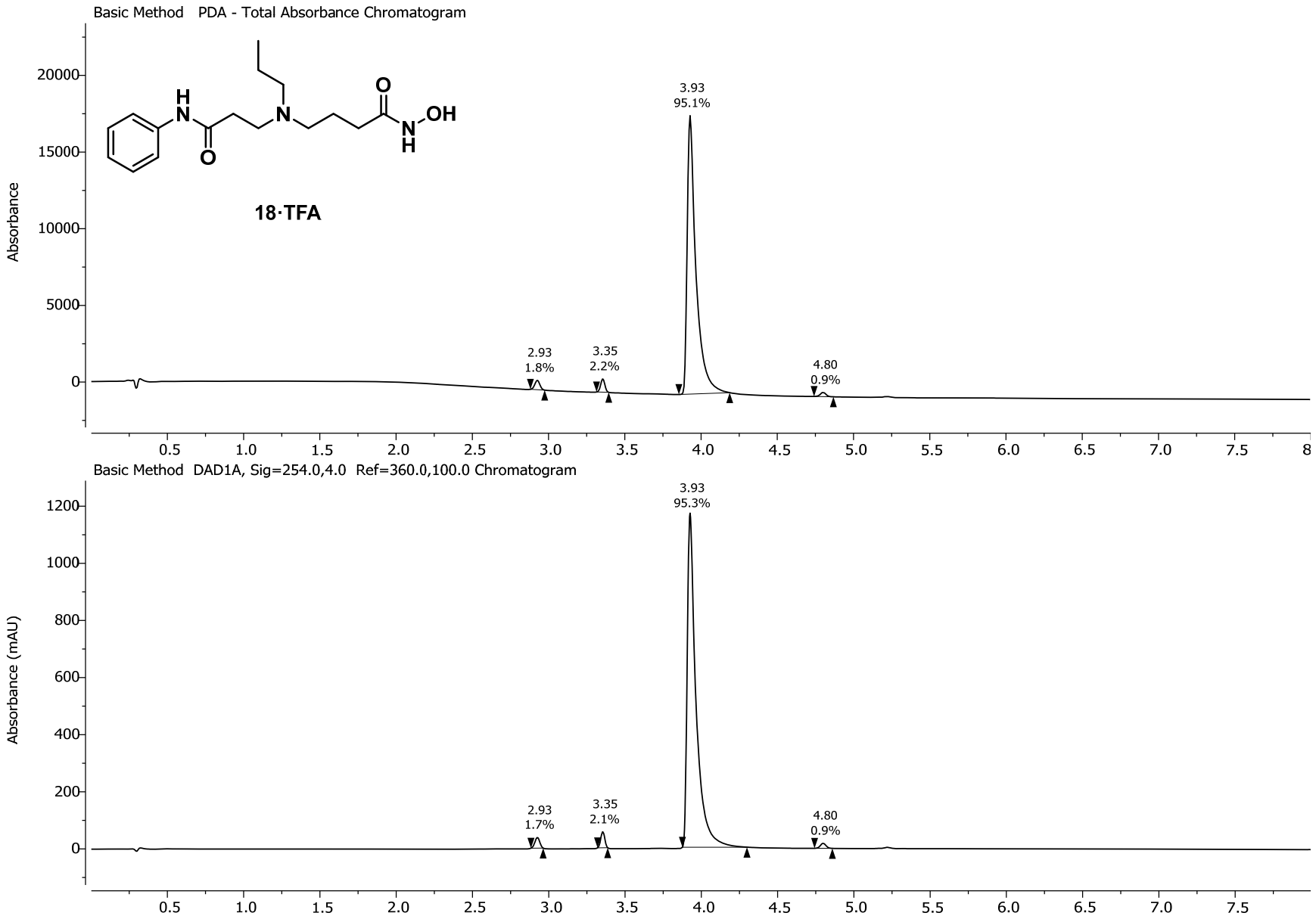

13C NMR, 100.62 MHz, 298.1 K
in D2O
174.06
173.06
166.05
165.70
139.36
132.15
128.66
124.72
120.67
117.77
114.87
58.07
53.96
40.39
33.28
31.98
22.35
17.40
7.30
17·TFA
f1 (ppm)

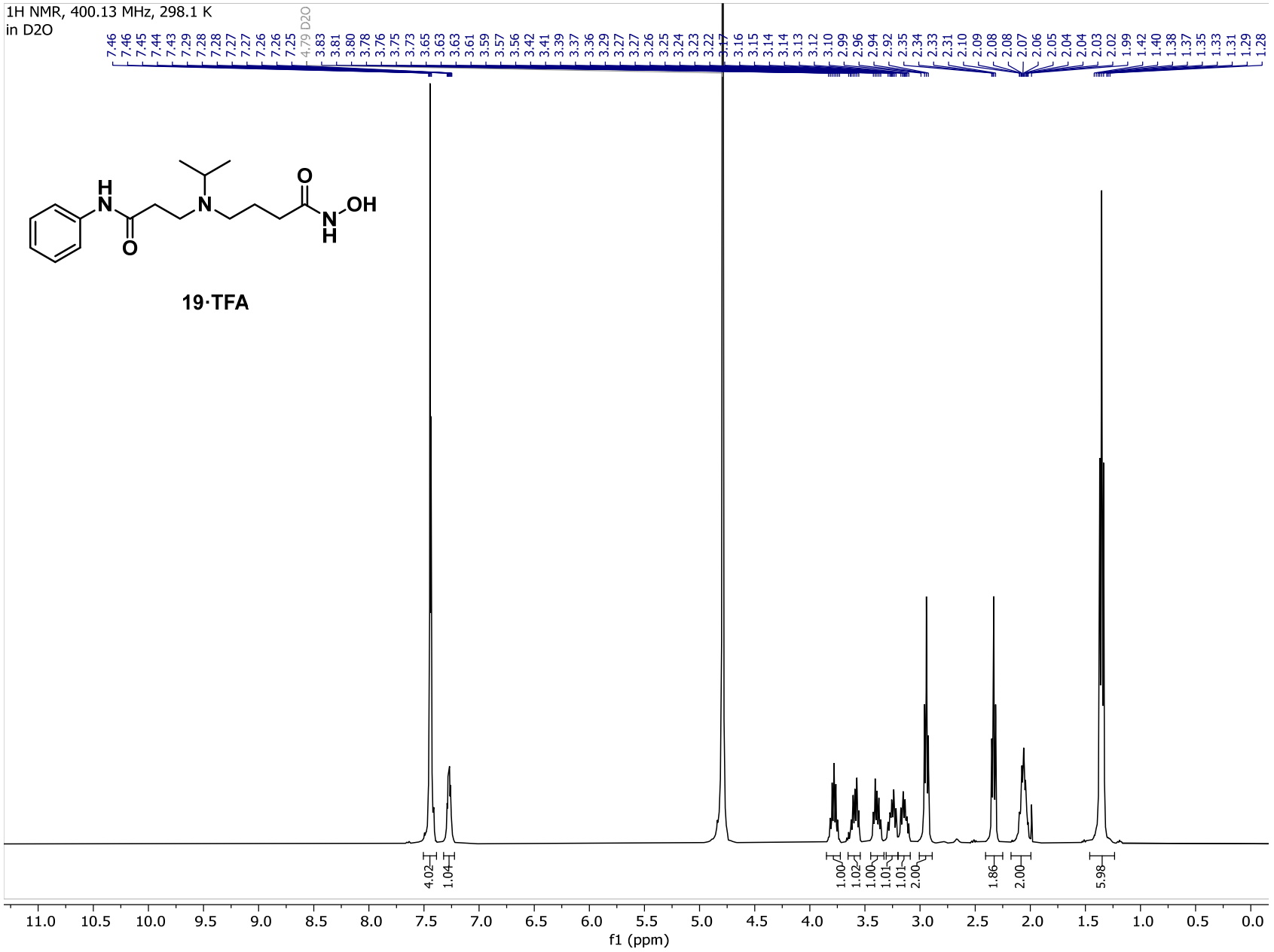

Basic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
3.73
97.4%
3.37
2.6%
17·TFA
Absorbance (mAU)
Basic Method DAD1C, Sig=214.0,4.0 Ref=360.0,100.0 Chromatogram
3.73
97.2%
3.37
2.2%
3.60
0.6%
Absorbance (mAU)

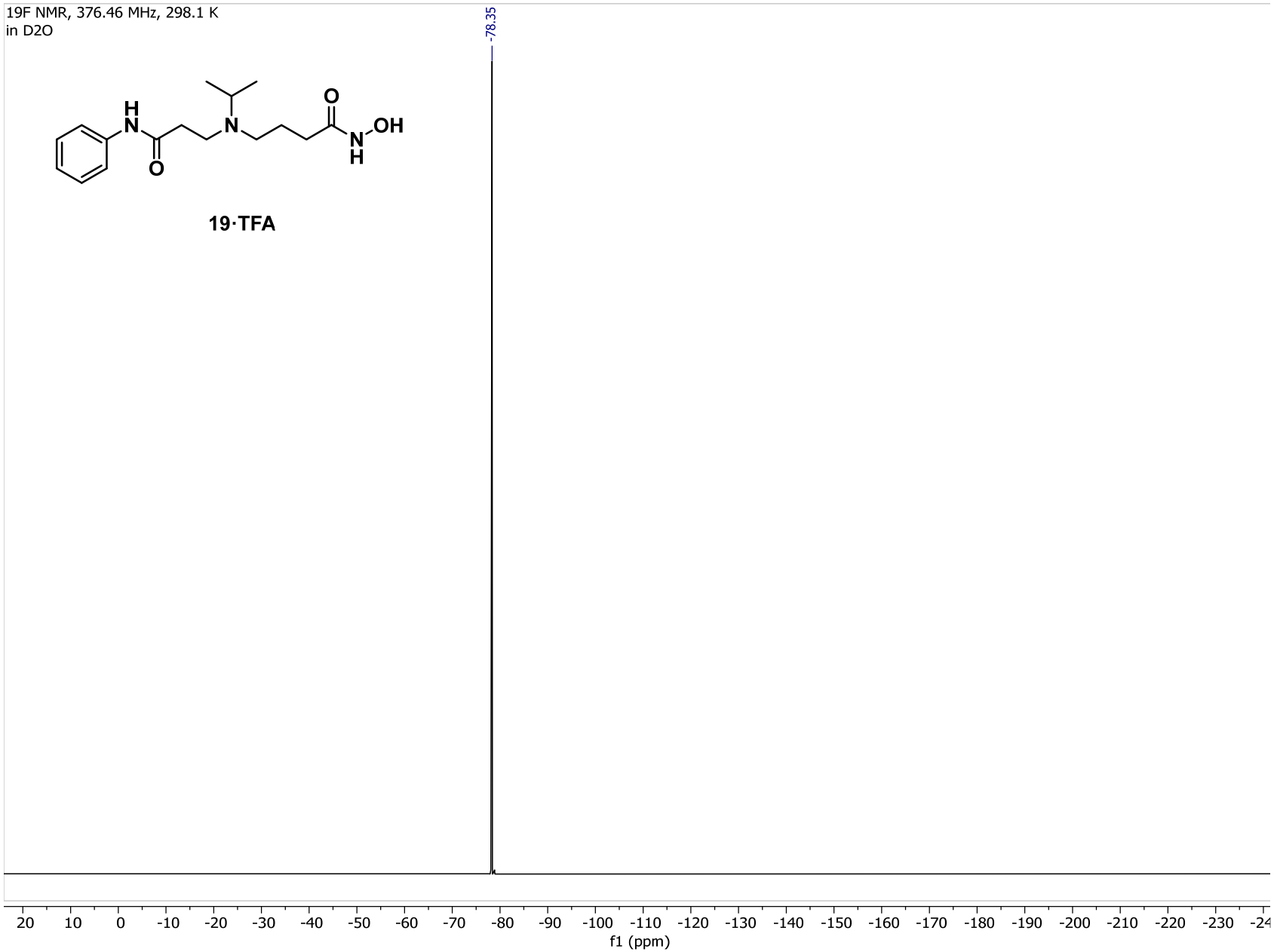

1H NMR, 600.18 MHz, 295.2 K
in D2O
4.79 D2O
18·TFA
f1 (ppm)

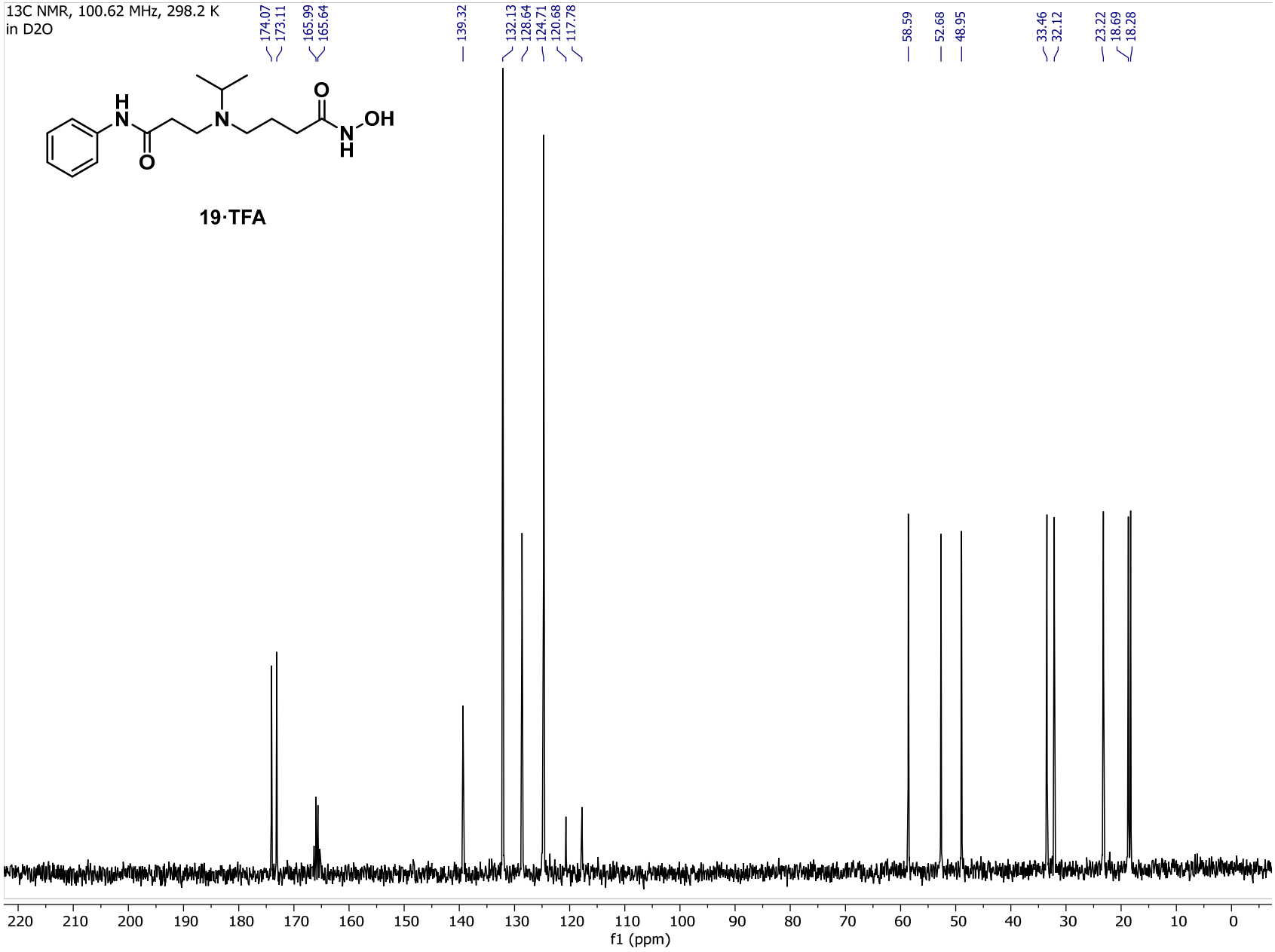

19F NMR, 376.23 MHz, 295.6 K
in D2O
-75.59
H
N
O
N
O
N
H
OH
18·TFA
30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210 -220 -230 -240 -250 -260 -270
f1 (ppm)

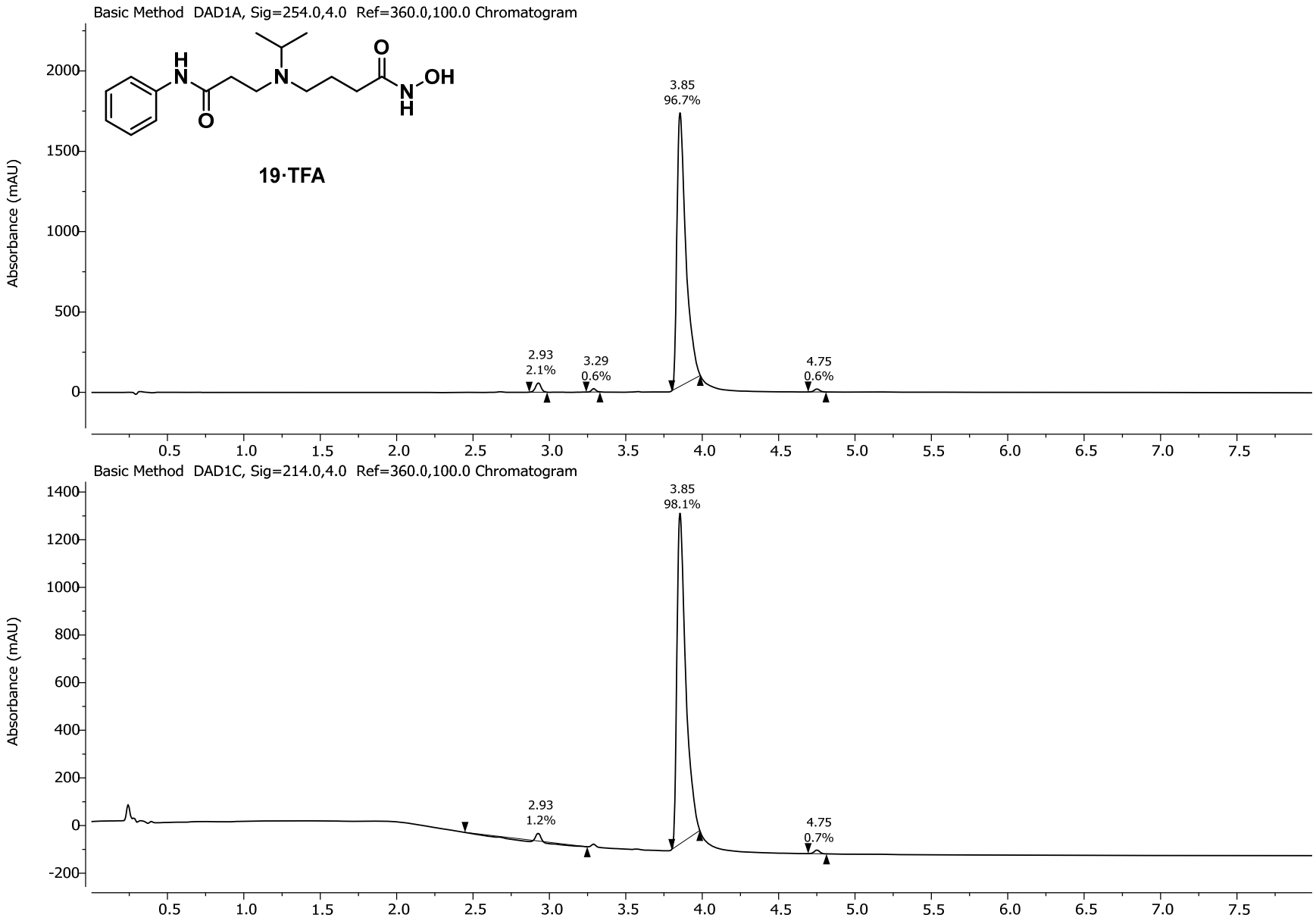

13C NMR, 150.93 MHz, 295.2 K
in D2O
171.05
170.09
163.28
163.05
162.81
162.58
136.41
129.20
125.70
121.75
119.19
117.26
115.32
113.39
54.85
52.25
48.72
29.86
28.93
19.12
16.81
9.97
18·TFA
f1 (ppm)

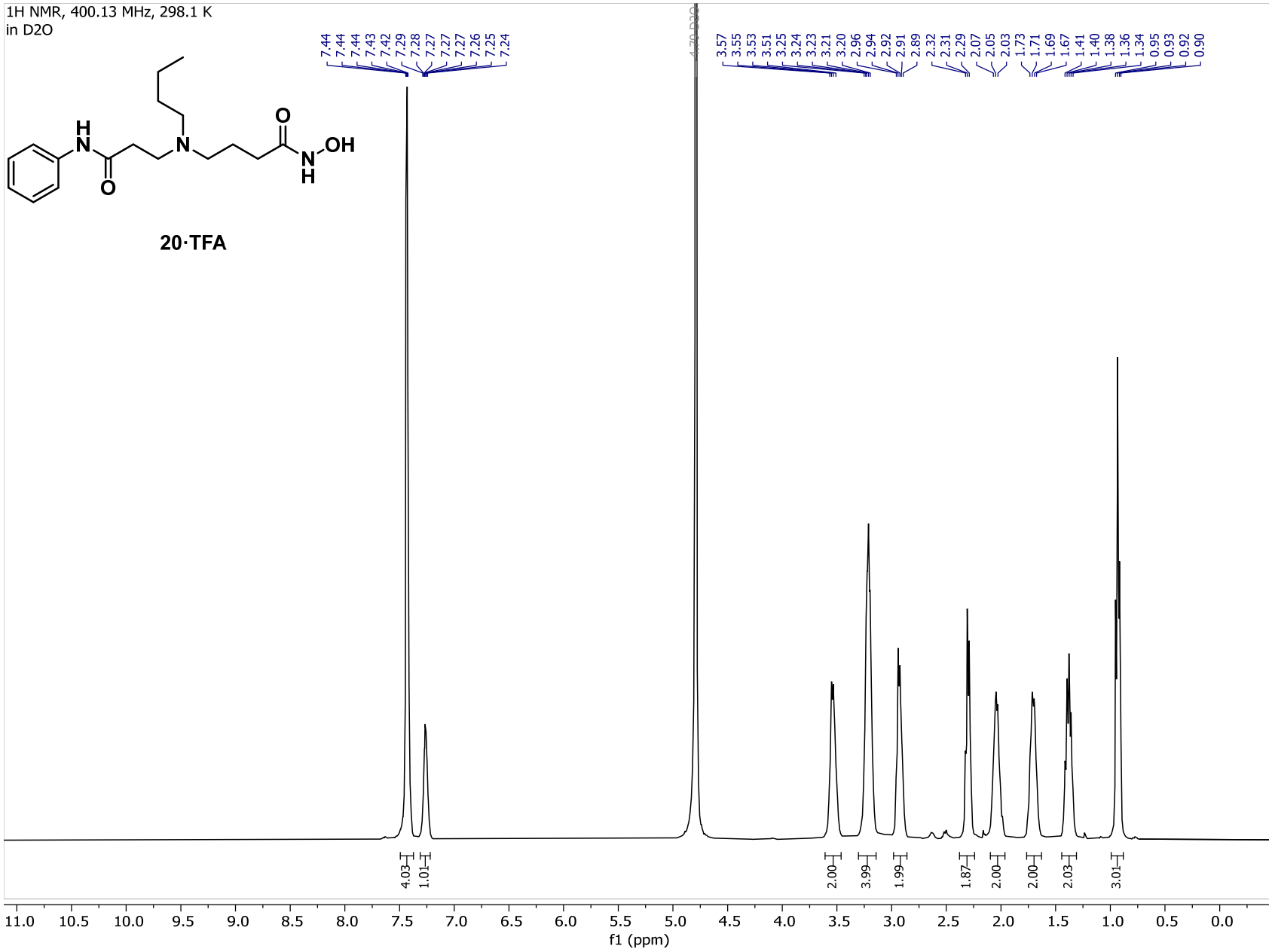

Basic Method PDA - Total Absorbance Chromatogram
Absorbance
20000
15000
10000
5000
0
3.93
95.1%
2.93
1.8%
3.35
2.2%
4.80
0.9%
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8
18·TFA
Basic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
Absorbance (mAU)
1200
1000
800
600
400
200
0
3.93
95.3%
2.93
1.7%
3.35
2.1%
4.80
0.9%
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5

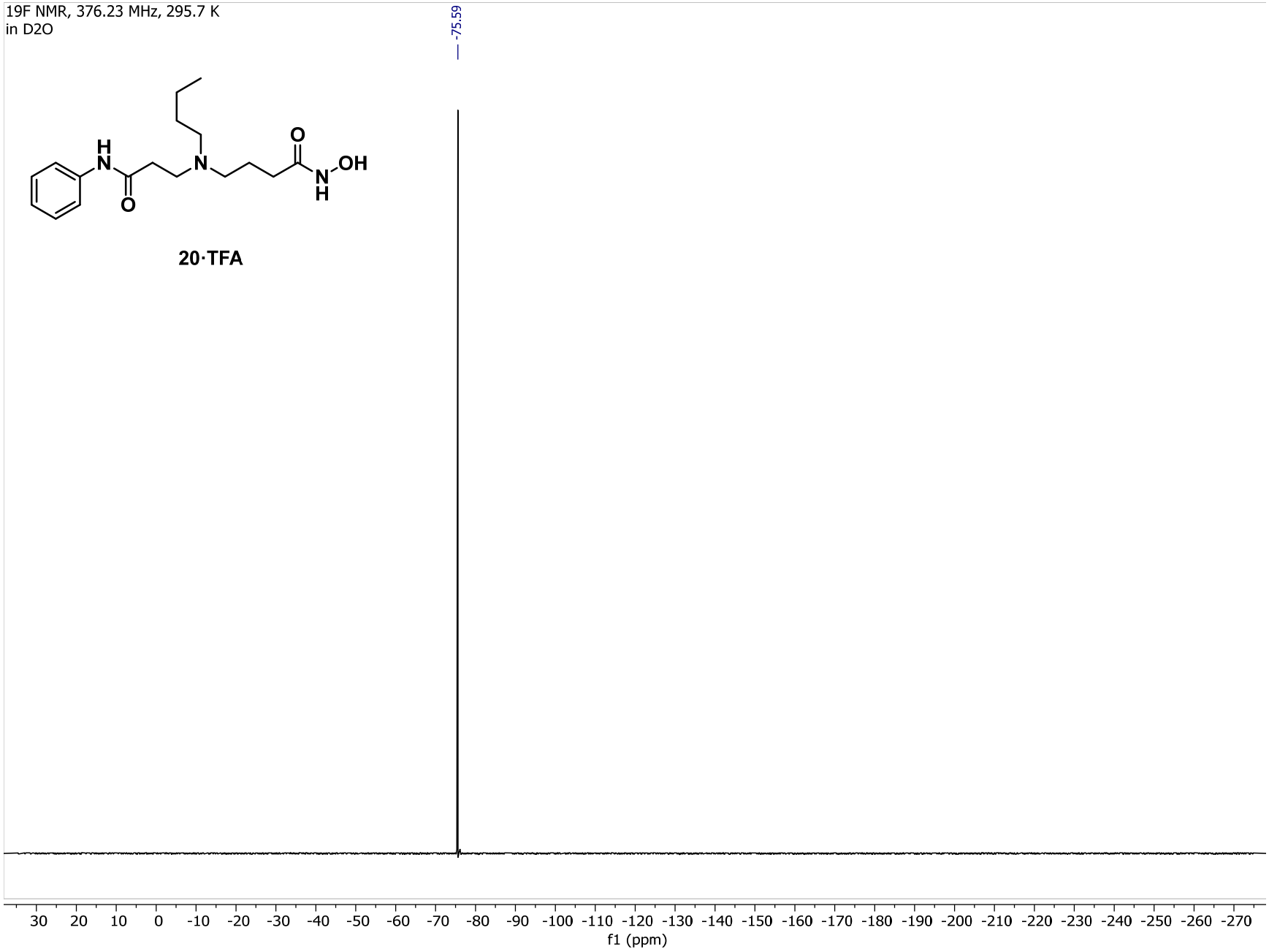

1H NMR, 400.13 MHz, 298.1 K
in D2O
19·TFA
f1 (ppm)

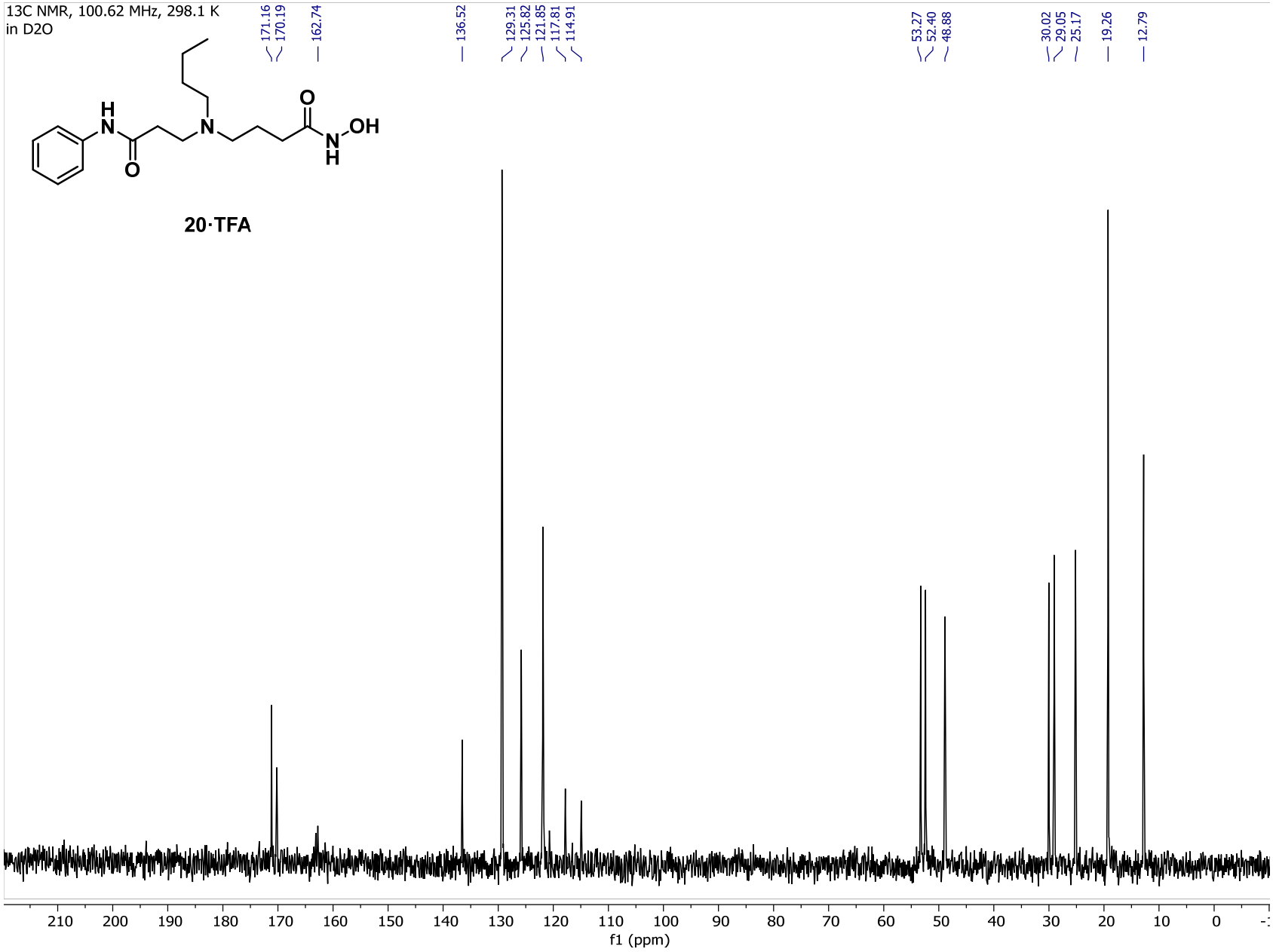

19F NMR, 376.46 MHz, 298.1 K
in D2O
-78.35
19·TFA
20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210 -220 -230
f1 (ppm)

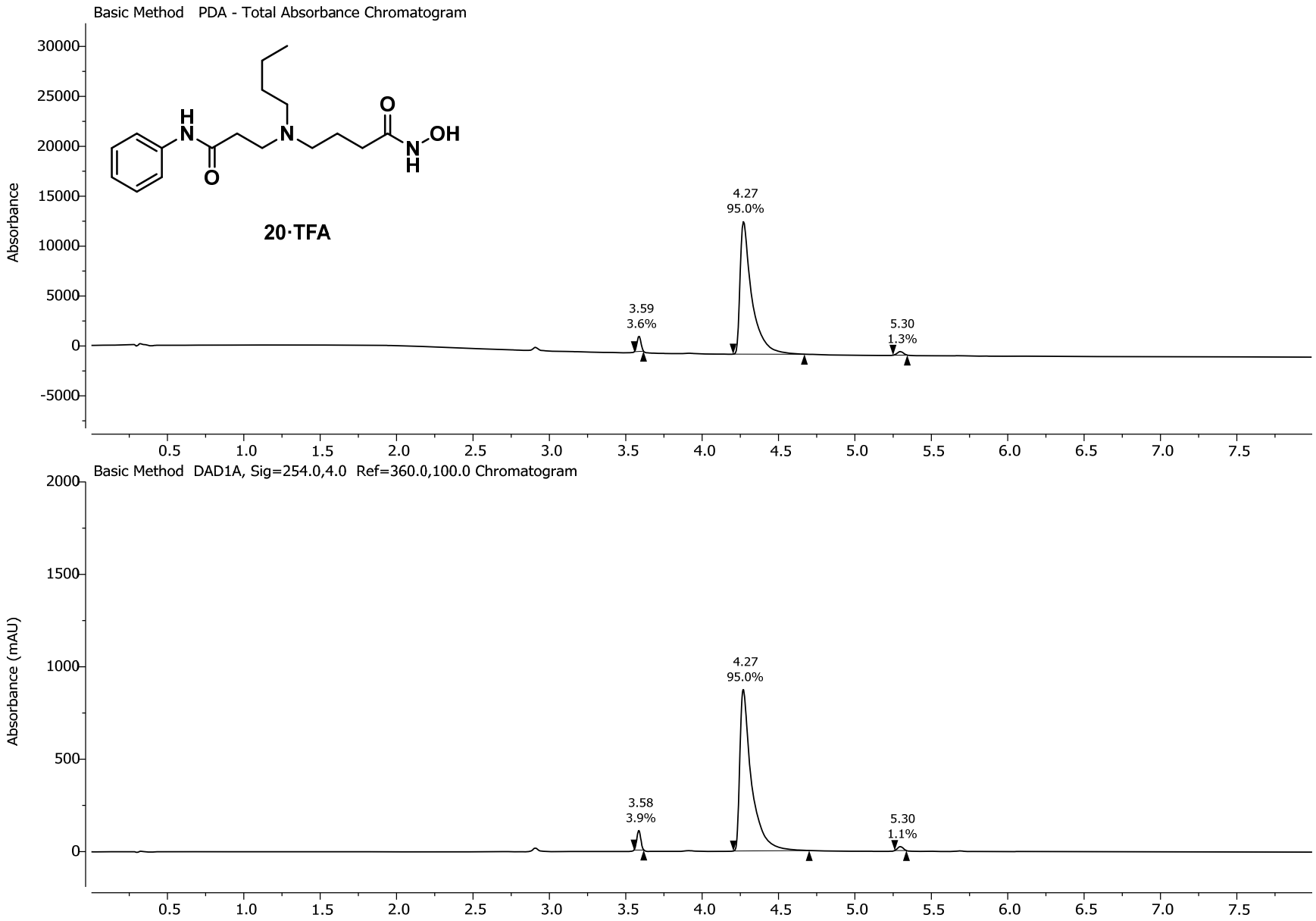

13C NMR, 100.62 MHz, 298.2 K
in D2O
174.07
173.11
165.99
165.64
139.32
132.13
128.64
124.71
120.68
117.78
58.59
52.68
48.95
33.46
32.12
23.22
18.69
18.28
19·TFA
f1 (ppm)

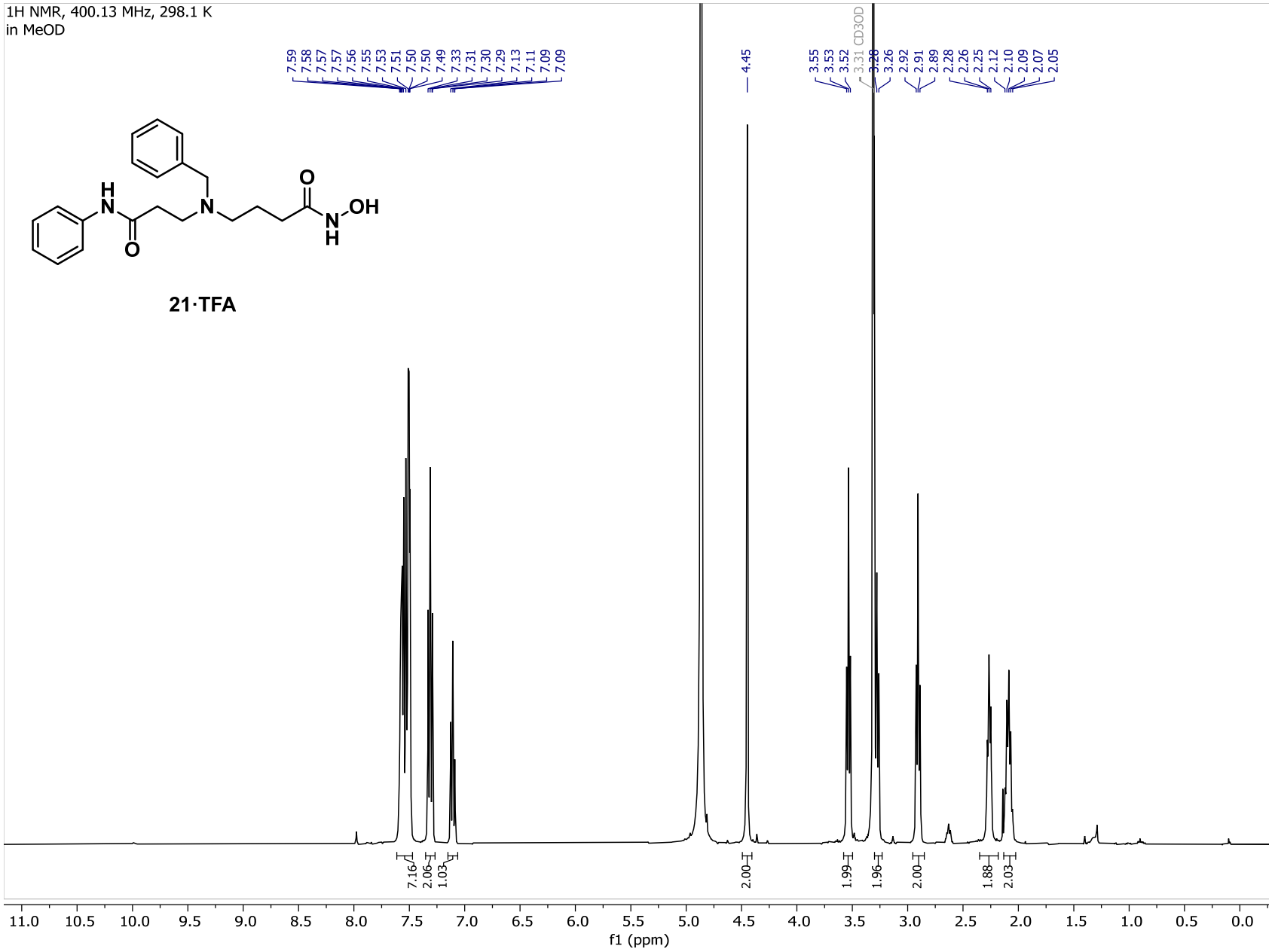

Basic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
19·TFA
Absorbance (mAU)
3.85
96.7%
2.93
2.1%
3.29
0.6%
4.75
0.6%
Basic Method DAD1C, Sig=214.0,4.0 Ref=360.0,100.0 Chromatogram
Absorbance (mAU)
3.85
98.1%
2.93
1.2%
4.75
0.7%

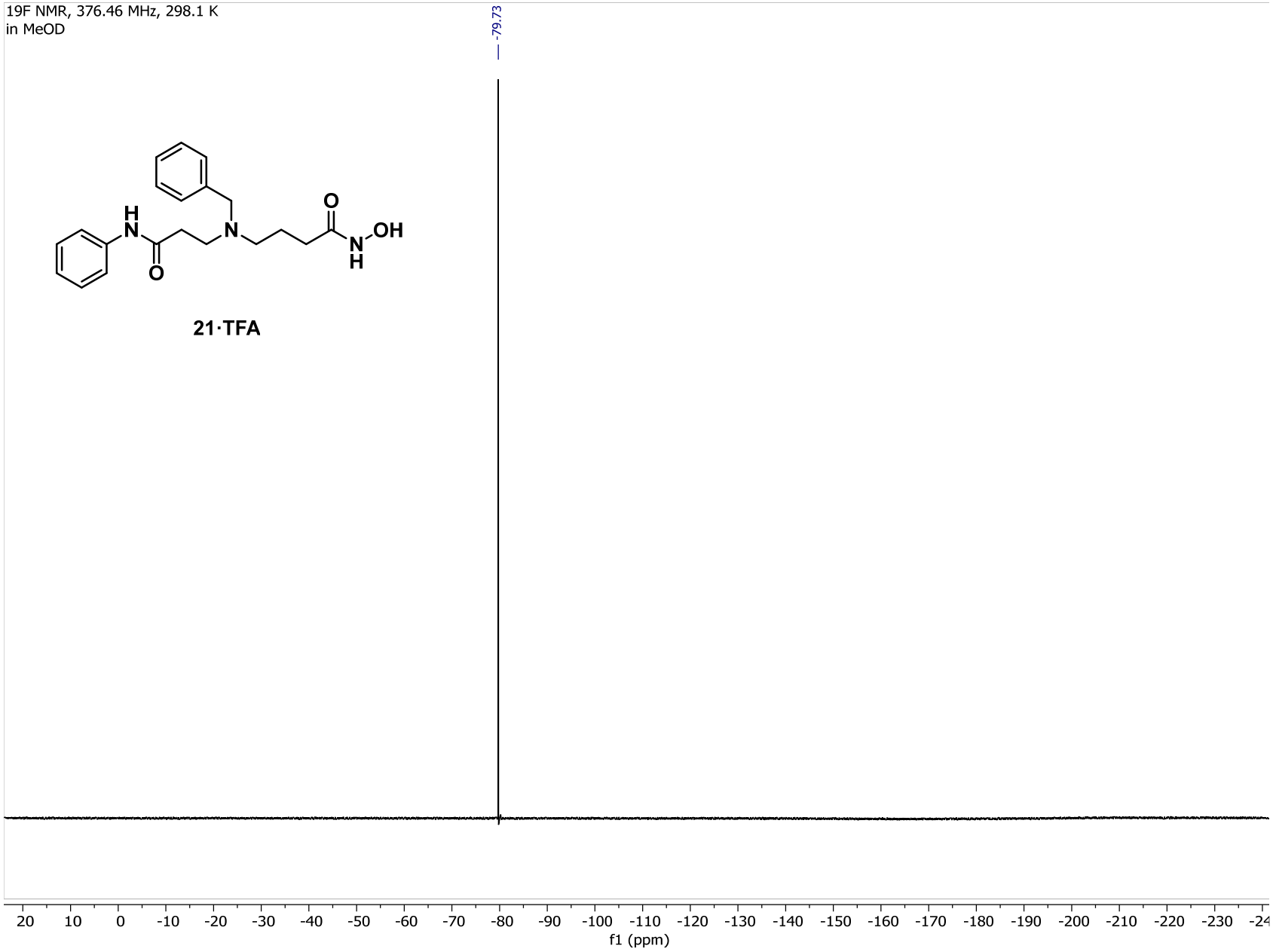

1H NMR, 400.13 MHz, 298.1 K
in D2O
20·TFA
f1 (ppm)

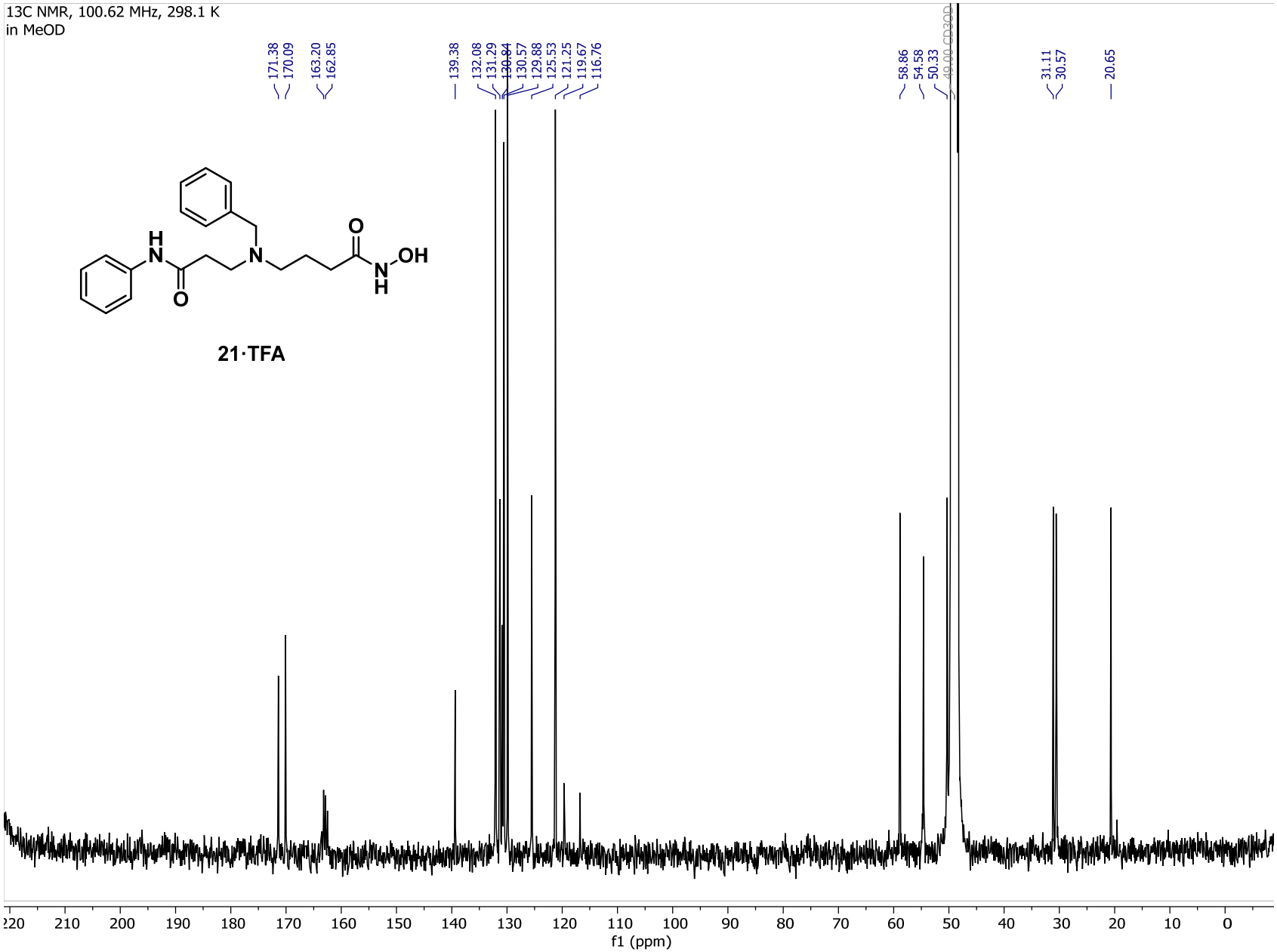

19F NMR, 376.23 MHz, 295.7 K
in D2O
-75.59
H
N
O
N
O
N
H
OH
20·TFA
30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210 -220 -230 -240 -250 -260 -270
f1 (ppm)

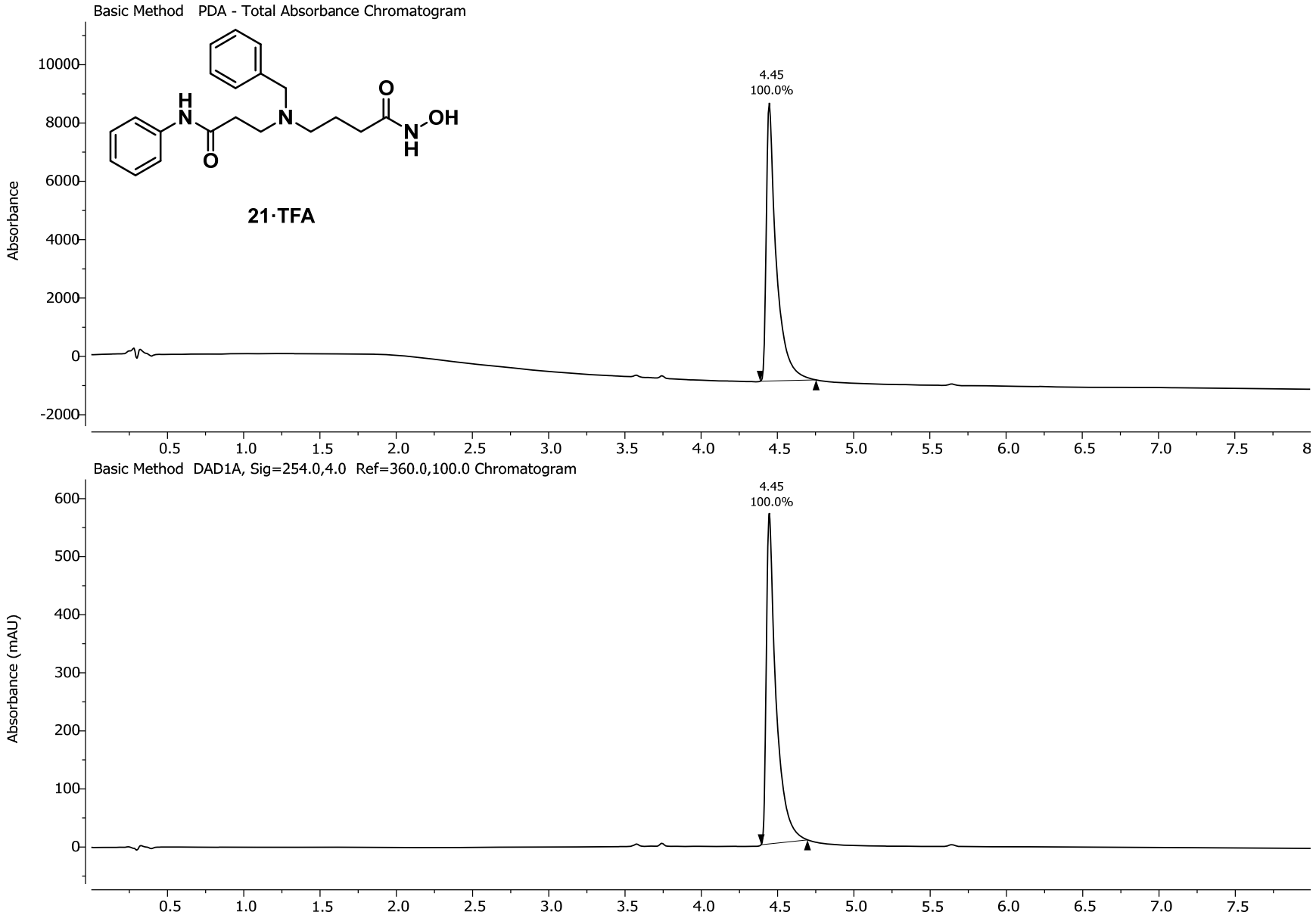

13C NMR, 100.62 MHz, 298.1 K
in D2O
171.16
170.19
162.74
136.52
129.31
125.82
121.85
117.81
114.91
53.27
52.40
48.88
30.02
29.05
25.17
19.26
12.79
20·TFA
f1 (ppm)

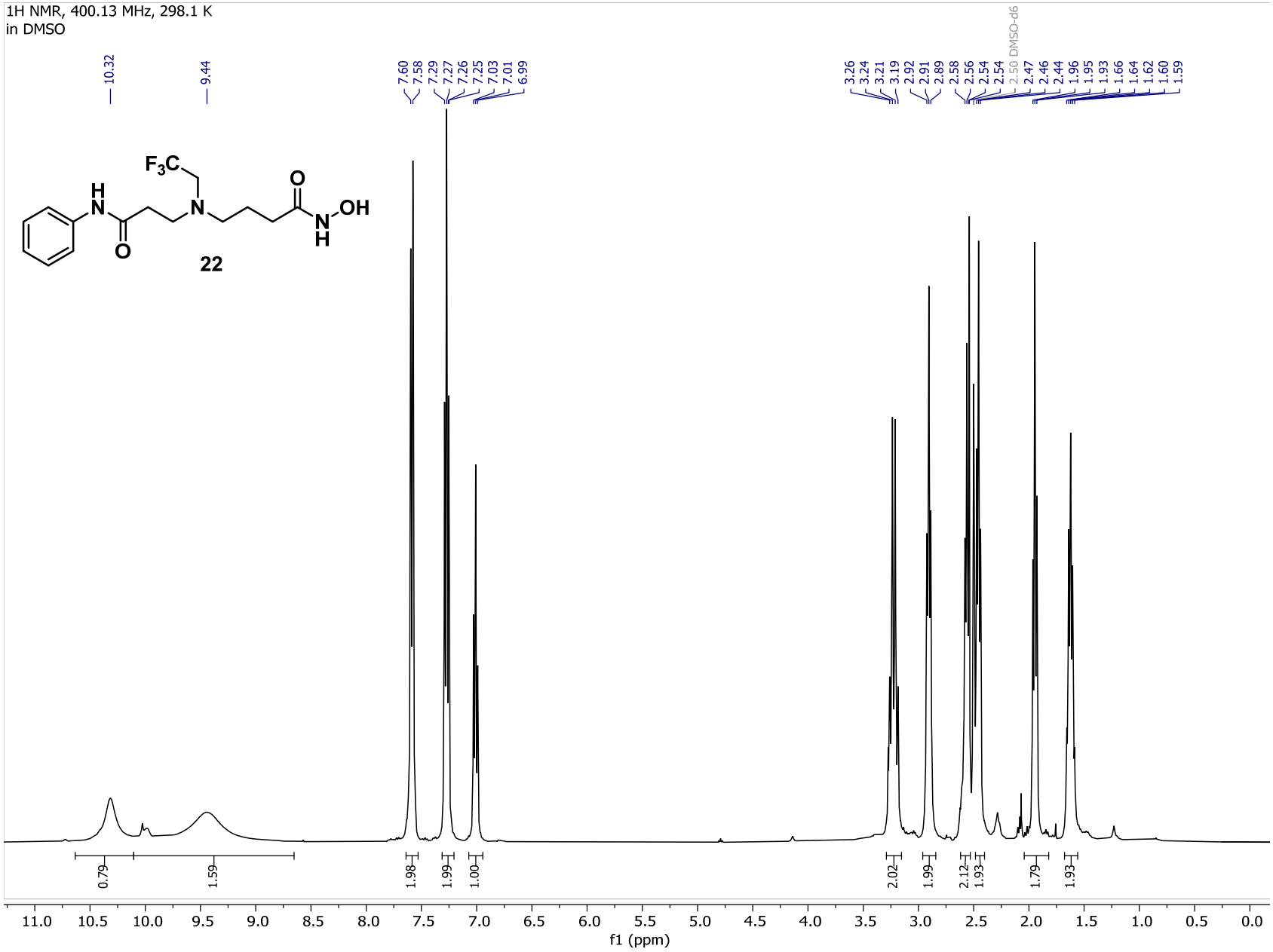

Basic Method PDA - Total Absorbance Chromatogram
Absorbance
30000
25000
20000
15000
10000
5000
0
-5000
20·TFA
4.27
95.0%
3.59
3.6%
5.30
1.3%
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
Basic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
Absorbance (mAU)
2000
1500
1000
500
0
4.27
95.0%
3.58
3.9%
5.30
1.1%
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5

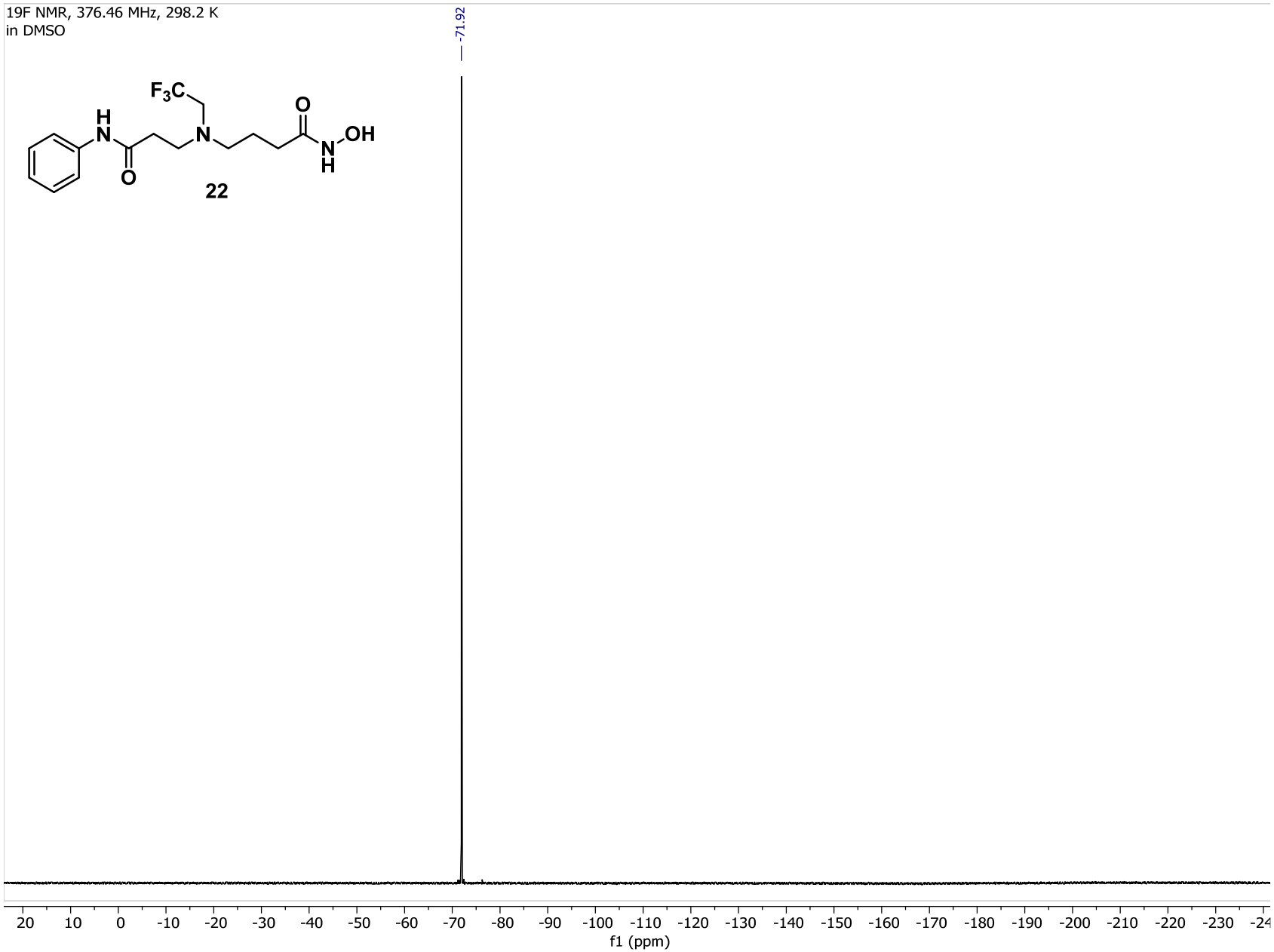

1H NMR, 400.13 MHz, 298.1 K
in MeOD
7.59
7.58
7.57
7.57
7.56
7.55
7.53
7.51
7.50
7.50
7.49
7.33
7.31
7.30
7.29
7.13
7.11
7.09
7.09
4.45
3.55
3.53
3.52
3.31 CD3OD
3.26
2.92
2.91
2.89
2.28
2.26
2.25
2.12
2.10
2.09
2.07
2.05
H
N
O
N
N
OH
H
O
21·TFA
7.16
2.06
1.03
2.00
1.99
1.96
2.00
1.88
2.03
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

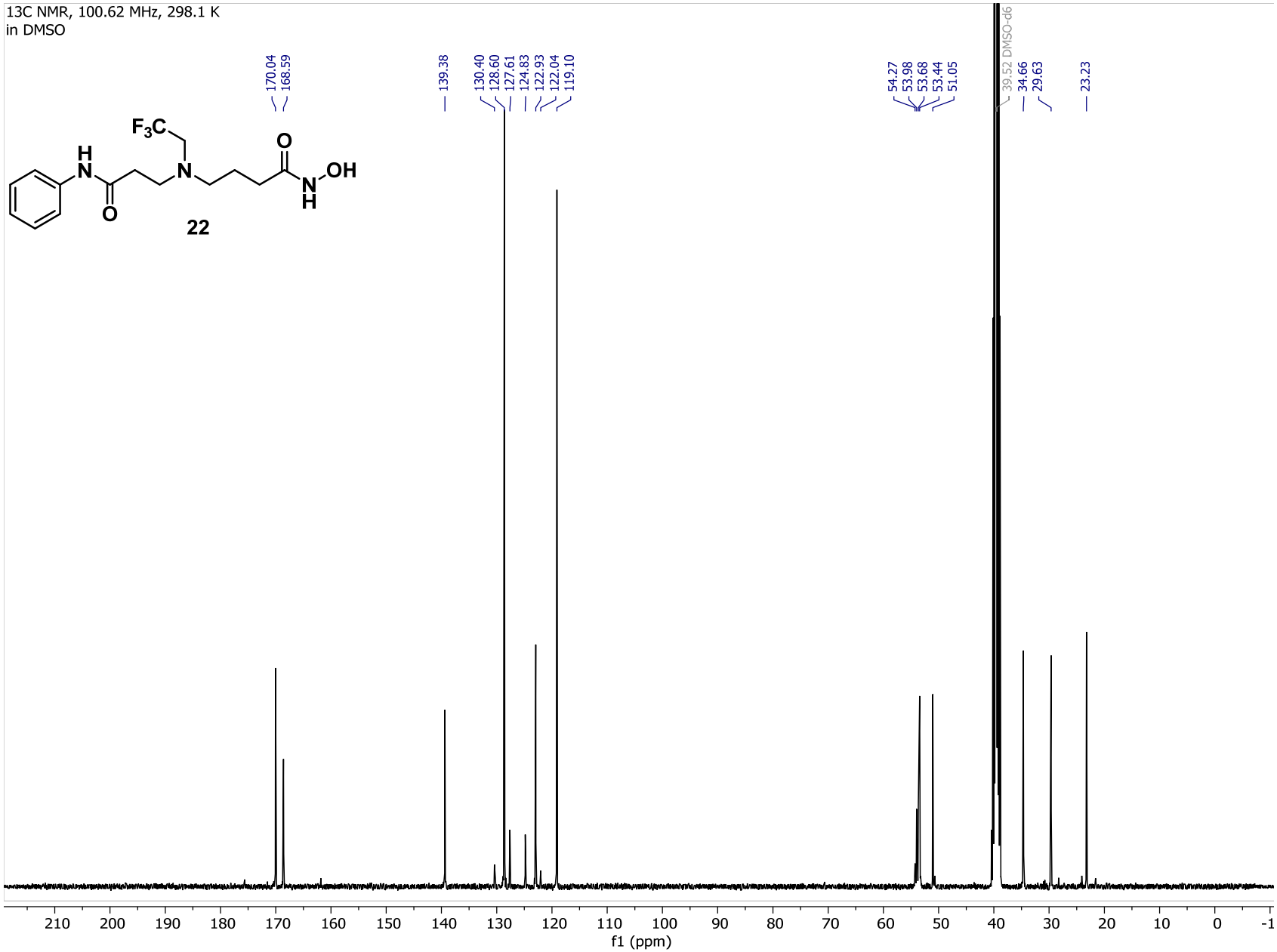

19F NMR, 376.46 MHz, 298.1 K
in MeOD
-79.73
H
N
O
N
O
N
H
OH
21·TFA
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
-210
-220
-230
f1 (ppm)

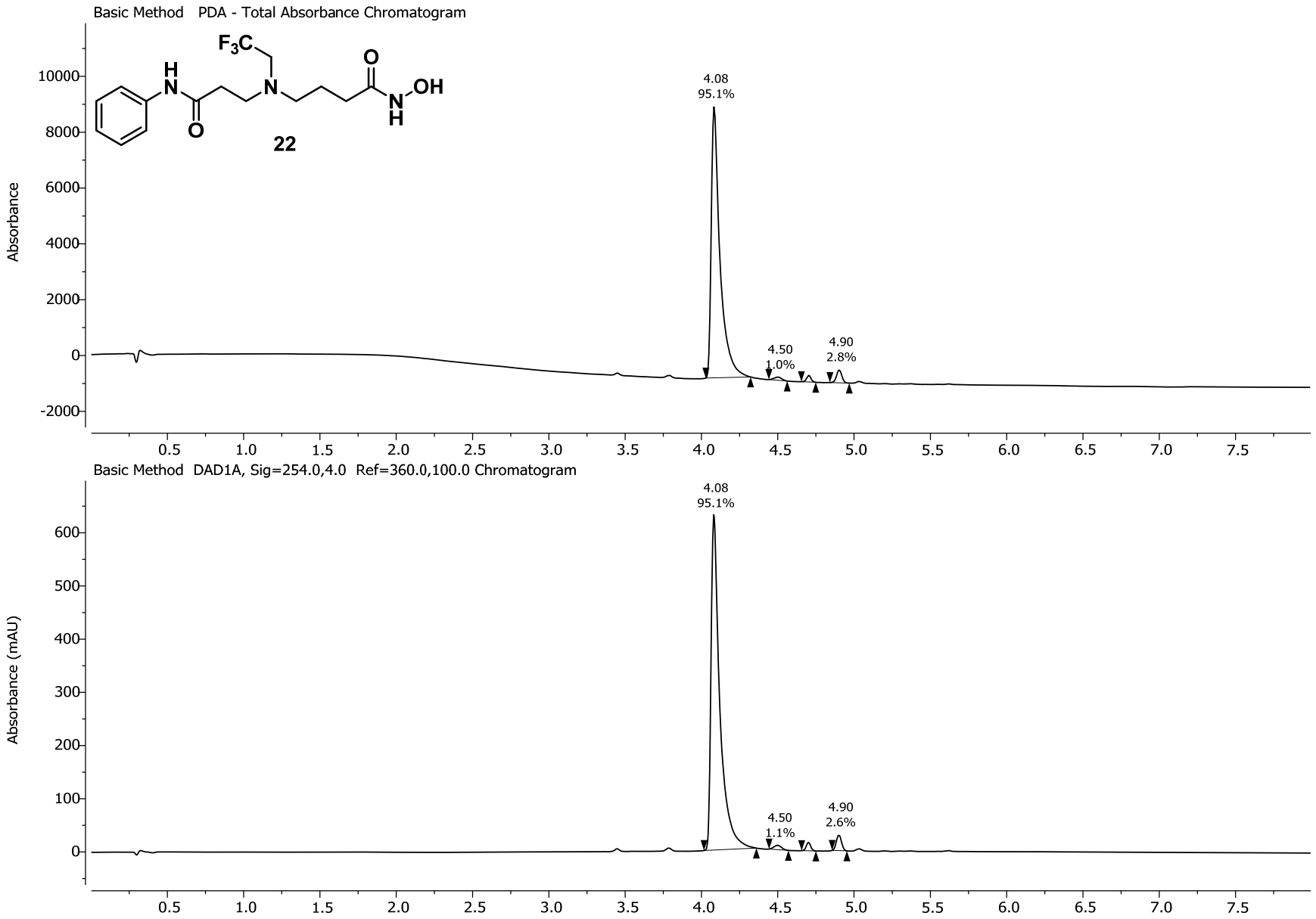

13C NMR, 100.62 MHz, 298.1 K
in MeOD
171.38
170.09
163.20
162.85
139.38
132.08
131.29
130.84
130.57
129.88
125.53
121.25
119.67
116.76
58.86
54.58
50.33
49.00 CD3OD
31.11
30.57
20.65
H
N
O
N
O
N
OH
H
21·TFA
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

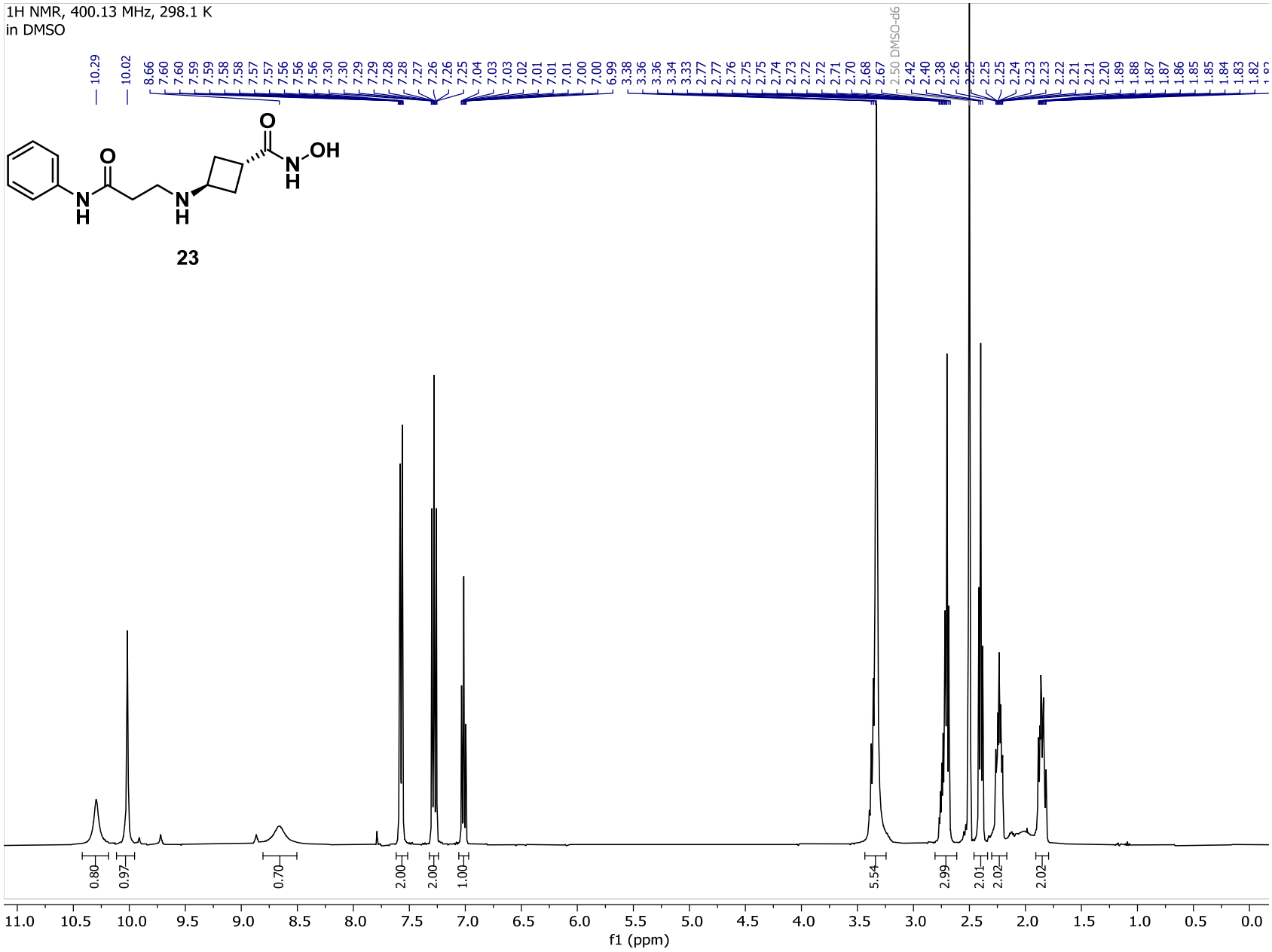

Basic Method PDA - Total Absorbance Chromatogram
Absorbance
10000
8000
6000
4000
2000
0
-2000
4.45
100.0%
21·TFA
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
Basic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
Absorbance (mAU)
600
500
400
300
200
100
0
4.45
100.0%

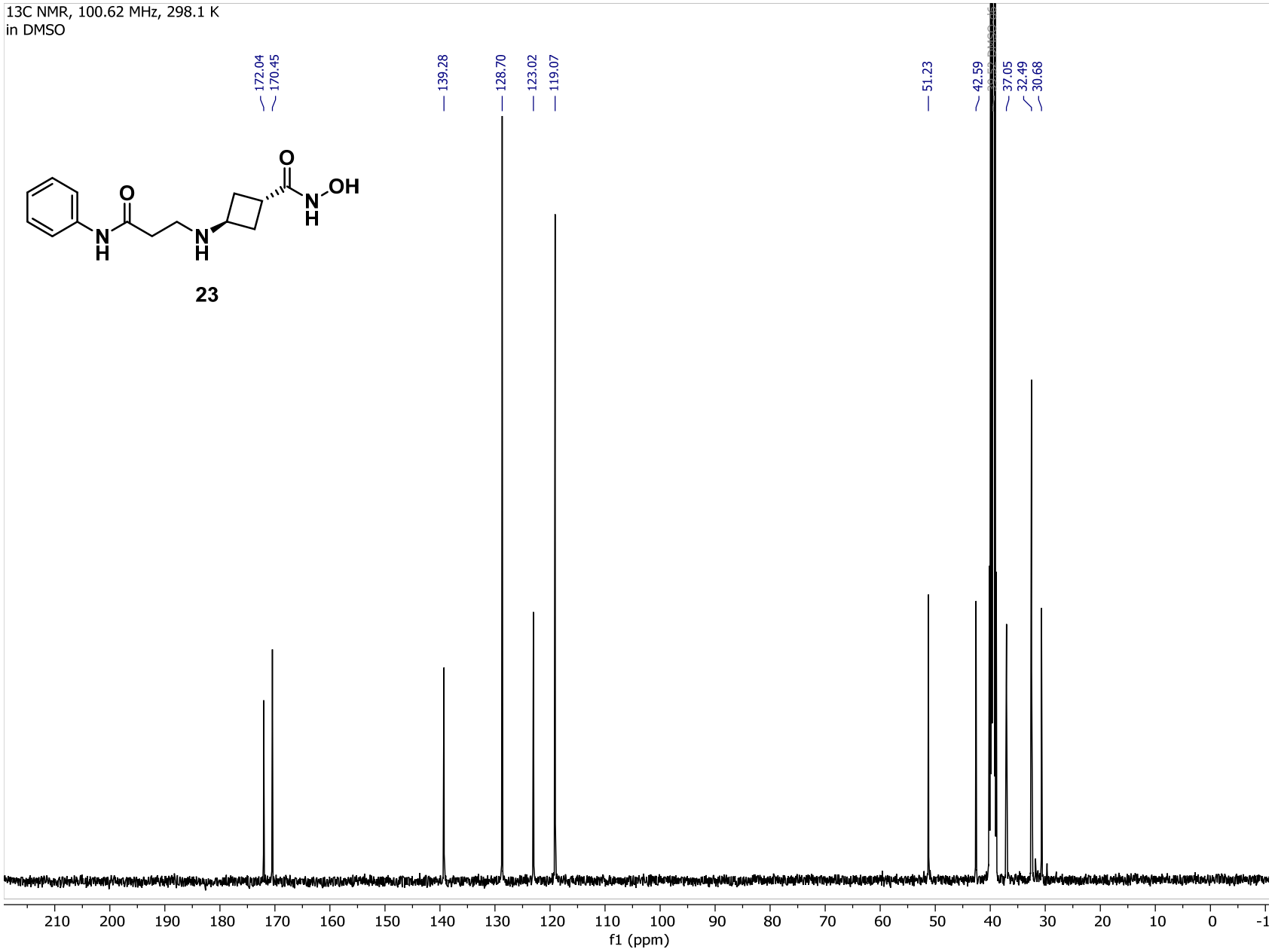

1H NMR, 400.13 MHz, 298.1 K
in DMSO
10.32
9.44
7.60
7.58
7.29
7.27
7.26
7.25
7.03
7.01
6.99
3.26
3.24
3.21
3.19
2.92
2.91
2.89
2.58
2.56
2.54
2.50 DMSO-d6
2.47
2.46
2.44
1.96
1.95
1.93
1.66
1.64
1.62
1.60
1.59
F3C
H
N
O
N
O
N
OH
H
22
0.79
1.59
1.98
1.99
1.00
2.02
1.99
2.12
1.93
1.79
1.93
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

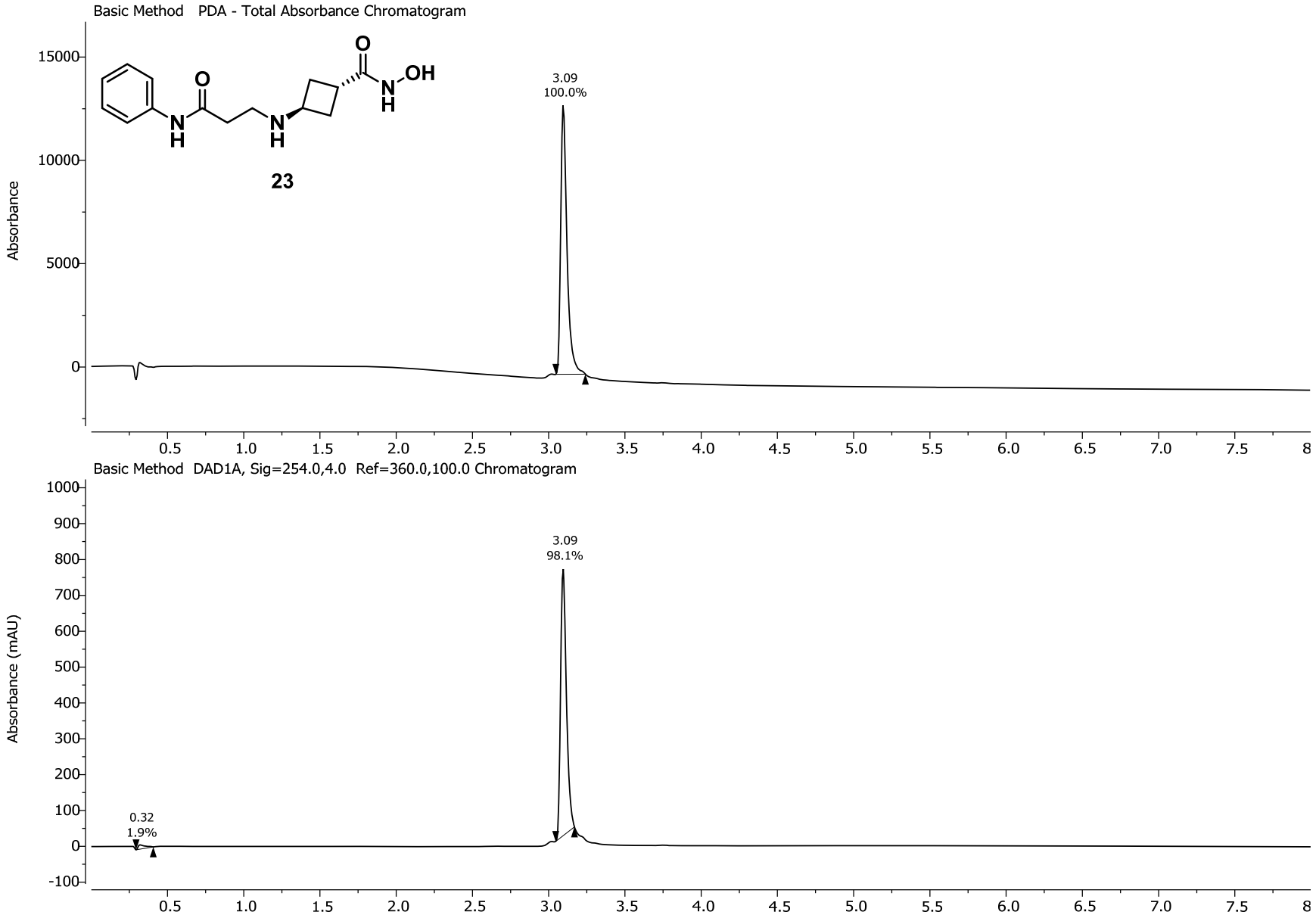

19F NMR, 376.46 MHz, 298.2 K
in DMSO
-71.92
F3C
H
N
O
N
O
N
H
OH
22
20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210 -220 -230
f1 (ppm)

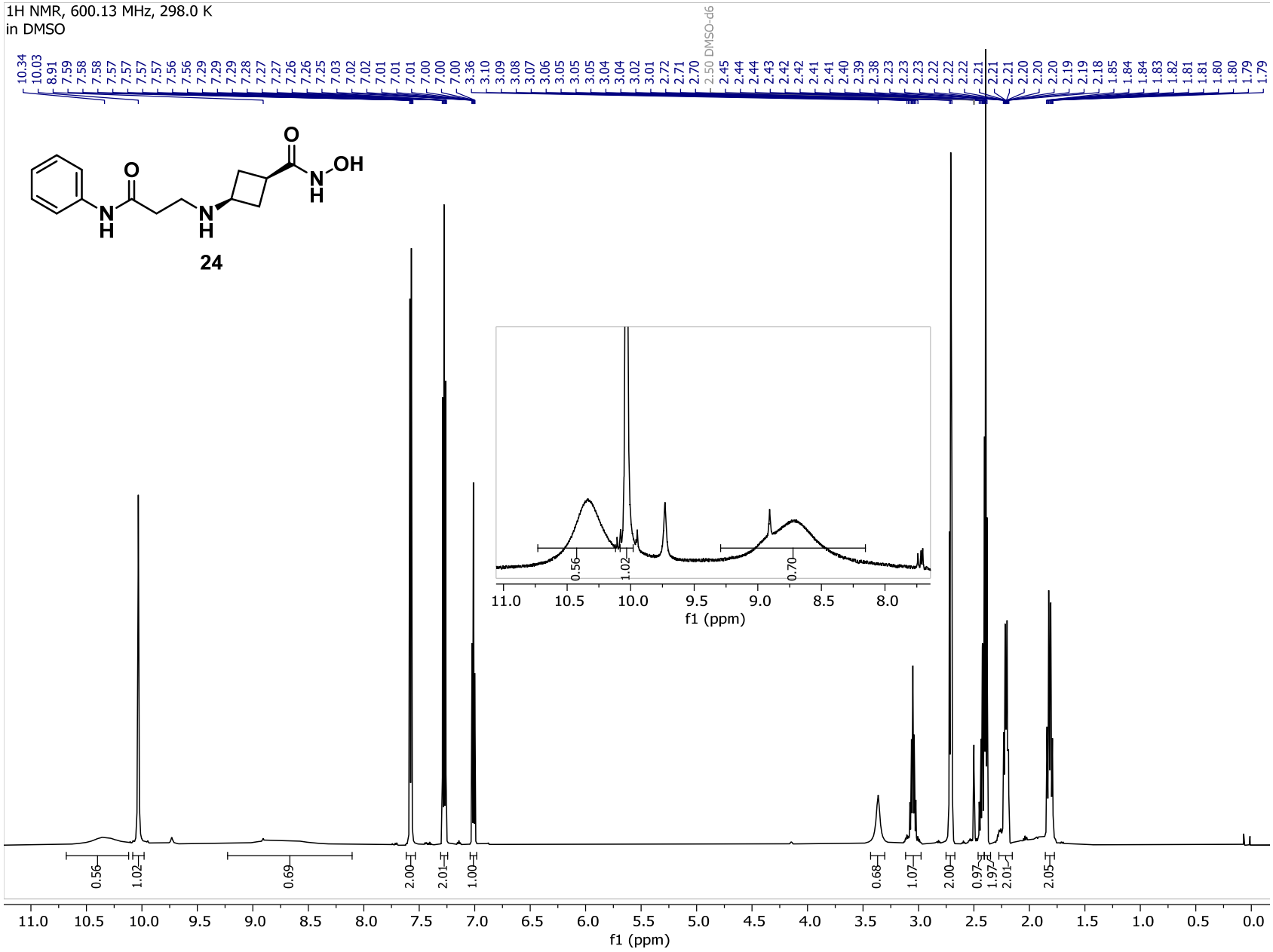

13C NMR, 100.62 MHz, 298.1 K
in DMSO
170.04
168.59
139.38
130.40
128.60
127.61
124.83
122.93
122.04
119.10
54.27
53.98
53.68
53.44
51.05
39.52 DMSO-d6
34.66
29.63
23.23
22
f1 (ppm)

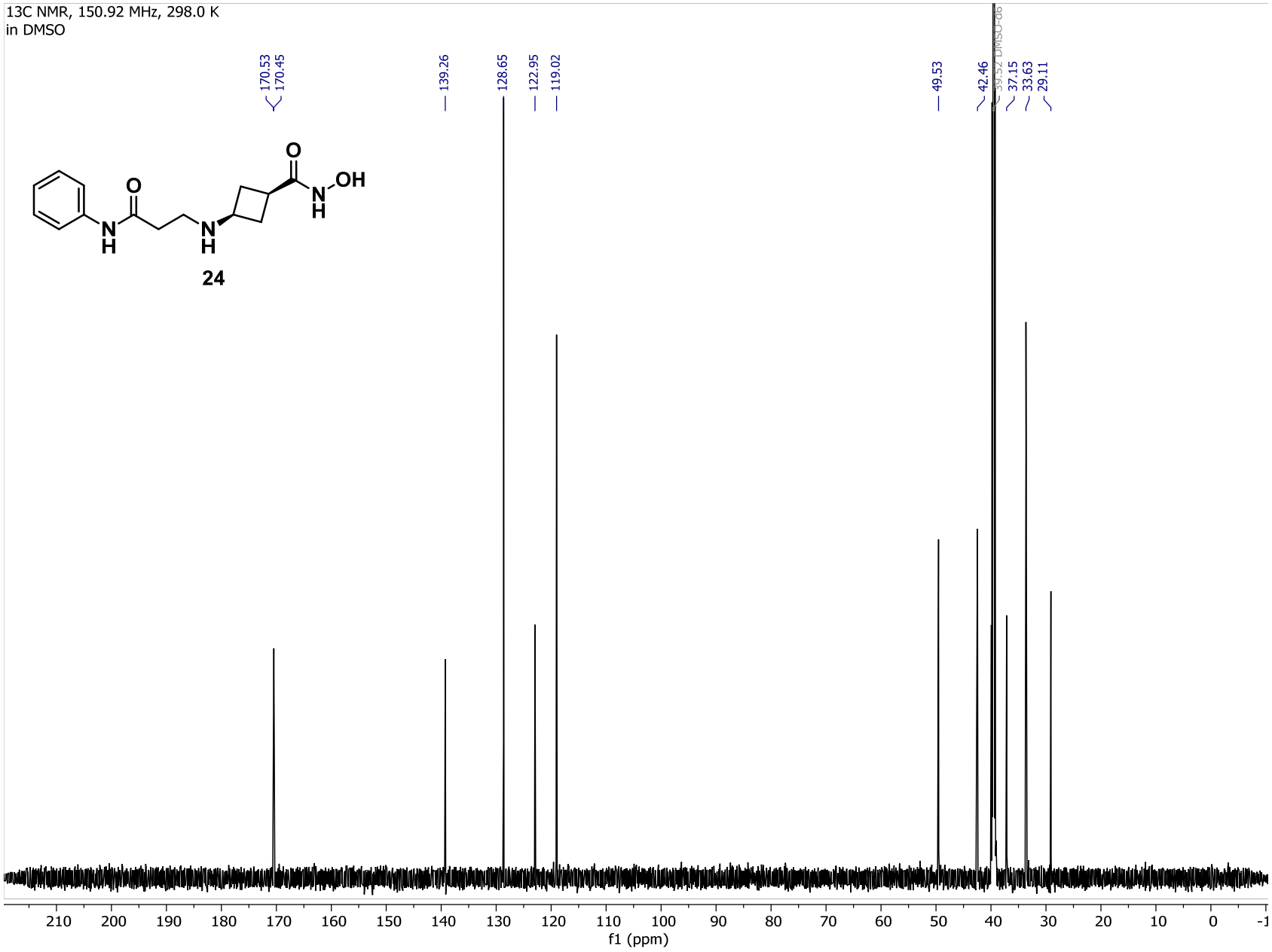

Basic Method PDA - Total Absorbance Chromatogram
F3C
H
N
O
N
O
N
OH
H
22
Absorbance
10000
8000
6000
4000
2000
0
-2000
4.08
95.1%
4.50
1.0%
4.90
2.8%
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
Basic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
Absorbance (mAU)
600
500
400
300
200
100
0
4.08
95.1%
4.50
1.1%
4.90
2.6%
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5

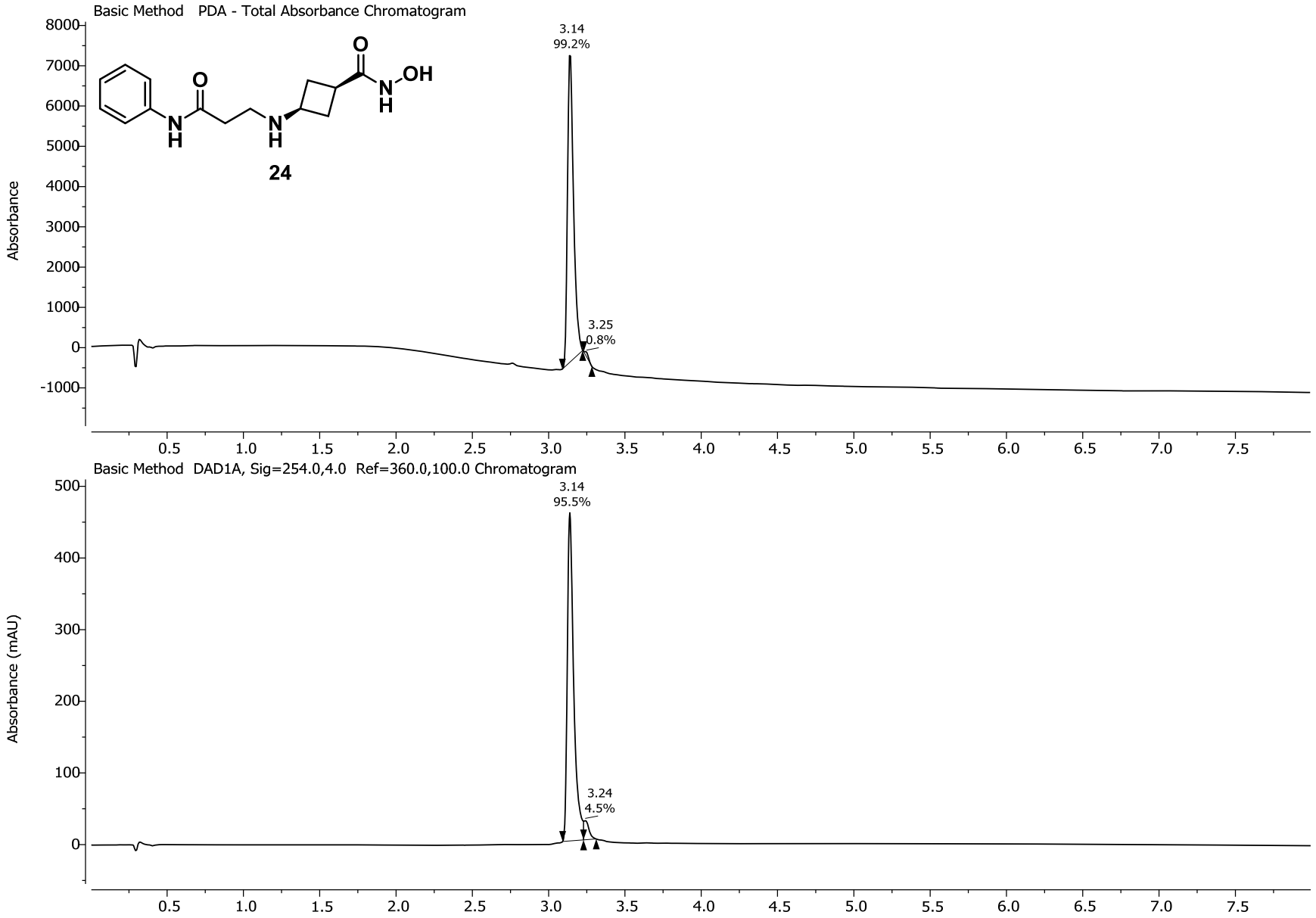

1H NMR, 400.13 MHz, 298.1 K
in DMSO
DMSO-d6
23
f1 (ppm)

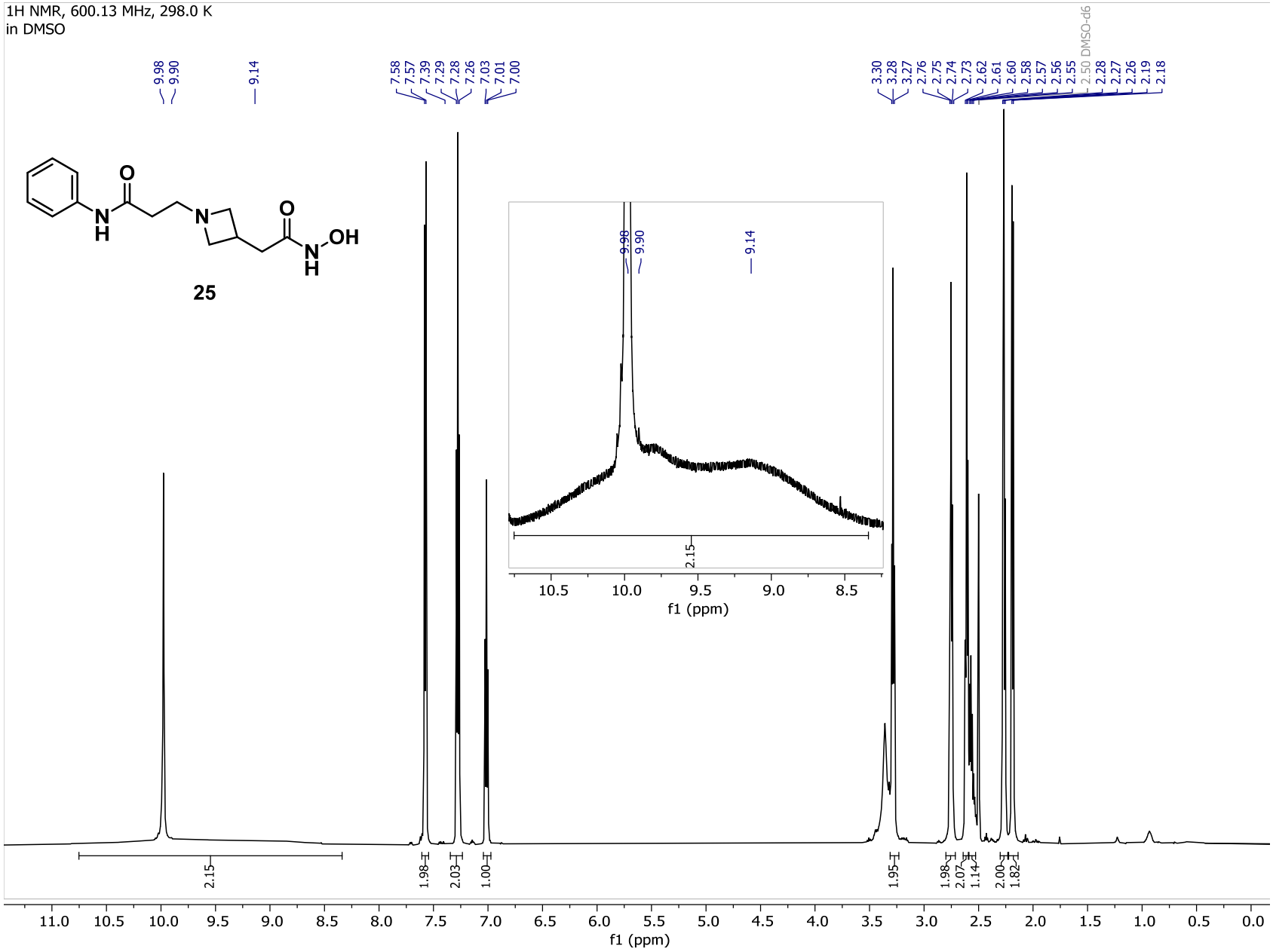

13C NMR, 100.62 MHz, 298.1 K
in DMSO
172.04
170.45
139.28
128.70
123.02
119.07
51.23
42.59
37.05
32.49
30.68
O
N
H
O
N
H
O
N
OH
H
23
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-1
f1 (ppm)

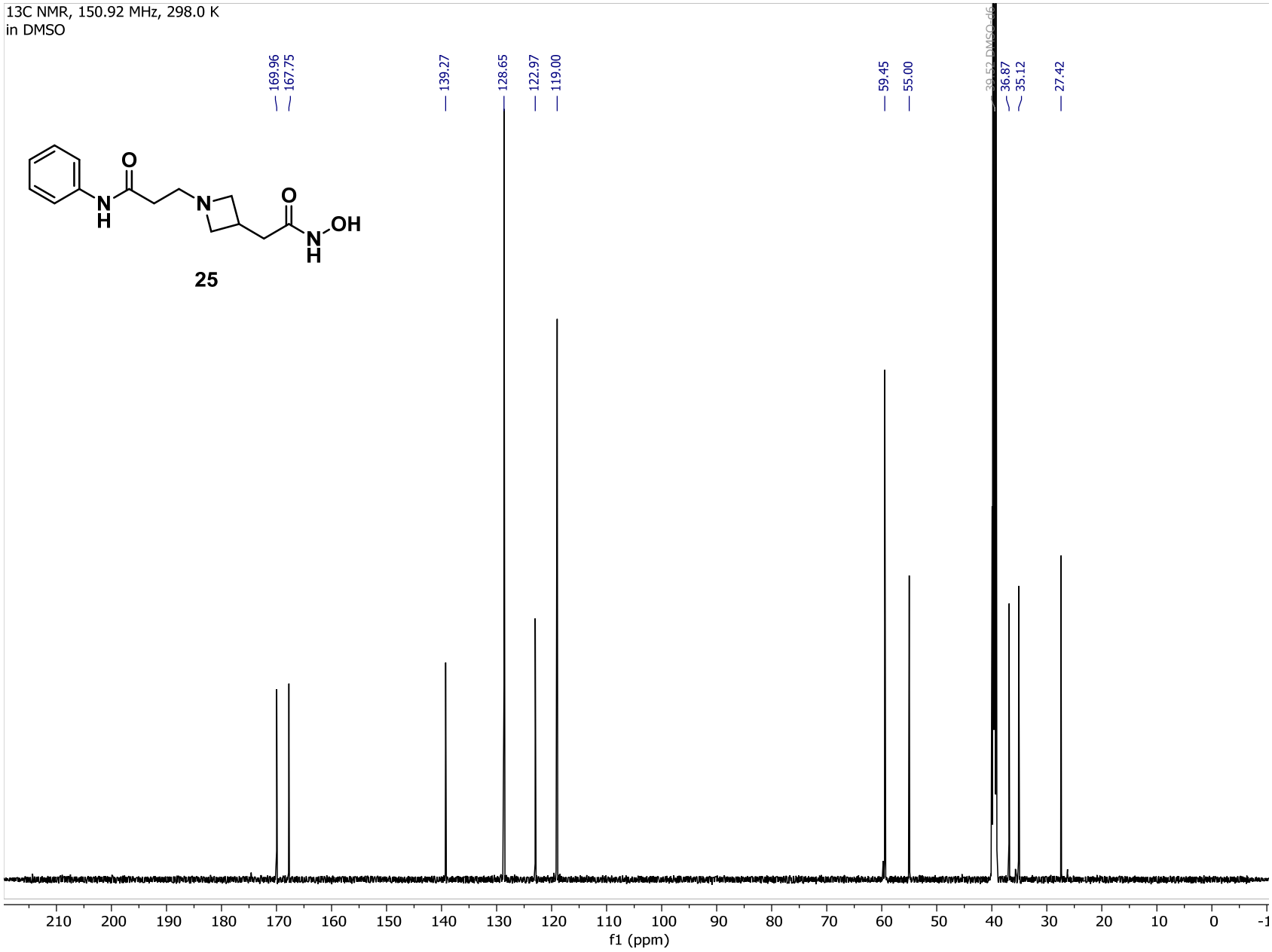

Basic Method PDA - Total Absorbance Chromatogram
Absorbance
15000
10000
5000
0
3.09
100.0%
23
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
Basic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
Absorbance (mAU)
1000
900
800
700
600
500
400
300
200
100
0
-100
3.09
98.1%
0.32
1.9%

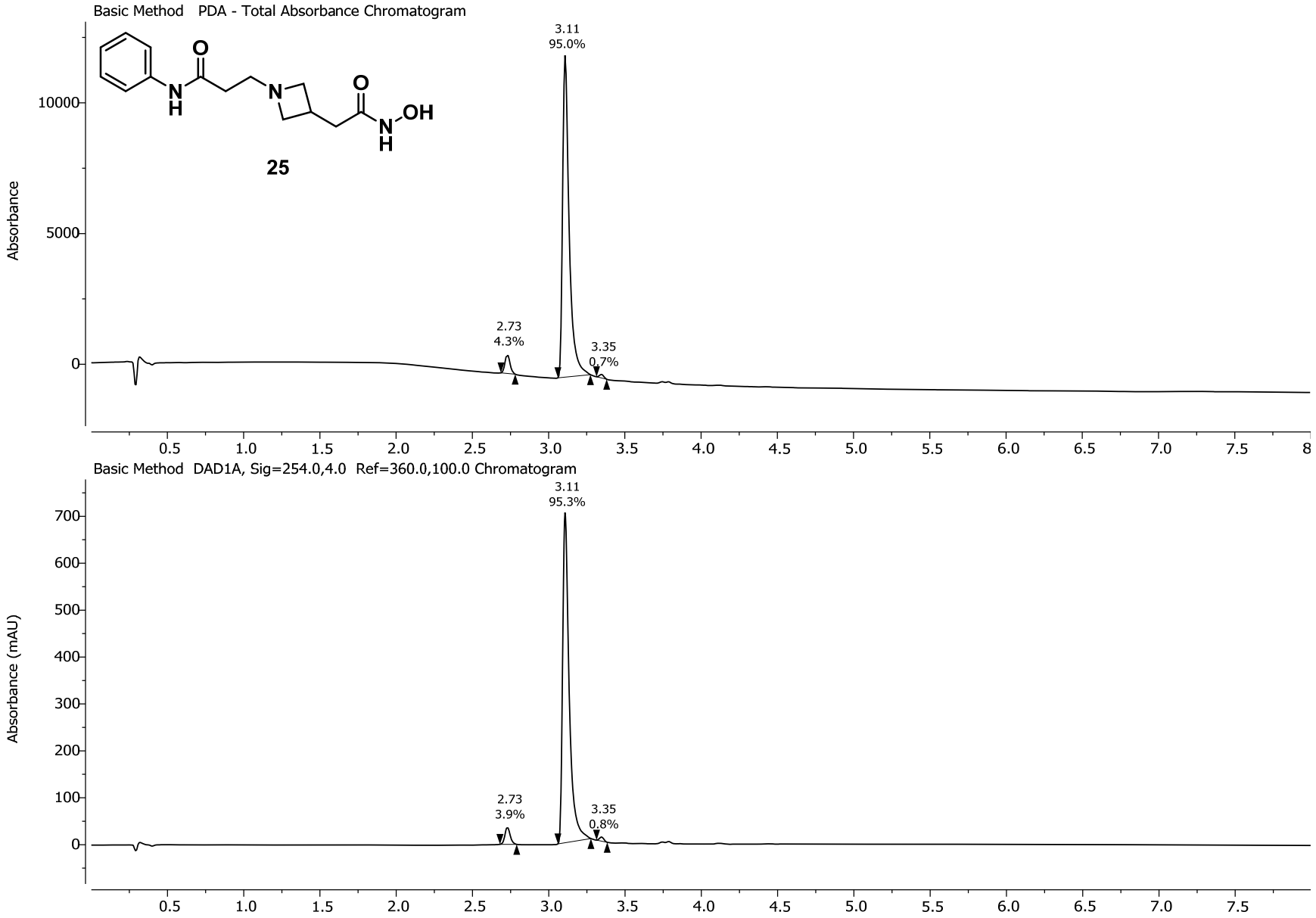

1H NMR, 600.13 MHz, 298.0 K
in DMSO
2.50 DMSO-d6
24
f1 (ppm)


13C NMR, 150.92 MHz, 298.0 K
in DMSO
170.53
170.45
139.26
128.65
122.95
119.02
49.53
42.46
39.52 DMSO-d6
37.15
33.63
29.11
O
N
H
O
N
H
O
N
OH
H
24
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-1
f1 (ppm)

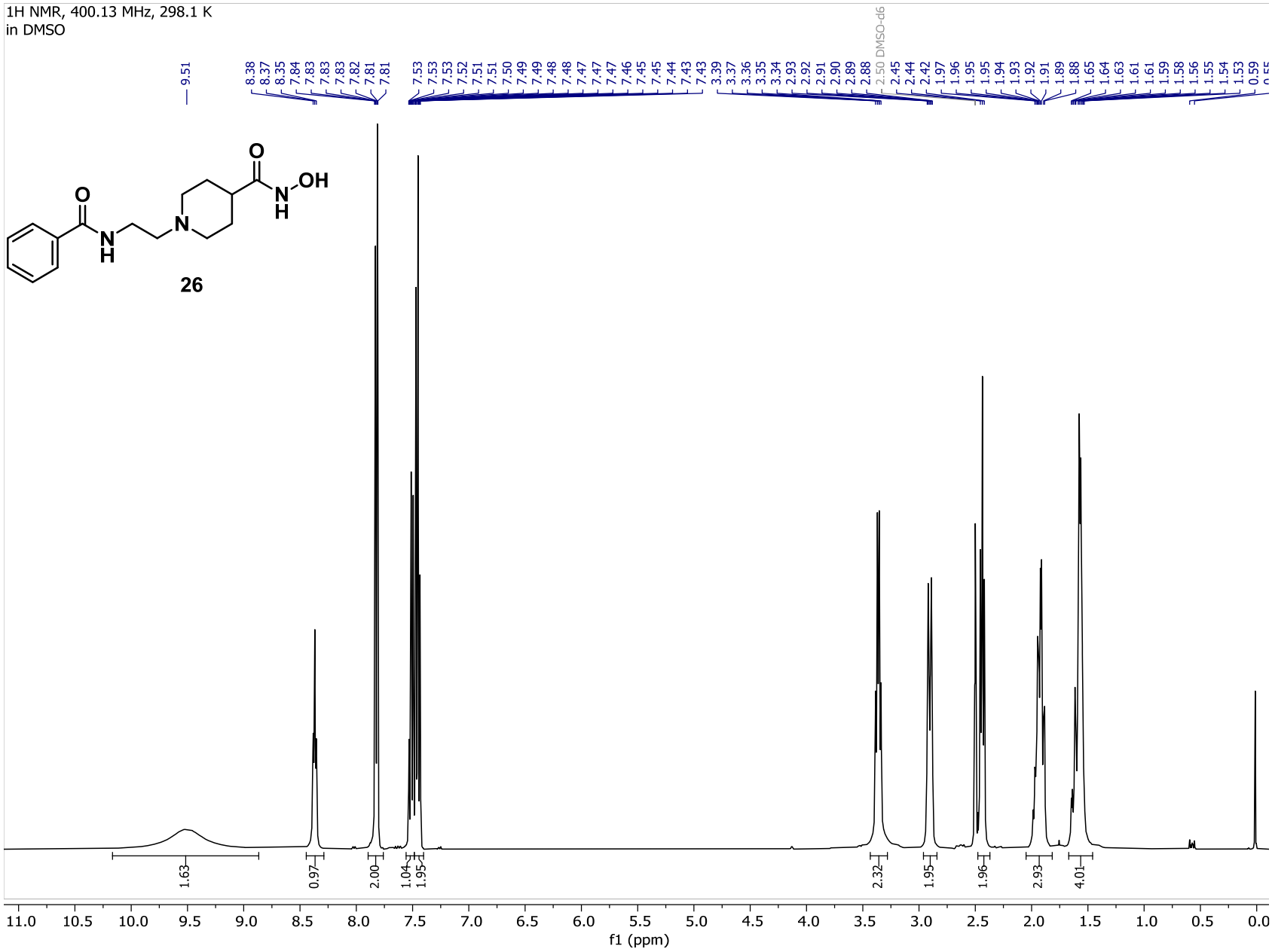

Basic Method PDA - Total Absorbance Chromatogram
Absorbance
3.14
99.2%
3.25
0.8%
24
Basic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
Absorbance (mAU)
3.14
95.5%
3.24
4.5%

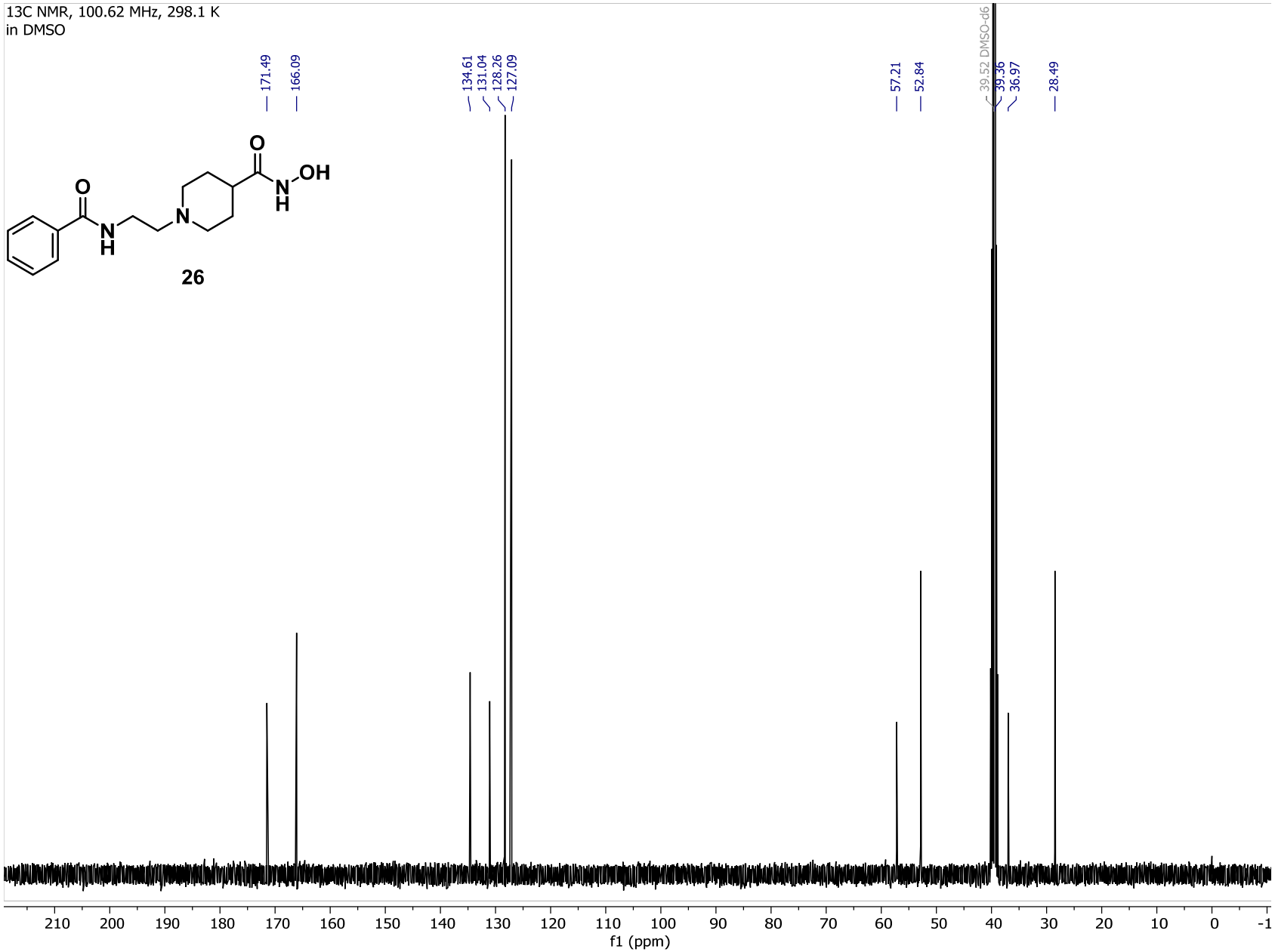

1H NMR, 600.13 MHz, 298.0 K
in DMSO
9.98
9.90
9.14
7.58
7.57
7.39
7.29
7.28
7.26
7.03
7.01
7.00
3.30
3.28
3.27
2.76
2.75
2.74
2.73
2.62
2.61
2.60
2.58
2.57
2.56
2.55
2.50 DMSO-d6
2.28
2.27
2.26
2.19
2.18
O
N
H
N
O
N
OH
H
25
2.15
1.98
2.03
1.00
1.95
1.98
2.07
1.14
2.00
1.82
f1 (ppm)

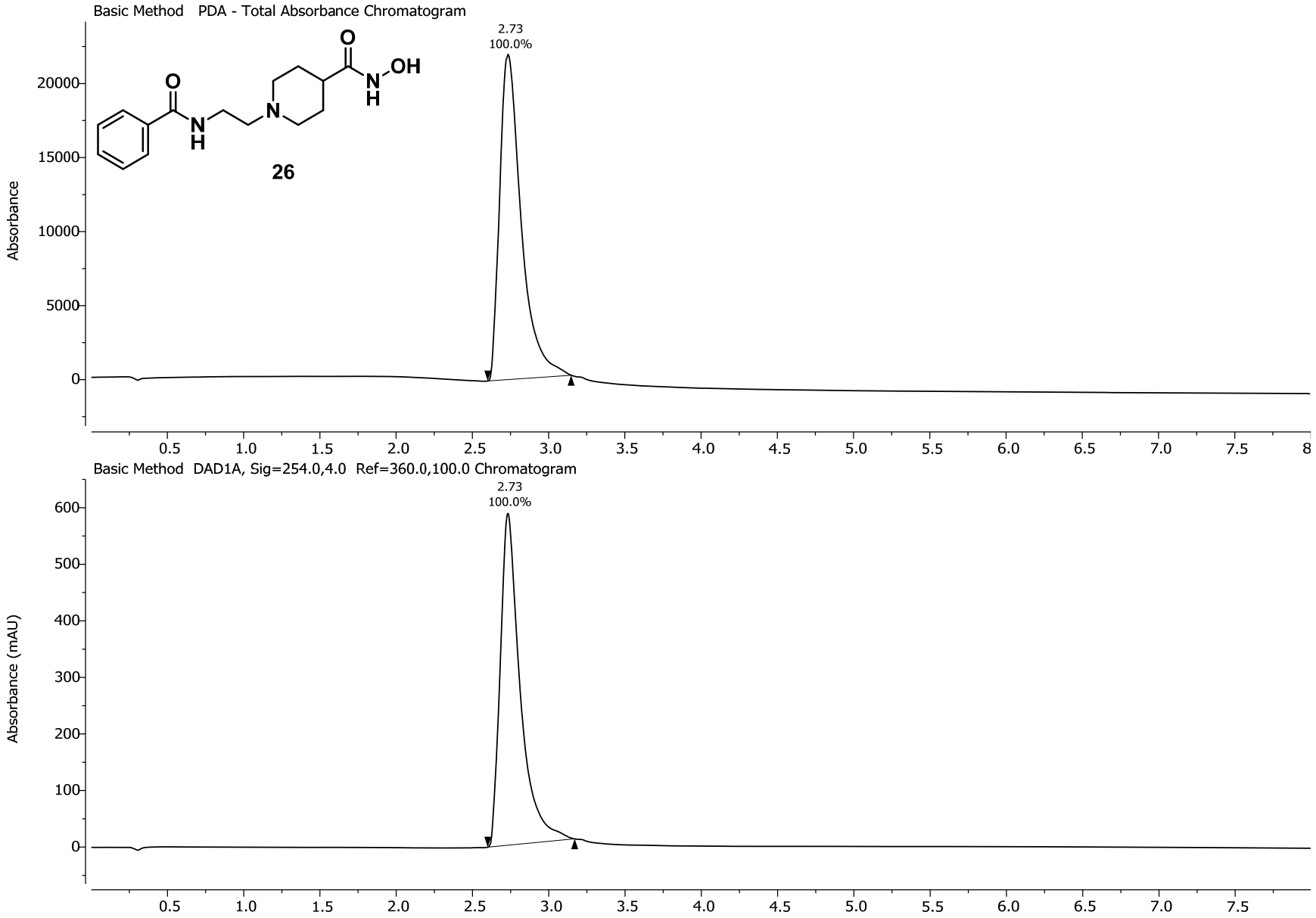

13C NMR, 150.92 MHz, 298.0 K
in DMSO
169.96
167.75
139.27
128.65
122.97
119.00
59.45
55.00
39.52 DMSO-d6
36.87
35.12
27.42
O
N
H
N
O
N
OH
H
25
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -1
f1 (ppm)

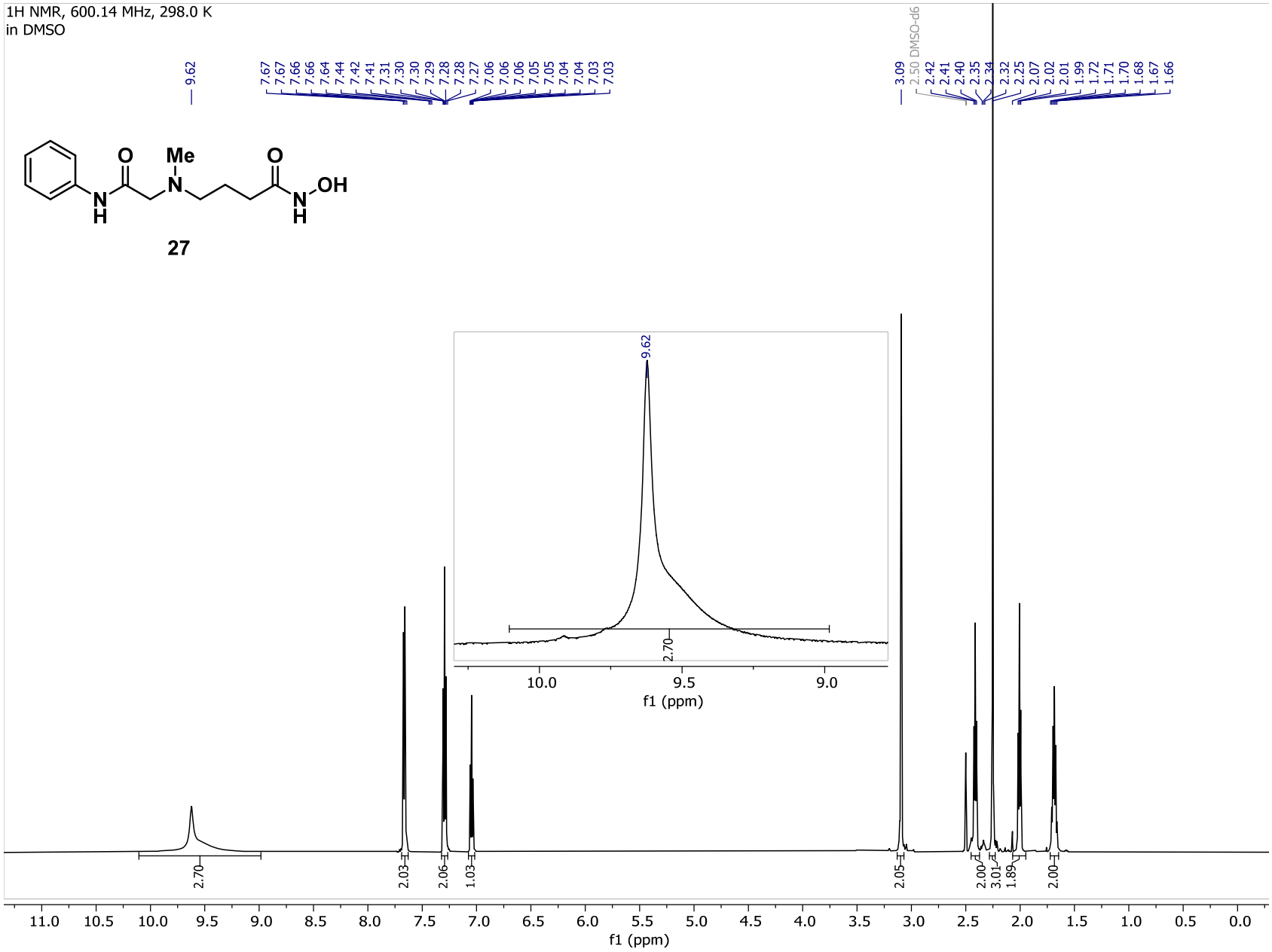

Basic Method PDA - Total Absorbance Chromatogram
Absorbance
10000
5000
0
3.11
95.0%
2.73
4.3%
3.35
0.7%
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8
Basic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
Absorbance (mAU)
700
600
500
400
300
200
100
0
3.11
95.3%
2.73
3.9%
3.35
0.8%
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5

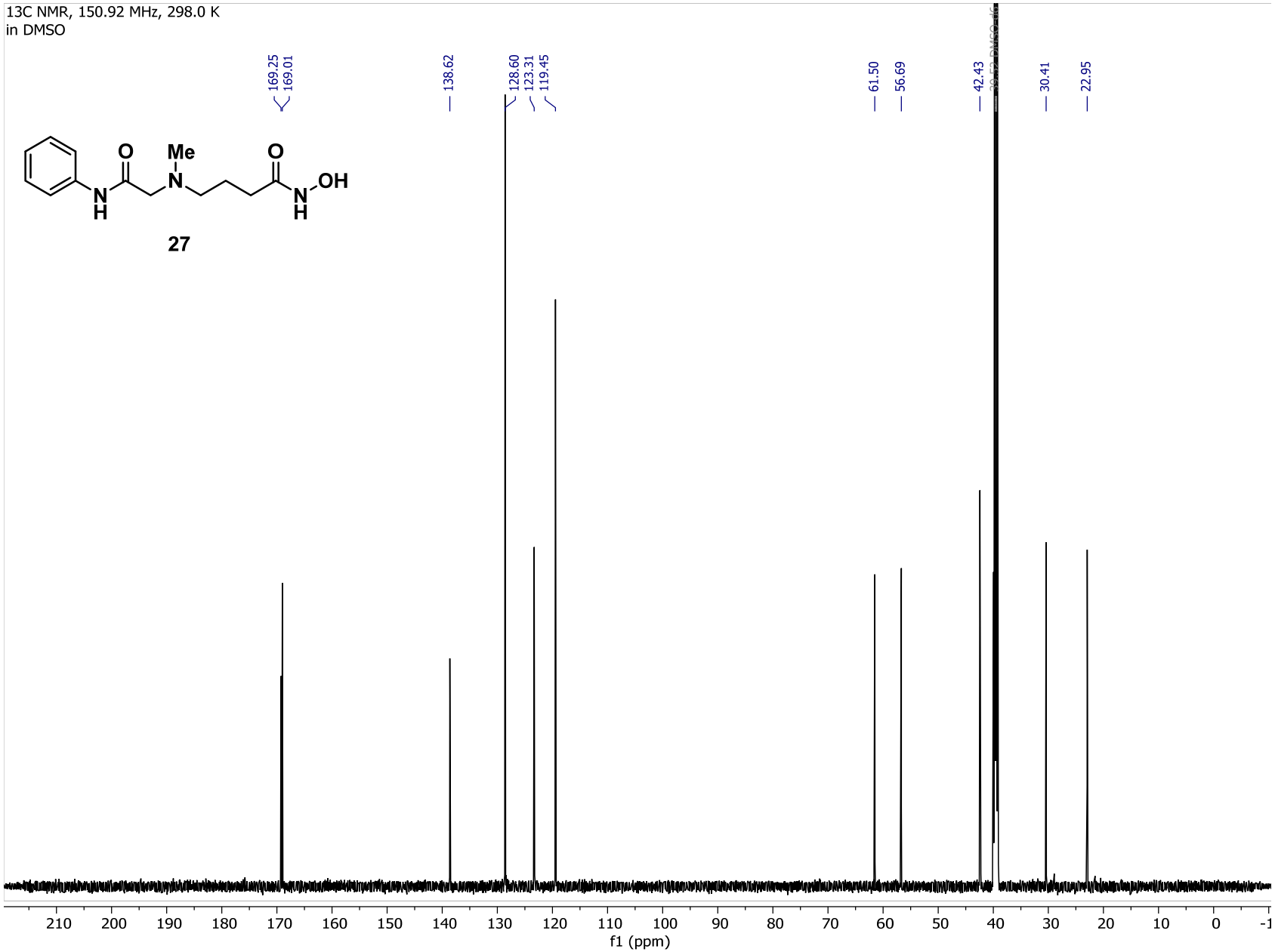
**25**

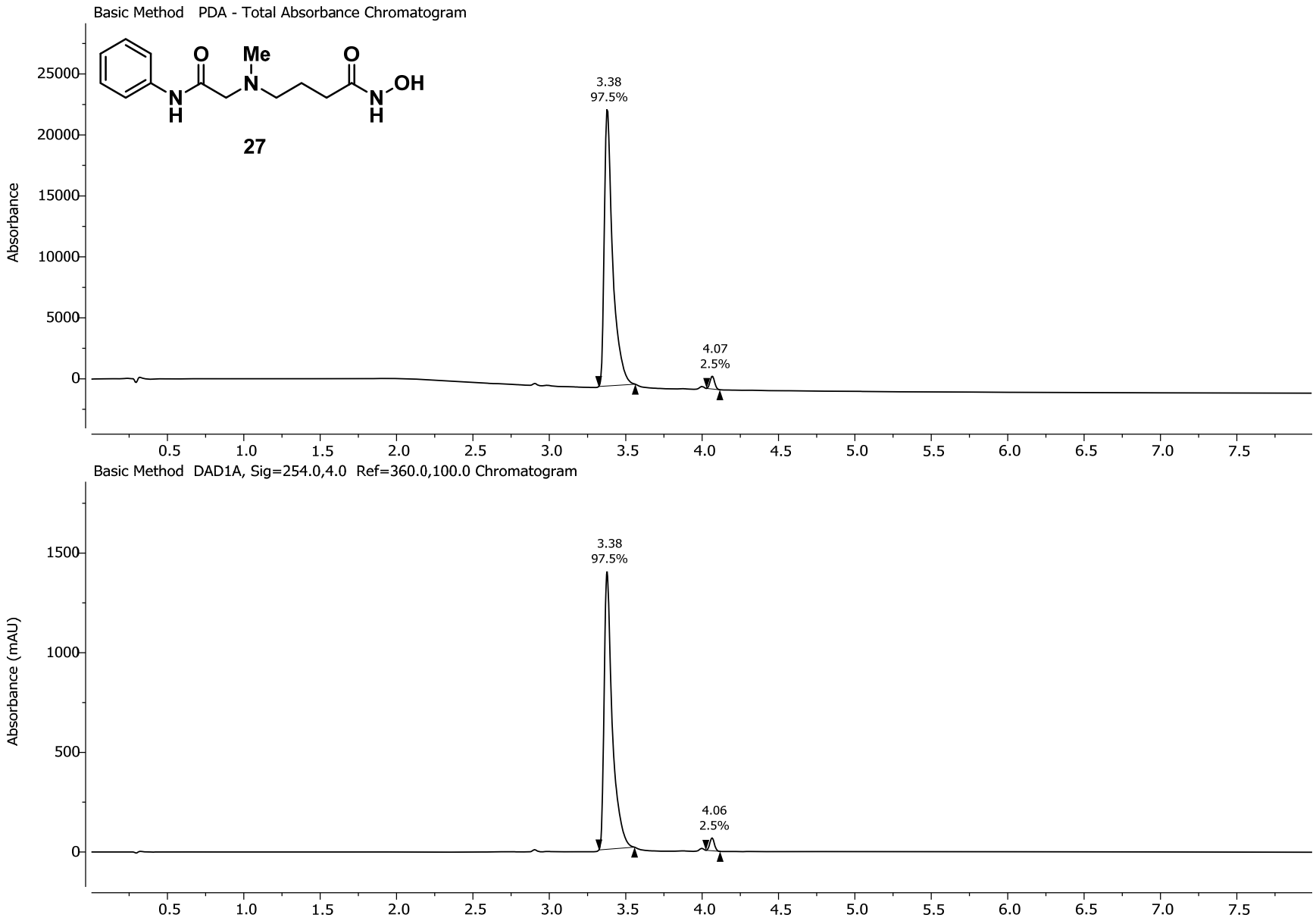

1H NMR, 400.13 MHz, 298.1 K
in DMSO
26
f1 (ppm)

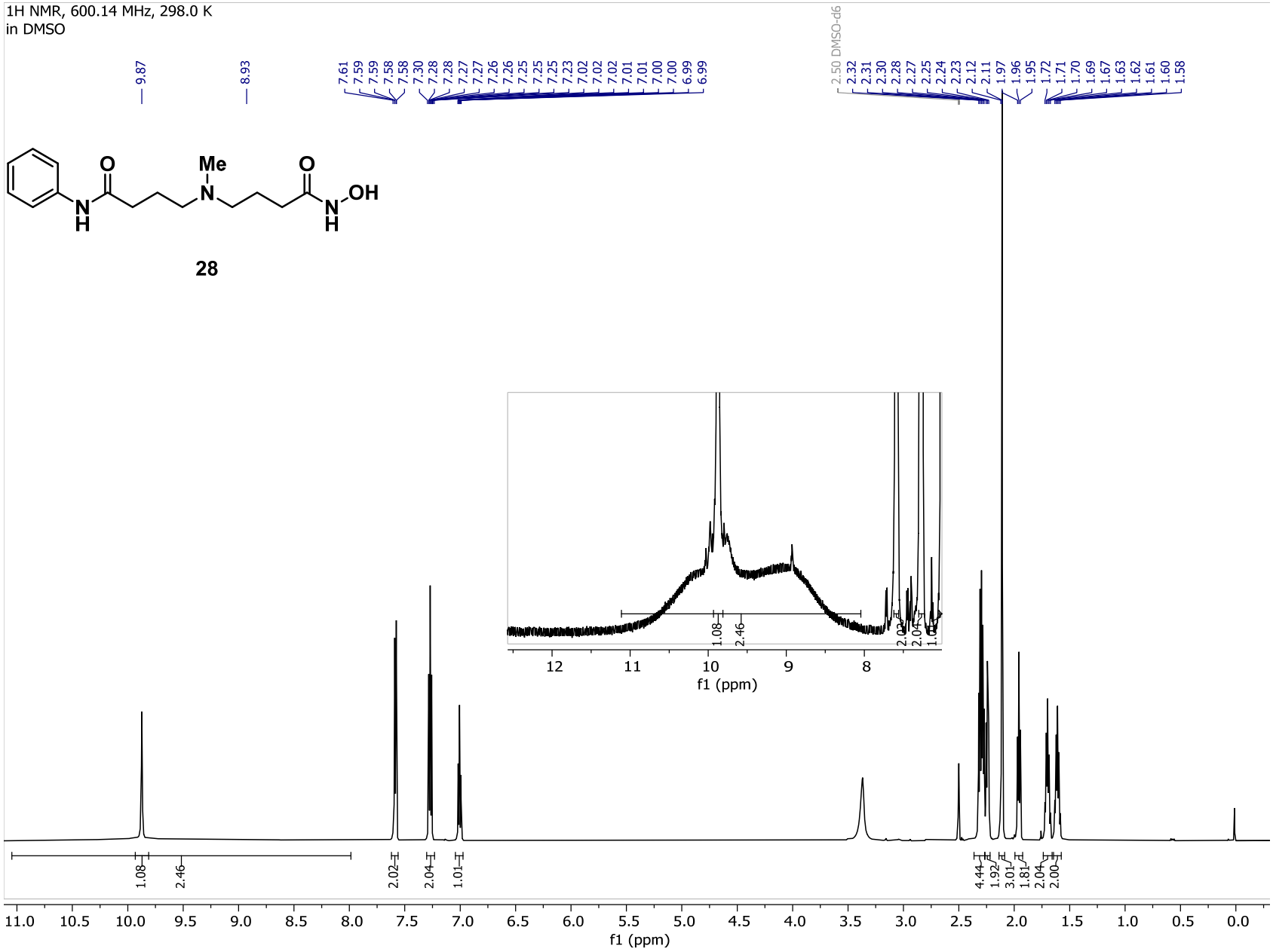

13C NMR, 100.62 MHz, 298.1 K
in DMSO
171.49
166.09
134.61
131.04
128.26
127.09
57.21
52.84
39.52 DMSO-d6
39.36
36.97
28.49
O
N
H
N
O
N
OH
H
26
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -1
f1 (ppm)

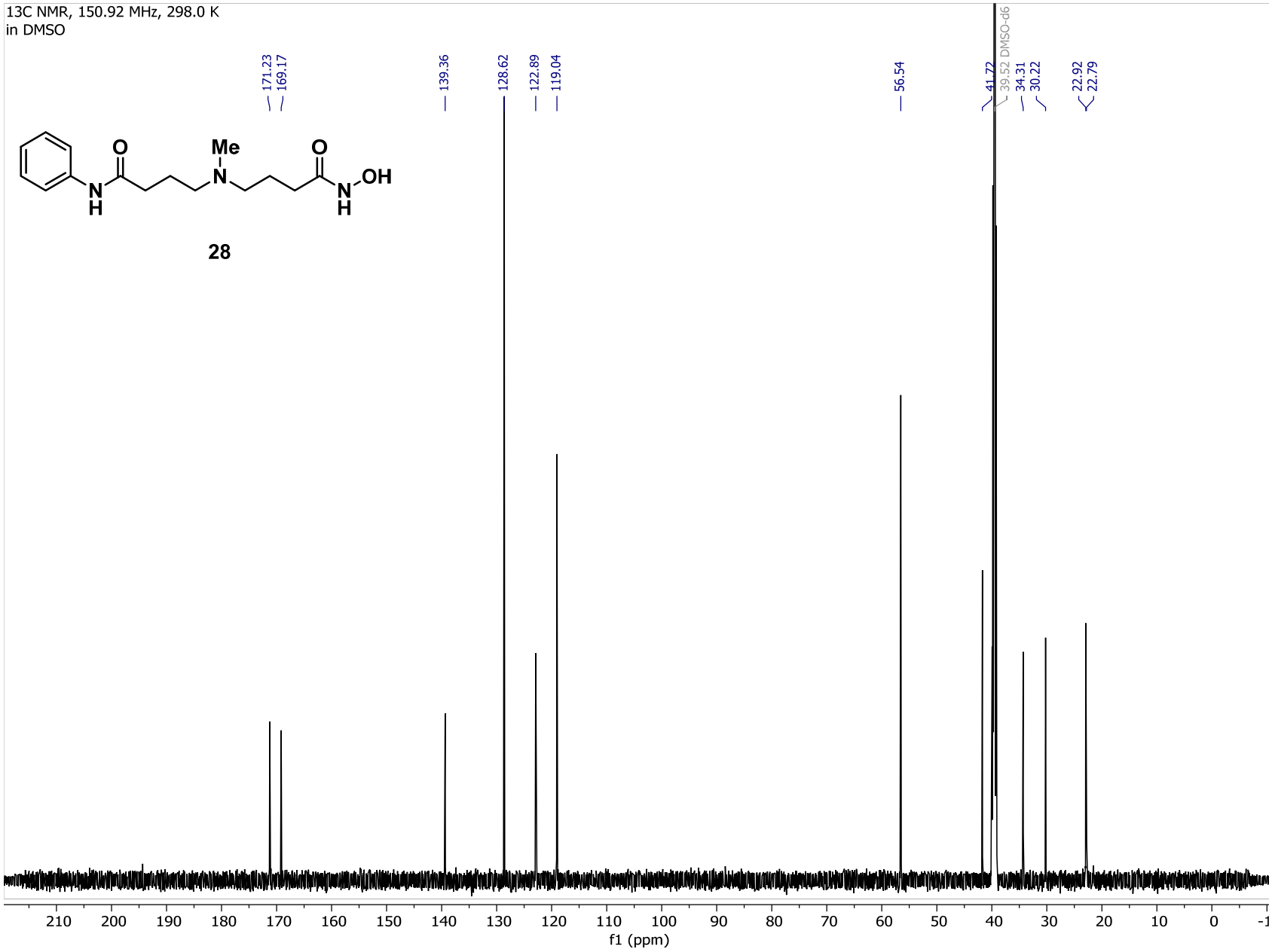

Basic Method PDA - Total Absorbance Chromatogram
2.73
100.0%
Absorbance
20000
15000
10000
5000
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8
O
N
H
OH
N
O
N
H
26
Basic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
2.73
100.0%
Absorbance (mAU)
600
500
400
300
200
100
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5

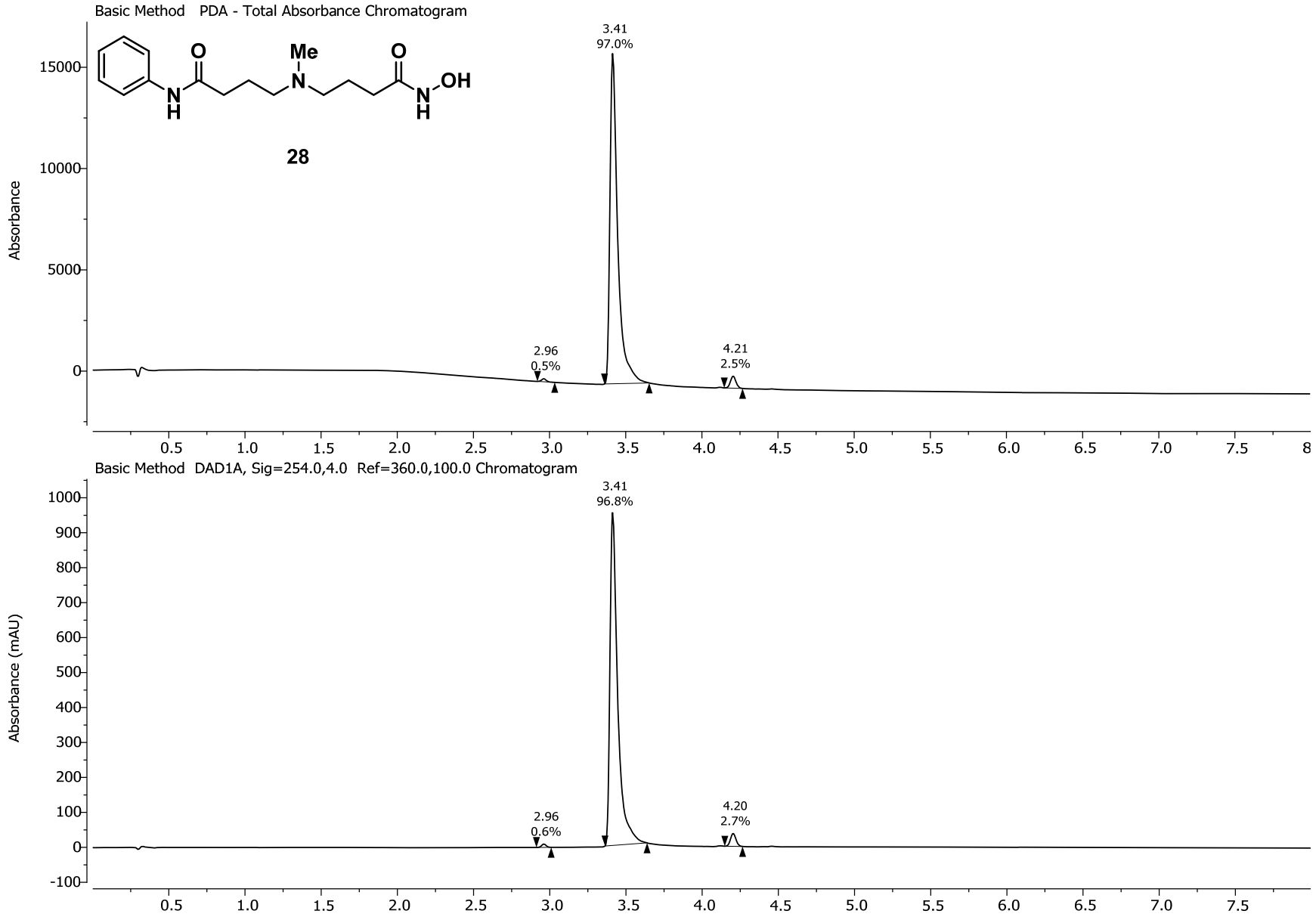

1H NMR, 600.14 MHz, 298.0 K
in DMSO
9.62
7.67 7.67 7.66 7.66 7.64 7.44 7.42 7.41 7.31 7.30 7.30 7.29 7.28 7.28 7.27 7.06 7.06 7.06 7.05 7.05 7.04 7.04 7.03 7.03
3.09
2.50 DMSO-d6
2.42 2.41 2.40 2.35 2.34 2.32 2.25 2.07 2.02 2.01 1.99 1.72 1.71 1.70 1.68 1.67 1.66
O
Me
O
N
N
OH
H
H
27
9.62
2.70
10.0
9.5
9.0
f1 (ppm)
2.70
2.03
2.06
1.03
2.05
2.00
3.01
1.89
2.00
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


13C NMR, 150.92 MHz, 298.0 K
in DMSO
169.25
169.01
138.62
128.60
123.31
119.45
61.50
56.69
42.43
30.41
22.95
O
Me
O
N
N
OH
H
H
27
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -1
f1 (ppm)

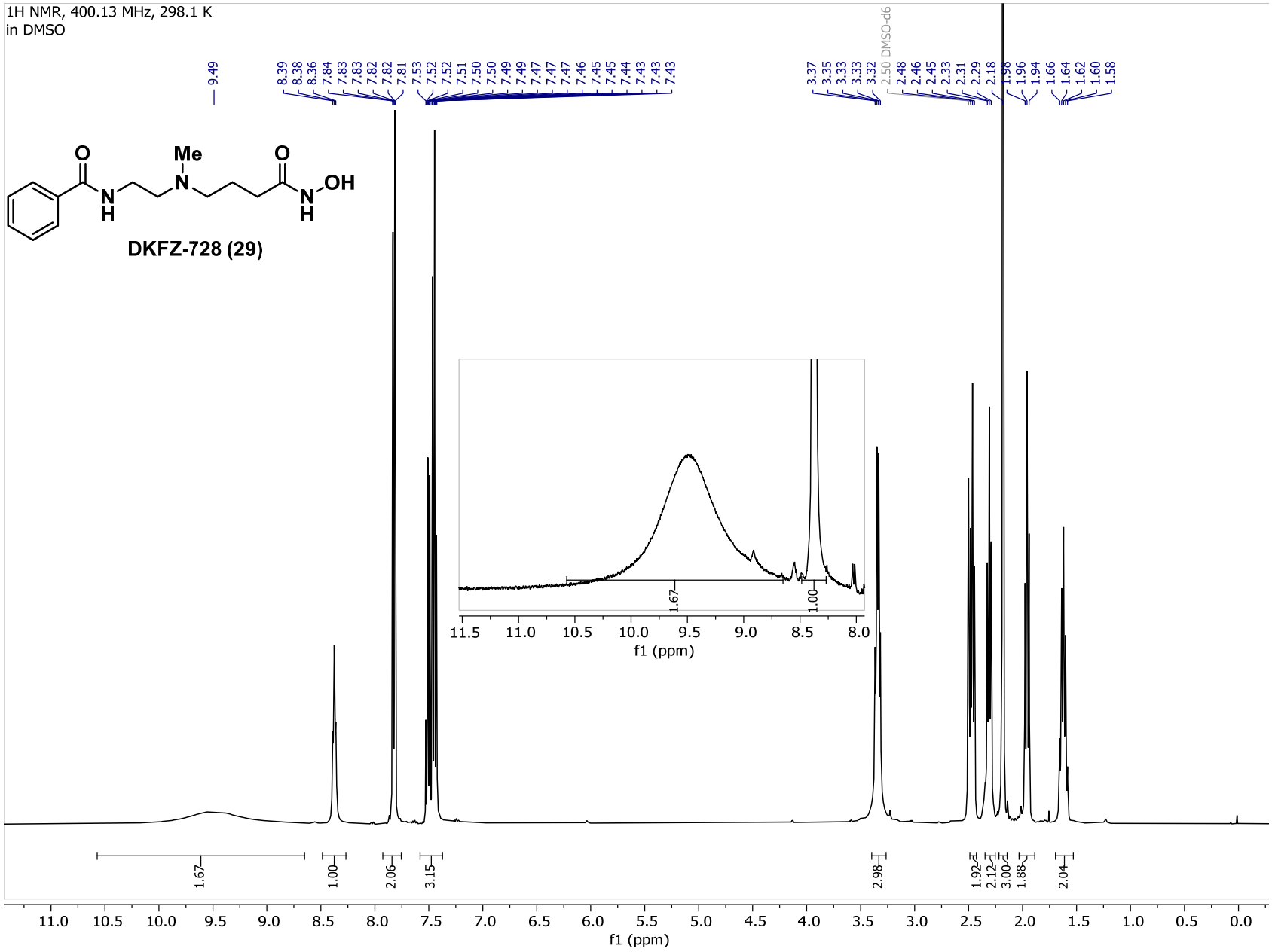

Basic Method PDA - Total Absorbance Chromatogram
O
Me
O
N
H
N
N
H
OH
27
Absorbance
25000
20000
15000
10000
5000
0
3.38
97.5%
4.07
2.5%
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
Basic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
Absorbance (mAU)
1500
1000
500
0
3.38
97.5%
4.06
2.5%
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5

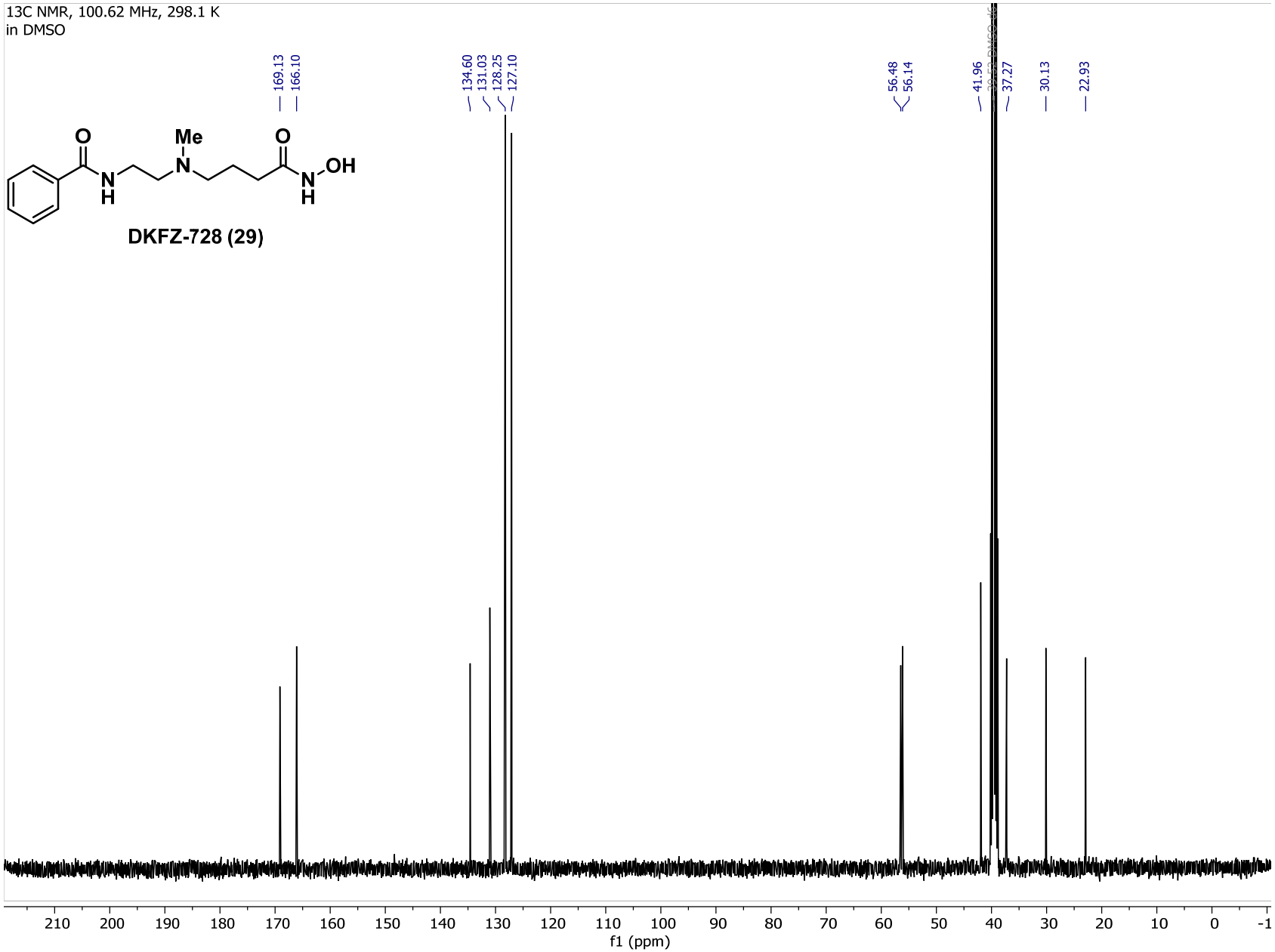

1H NMR, 600.14 MHz, 298.0 K
in DMSO
9.87
8.93
7.61
7.59
7.59
7.58
7.58
7.30
7.28
7.28
7.27
7.27
7.26
7.26
7.25
7.25
7.25
7.23
7.02
7.02
7.02
7.01
7.01
7.00
7.00
6.99
6.99
2.50 DMSO-d6
2.32
2.31
2.30
2.28
2.27
2.25
2.24
2.23
2.12
2.11
1.97
1.96
1.95
1.72
1.71
1.70
1.69
1.67
1.63
1.62
1.61
1.60
1.58
O
Me
O
N
N
OH
H
H
28
1.08
2.46
2.02
2.04
1.01
4.44
1.92
3.01
1.81
2.04
2.00
f1 (ppm)

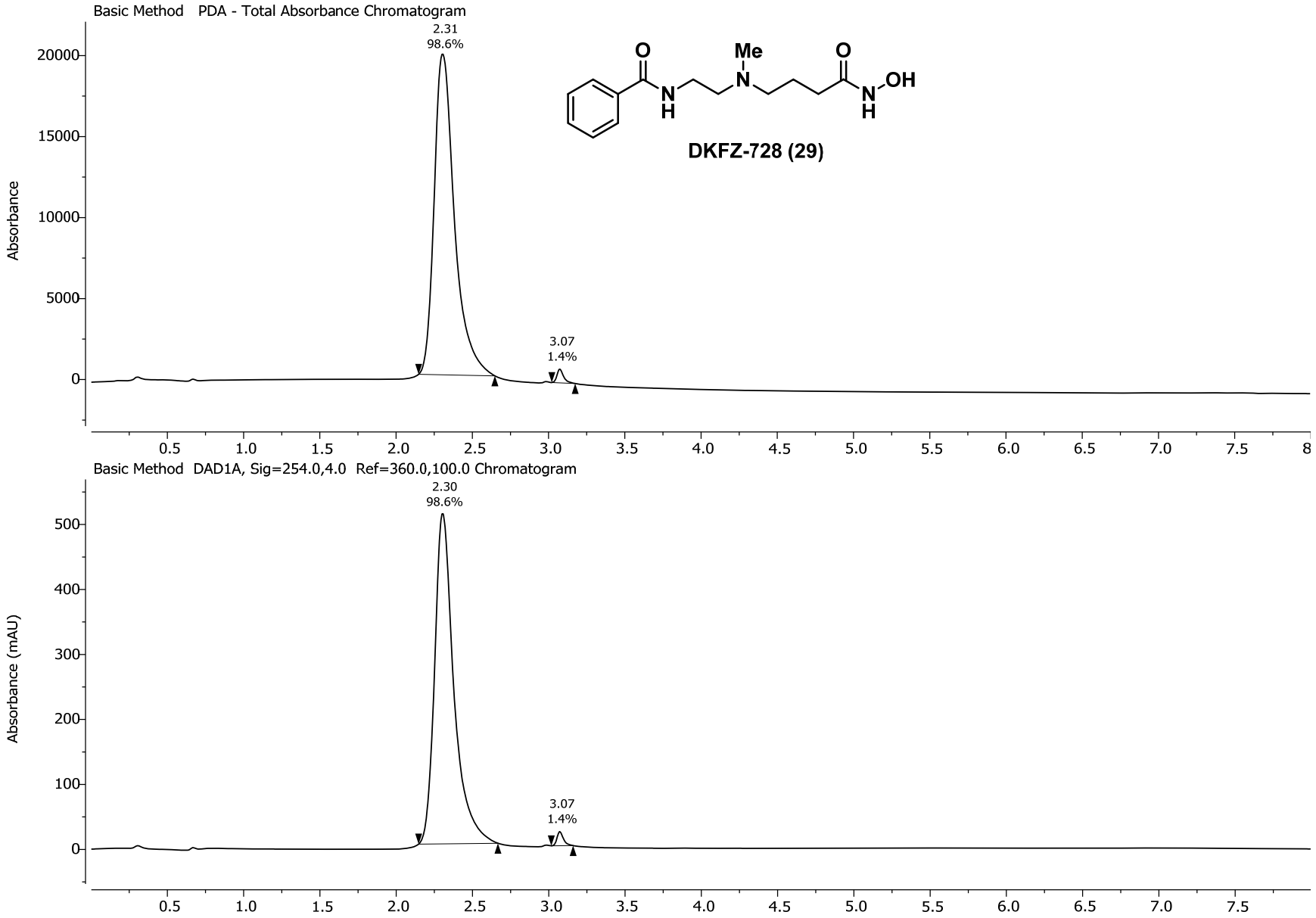

13C NMR, 150.92 MHz, 298.0 K
in DMSO
171.23
169.17
139.36
128.62
122.89
119.04
56.54
41.72
39.52 DMSO-d6
34.31
30.22
22.92
22.79
O
Me
O
N
H
N
N
H
OH
28
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-1
f1 (ppm)

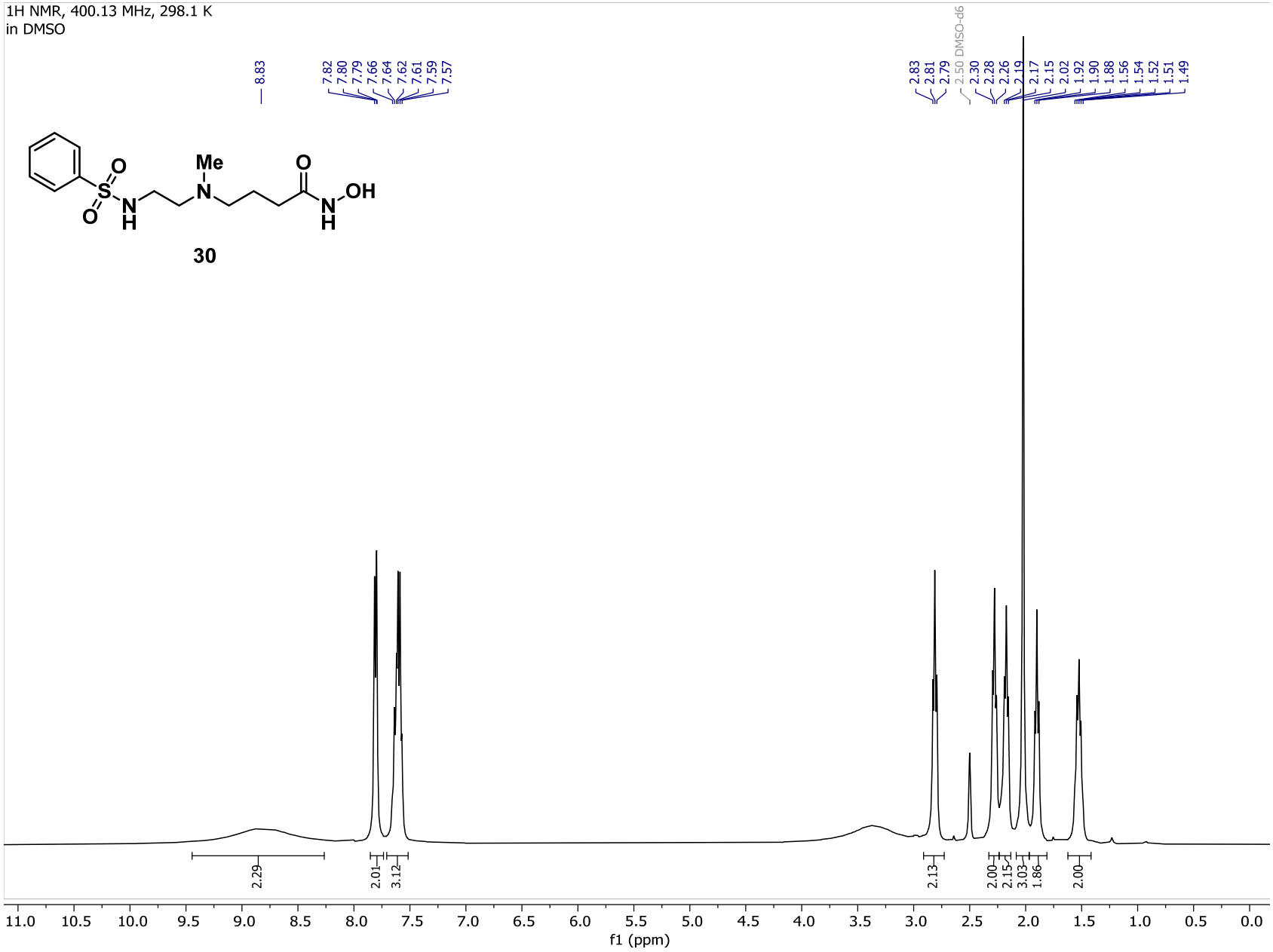

Basic Method PDA - Total Absorbance Chromatogram
Absorbance
15000
10000
5000
0
3.41
97.0%
2.96
0.5%
4.21
2.5%
0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5
Basic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
Absorbance (mAU)
1000
900
800
700
600
500
400
300
200
100
0
-100
3.41
96.8%
2.96
0.6%
4.20
2.7%
0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5

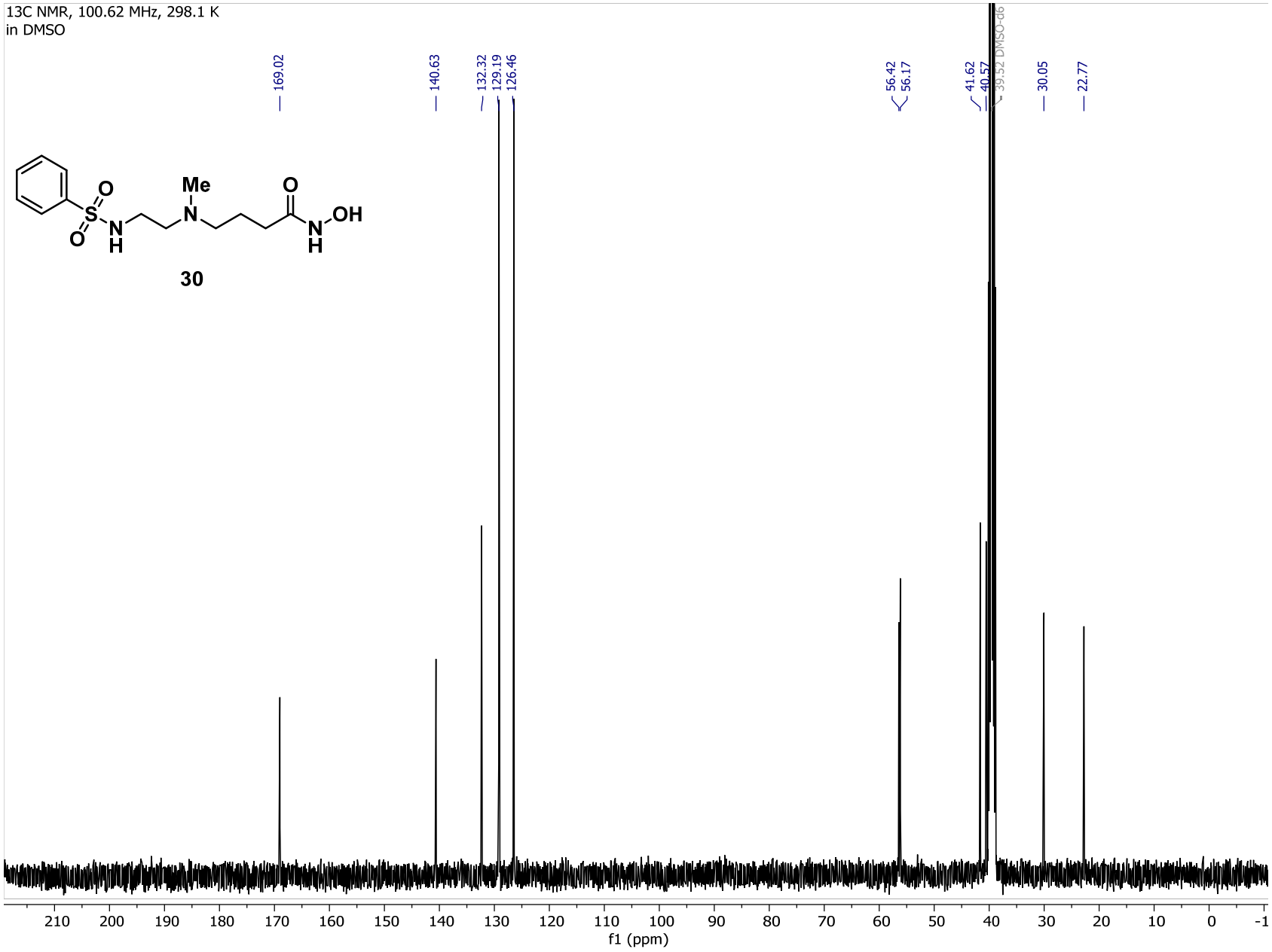
**28**

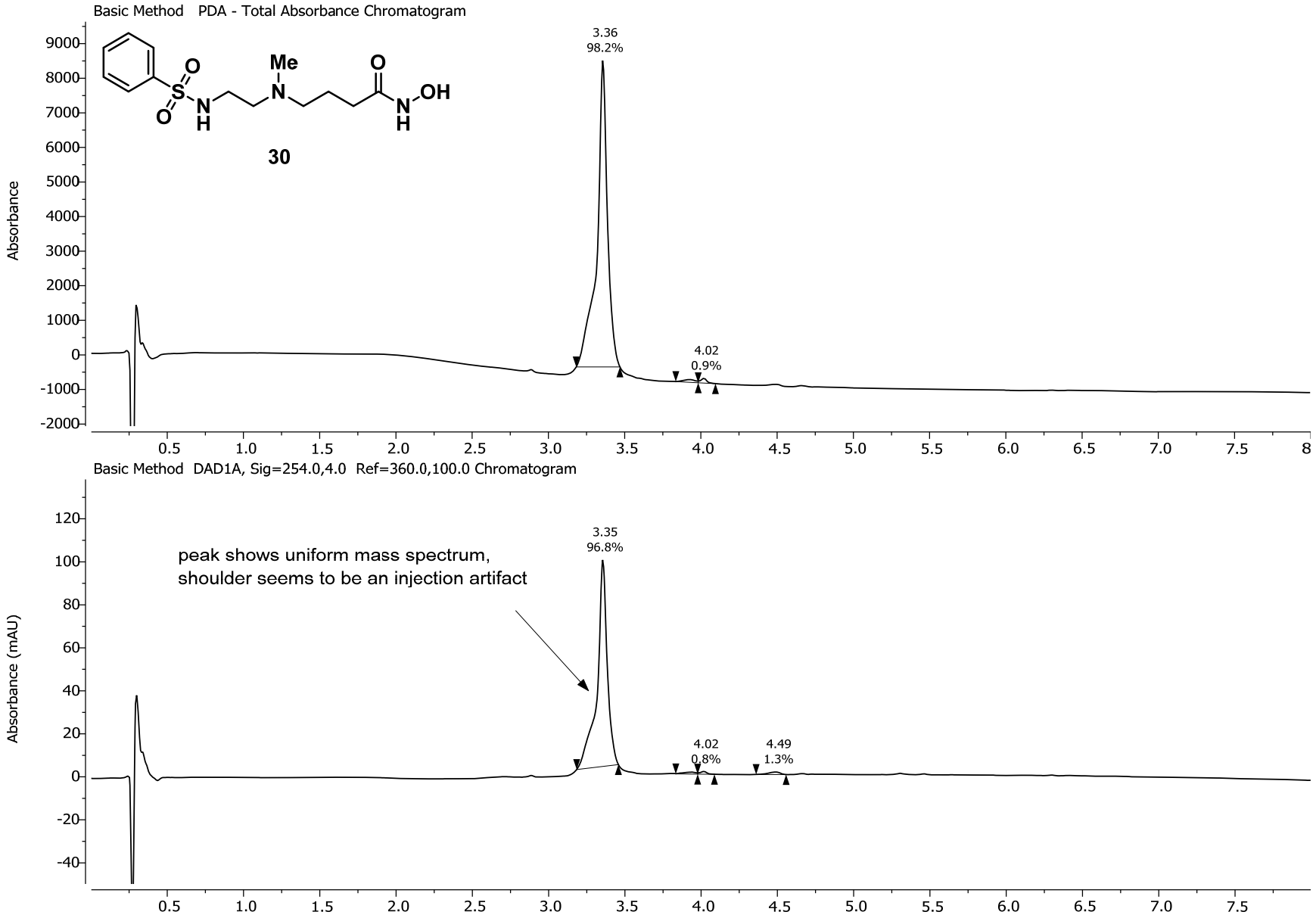

1H NMR, 400.13 MHz, 298.1 K
in DMSO
DKFZ-728 (29)
f1 (ppm)

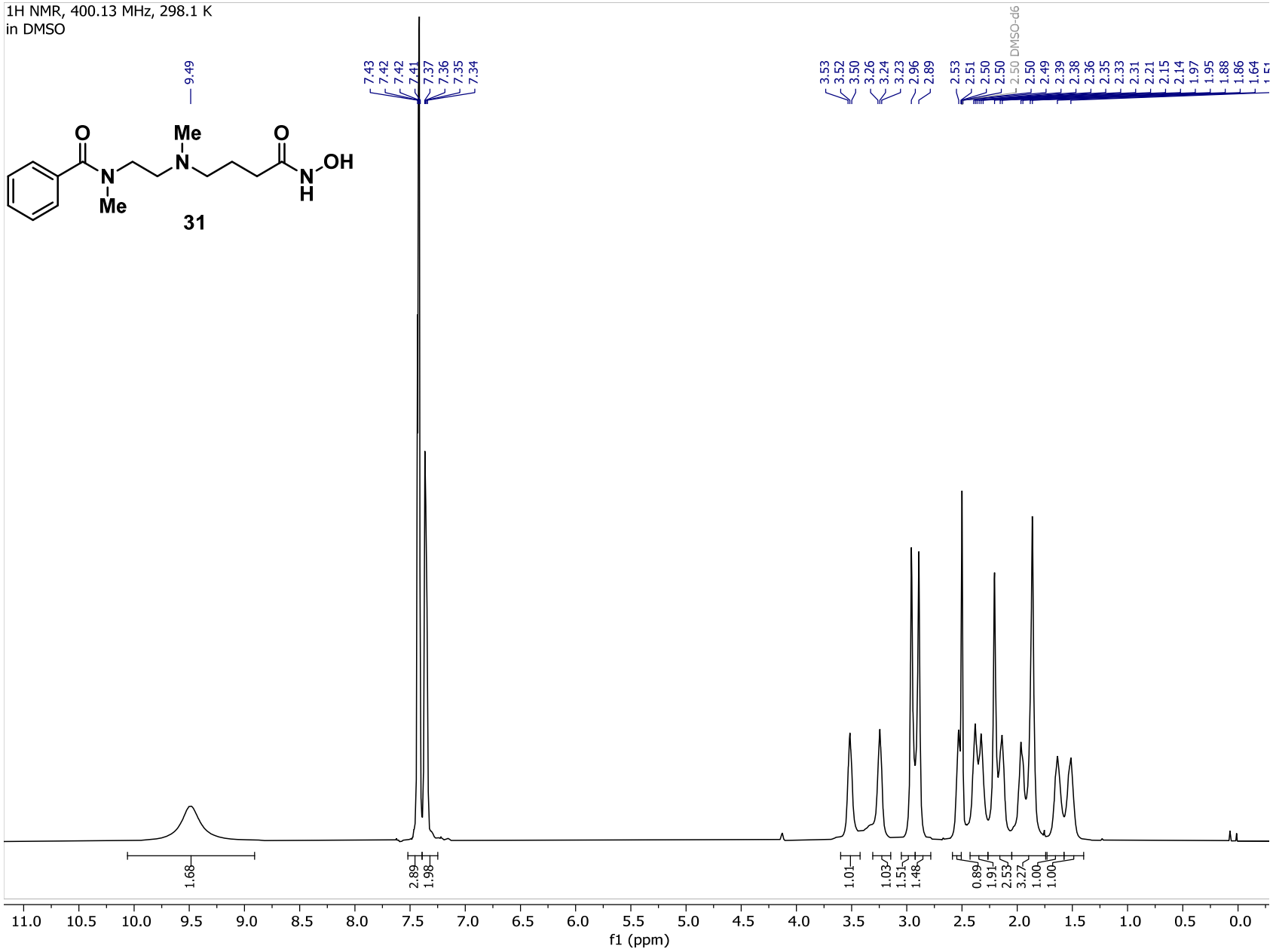

13C NMR, 100.62 MHz, 298.1 K
in DMSO
169.13
166.10
134.60
131.03
128.25
127.10
56.48
56.14
41.96
37.27
30.13
22.93
O
Me
O
N
N
N
OH
H
H
DKFZ-728 (29)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -1
f1 (ppm)

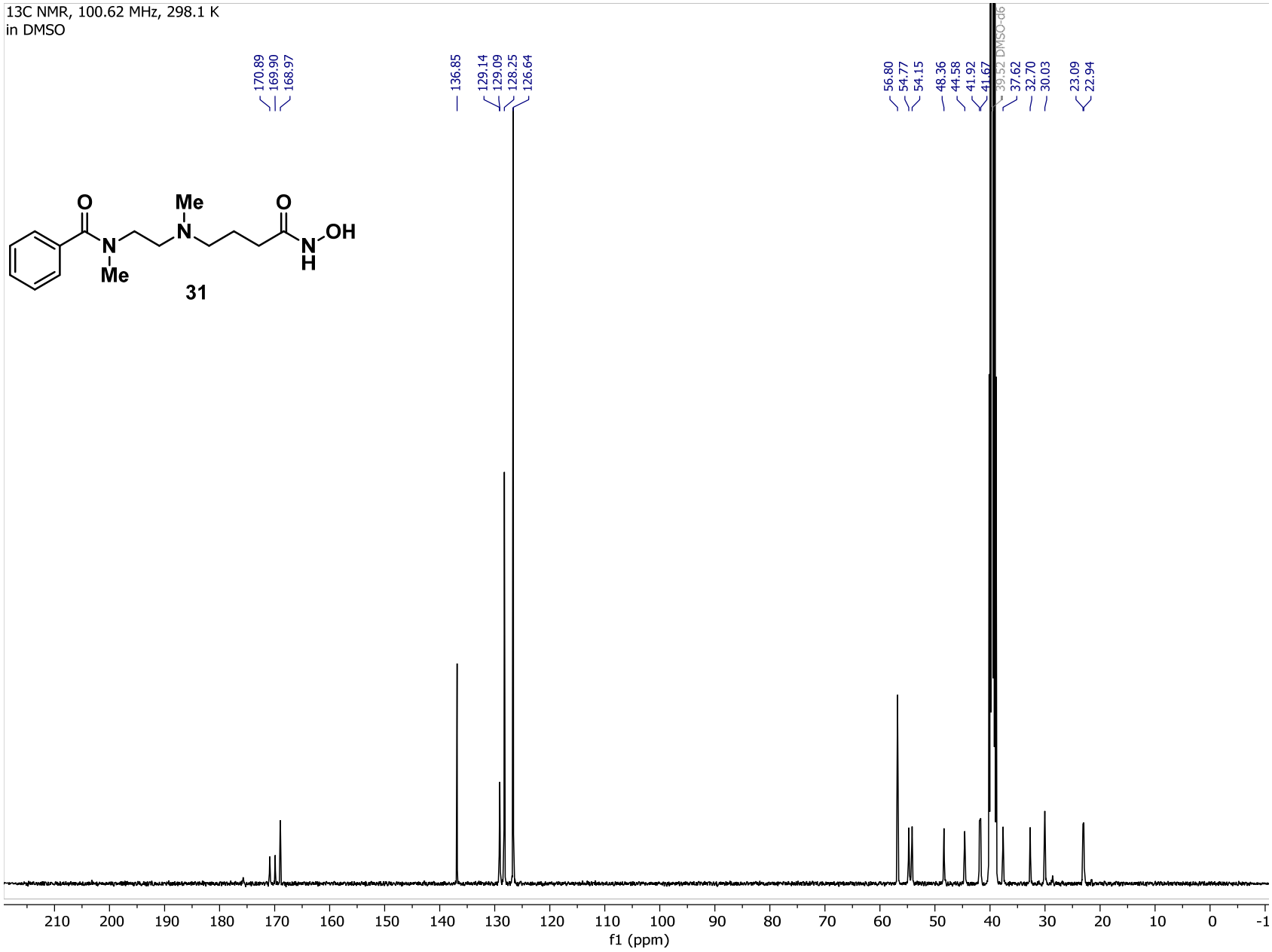

Basic Method PDA - Total Absorbance Chromatogram
2.31
98.6%
3.07
1.4%
Absorbance
20000
15000
10000
5000
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
O
Me
O
N
H
N
N
H
OH
DKFZ-728 (29)
Basic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
2.30
98.6%
3.07
1.4%
Absorbance (mAU)
500
400
300
200
100
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5

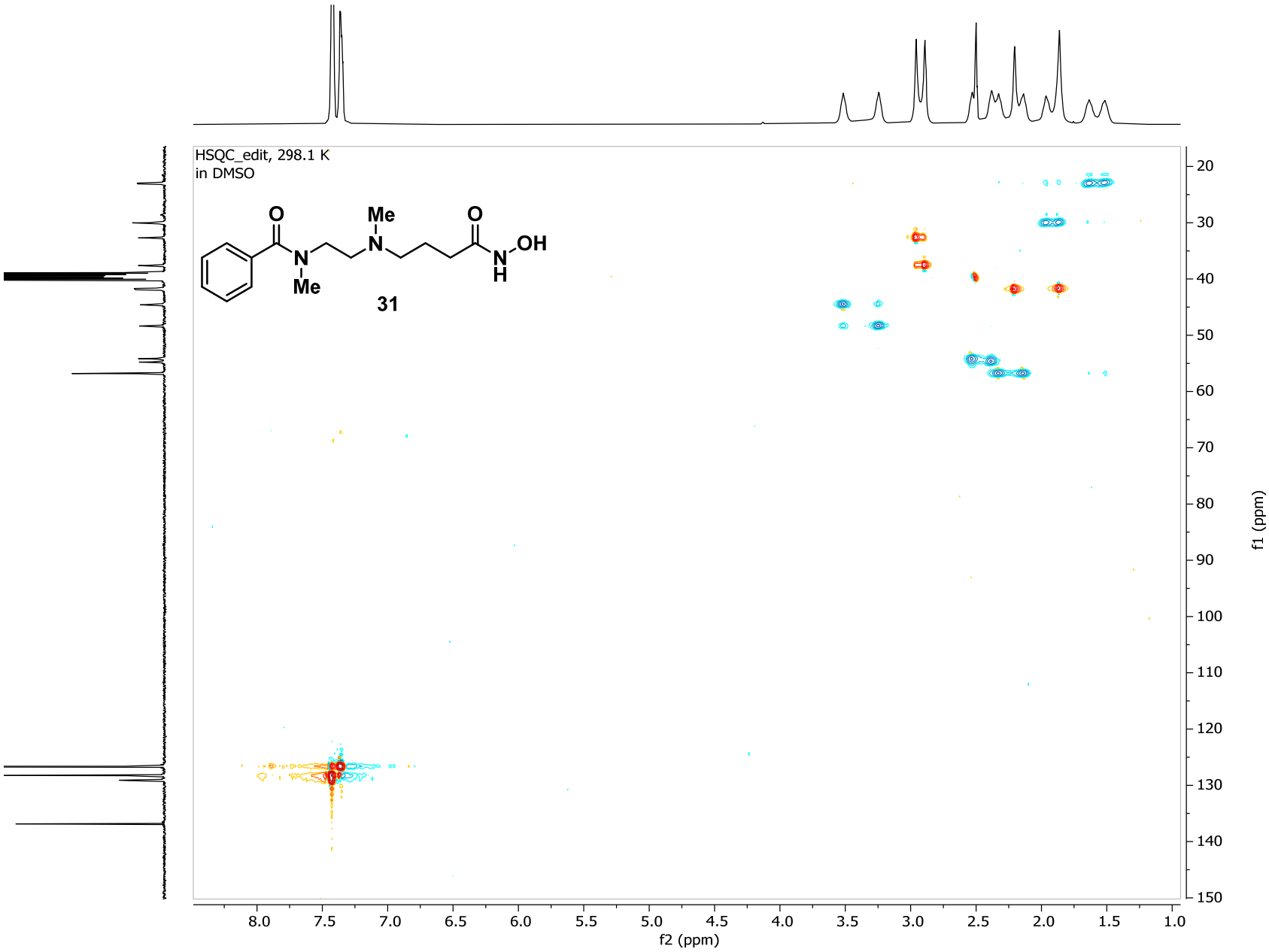

1H NMR, 400.13 MHz, 298.1 K
in DMSO
8.83
7.82
7.80
7.79
7.66
7.64
7.62
7.61
7.59
7.57
2.83
2.81
2.79
2.50 DMSO-d6
2.30
2.28
2.26
2.19
2.17
2.15
2.02
1.92
1.90
1.88
1.56
1.54
1.52
1.51
1.49
Me
O
S
O
O
N
H
N
O
N
H
OH
30
2.29
2.01
3.12
2.13
2.00
2.15
3.03
1.86
2.00
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

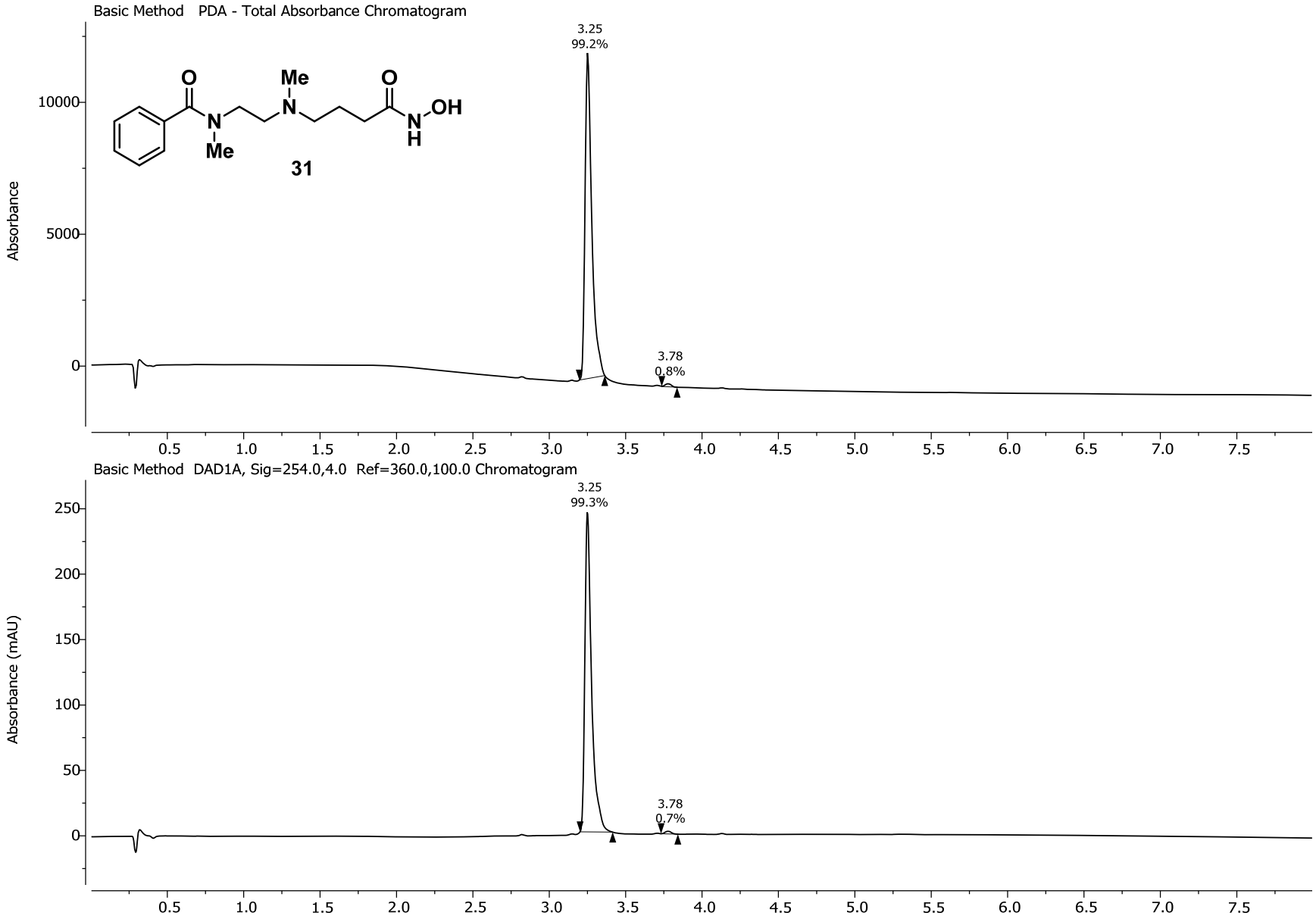

13C NMR, 100.62 MHz, 298.1 K
in DMSO
169.02
140.63
132.32
129.19
126.46
56.42
56.17
41.62
40.57
39.52 DMSO-d6
30.05
22.77
Me
O
O
S
O
N
H
N
O
N
H
OH
30
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-1
f1 (ppm)

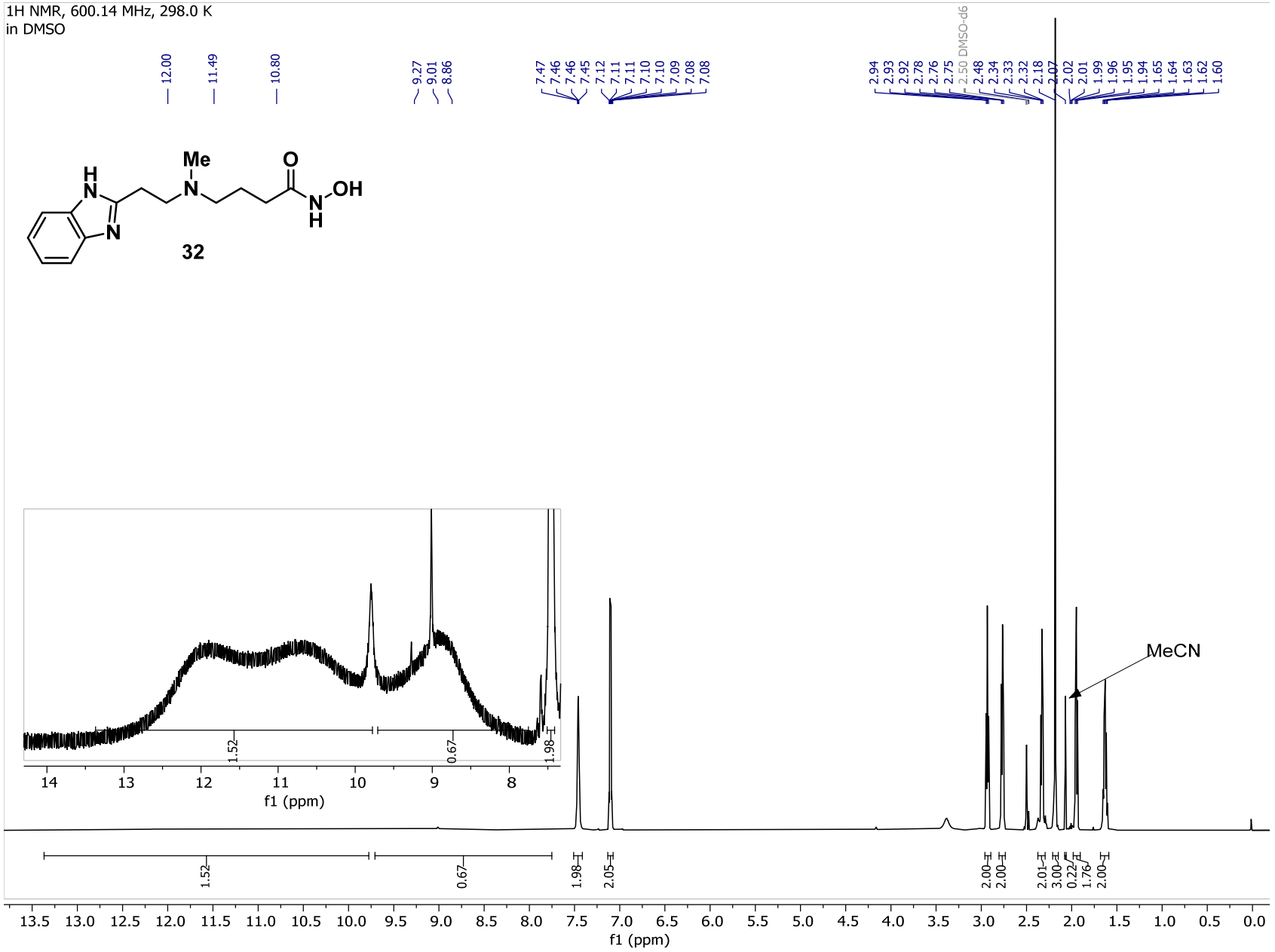

Basic Method PDA - Total Absorbance Chromatogram
Absorbance
3.36
98.2%
4.02
0.9%
30
Basic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
Absorbance (mAU)
peak shows uniform mass spectrum,
shoulder seems to be an injection artifact
3.35
96.8%
4.02
0.8%
4.49
1.3%

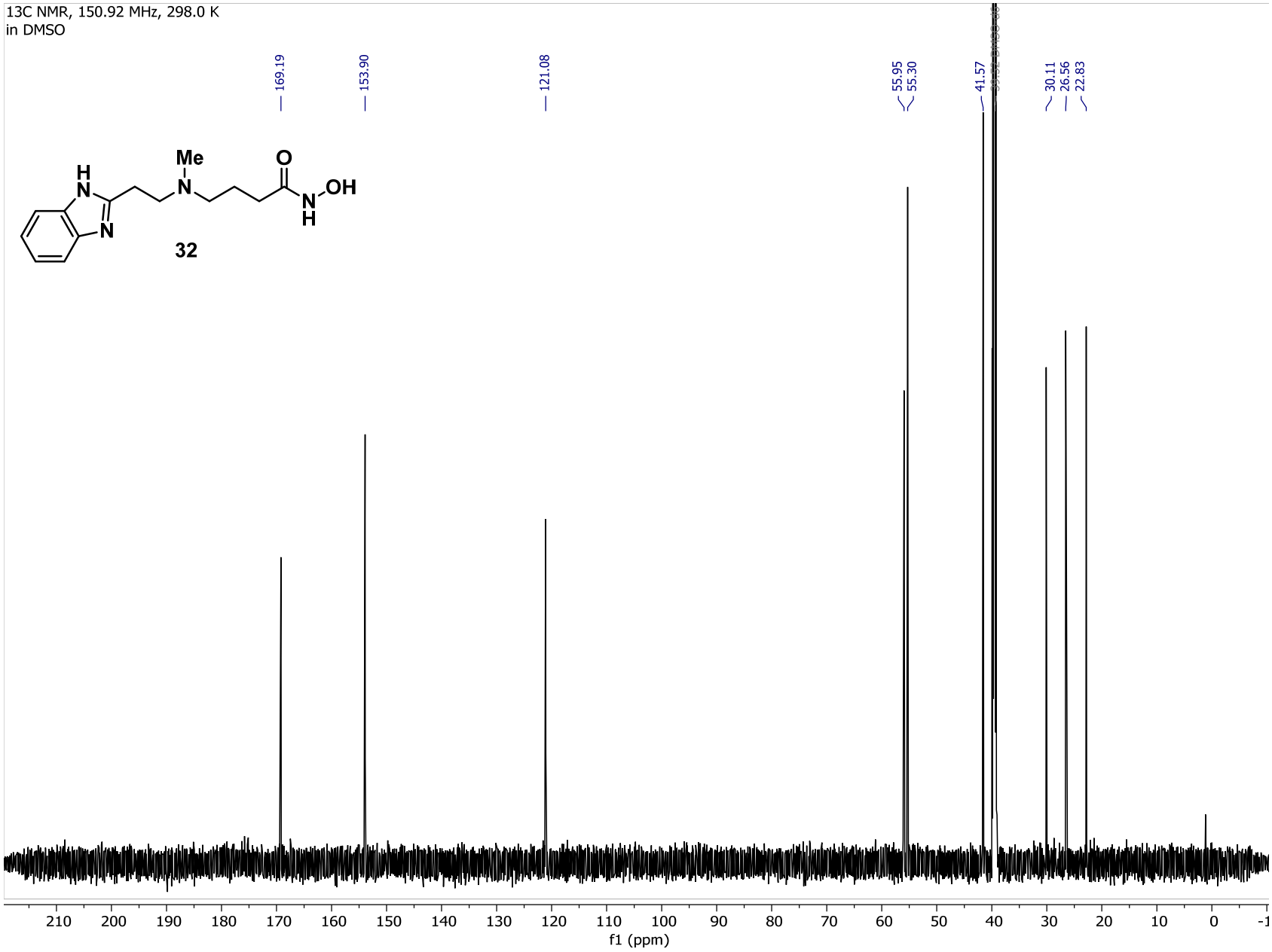

1H NMR, 400.13 MHz, 298.1 K
in DMSO
9.49
7.43
7.42
7.42
7.41
7.37
7.36
7.35
7.34
3.53
3.52
3.50
3.26
3.24
3.23
2.96
2.89
2.53
2.51
2.50
2.50
2.50 DMSO-d6
2.50
2.49
2.39
2.38
2.36
2.35
2.33
2.31
2.21
2.15
2.14
1.97
1.95
1.88
1.86
1.64
O
Me
O
N
N
N
OH
H
Me
31
1.68
2.89
1.98
1.01
1.03
1.51
1.48
0.89
1.91
2.53
3.27
1.00
1.00
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

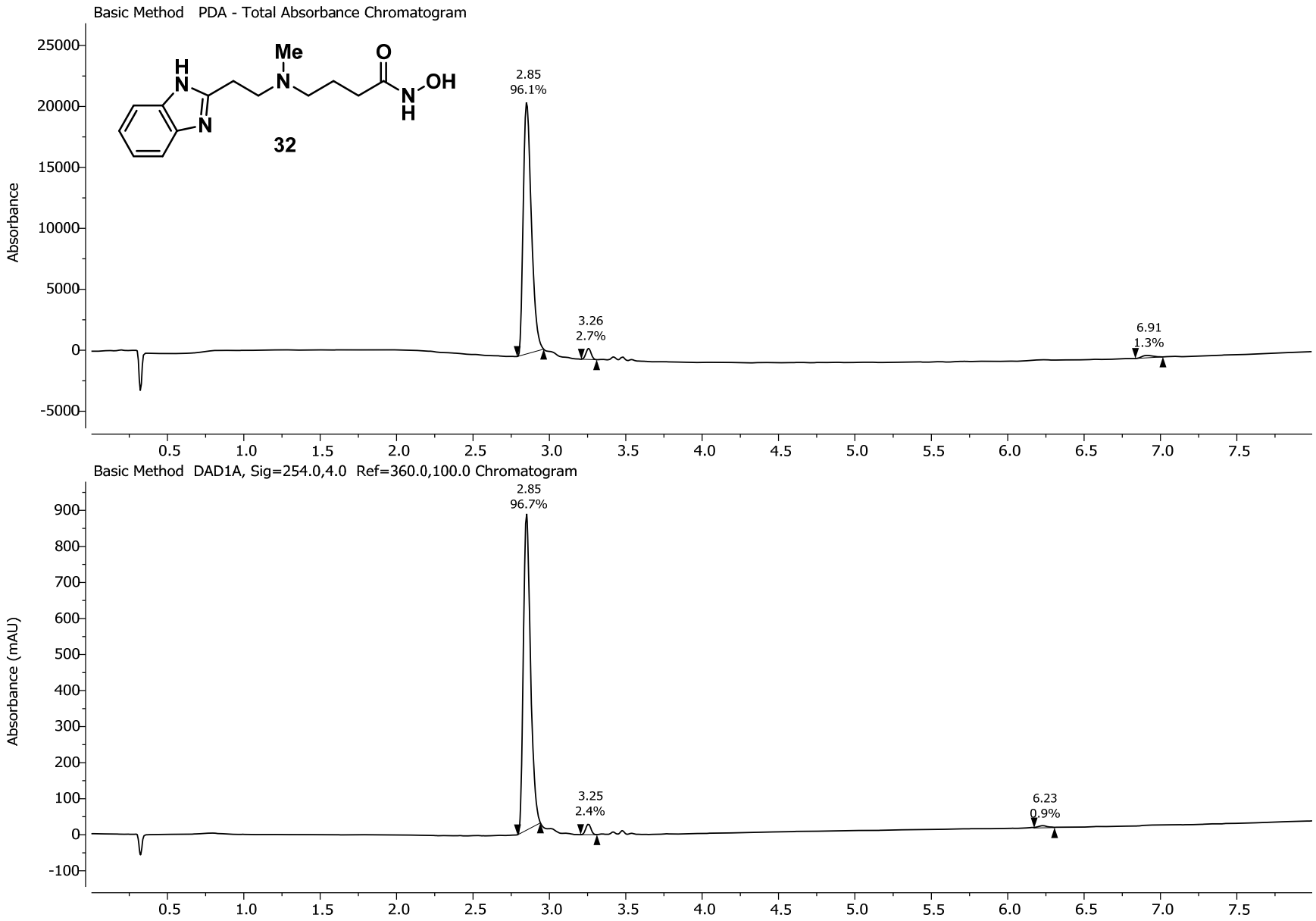

13C NMR, 100.62 MHz, 298.1 K
in DMSO
170.89
169.90
168.97
136.85
129.14
129.09
128.25
126.64
56.80
54.77
54.15
48.36
44.58
41.92
41.67
39.52 DMSO-d6
37.62
32.70
30.03
23.09
22.94
O
Me
O
N
N
N
OH
H
Me
31
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -1
f1 (ppm)

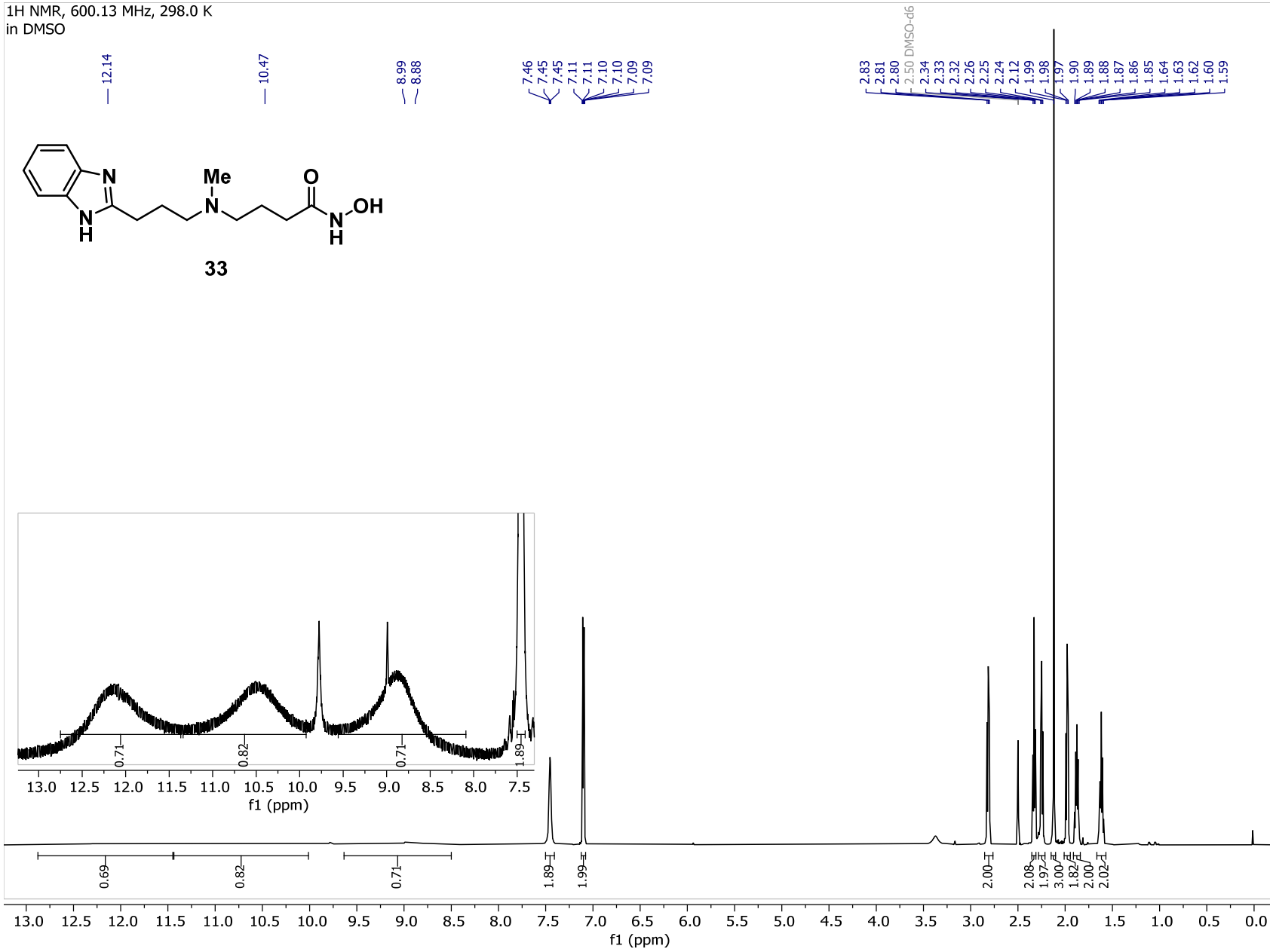

HSQC_edit, 298.1 K
in DMSO
O
Me
O
N
N
N
OH
Me
H
31
f2 (ppm)
f1 (ppm)

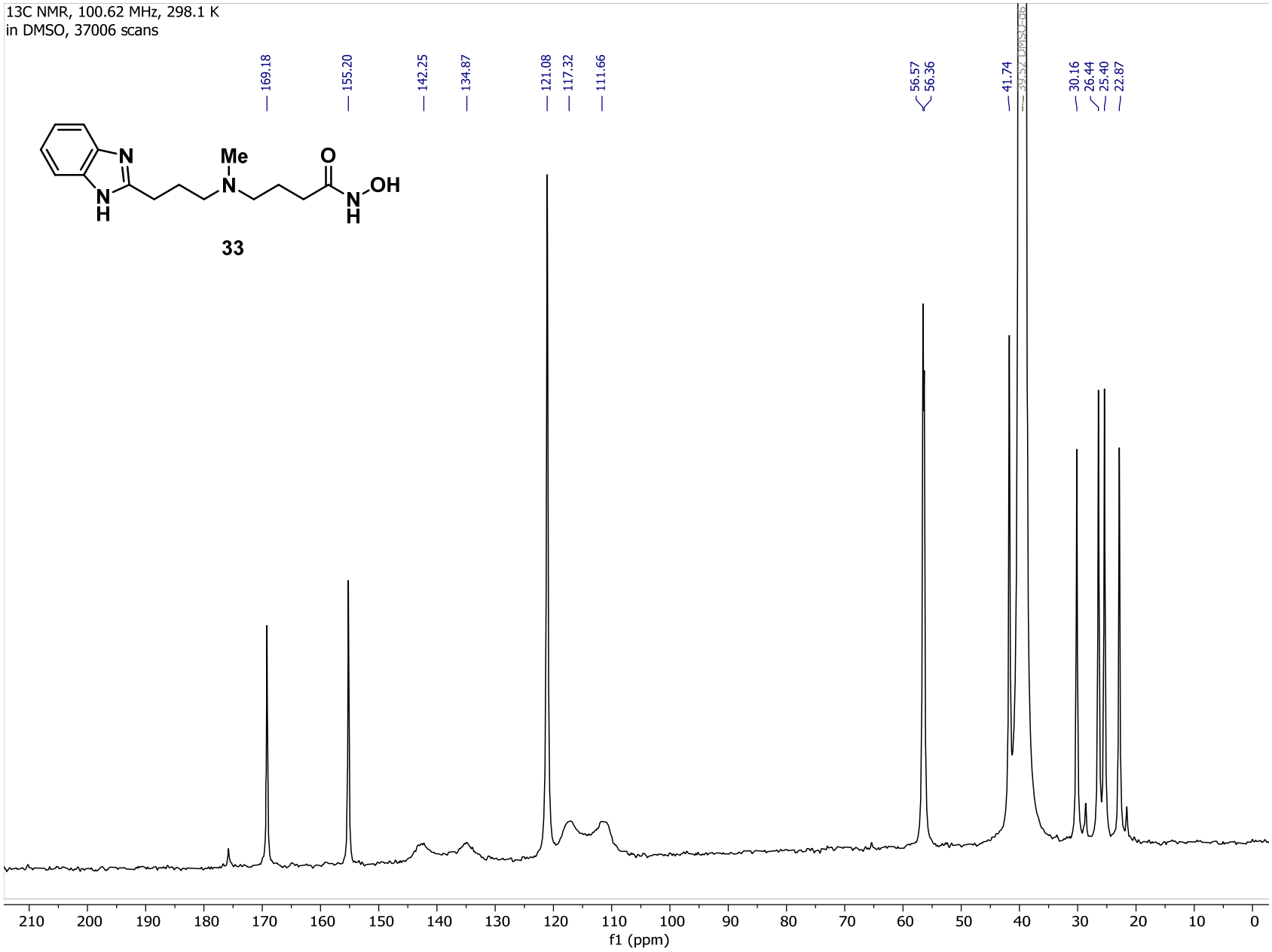

Basic Method PDA - Total Absorbance Chromatogram
3.25
99.2%
3.78
0.8%
Absorbance
10000
5000
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
O
Me
O
N
N
N
OH
H
Me
31
Basic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
3.25
99.3%
3.78
0.7%
Absorbance (mAU)
250
200
150
100
50
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5

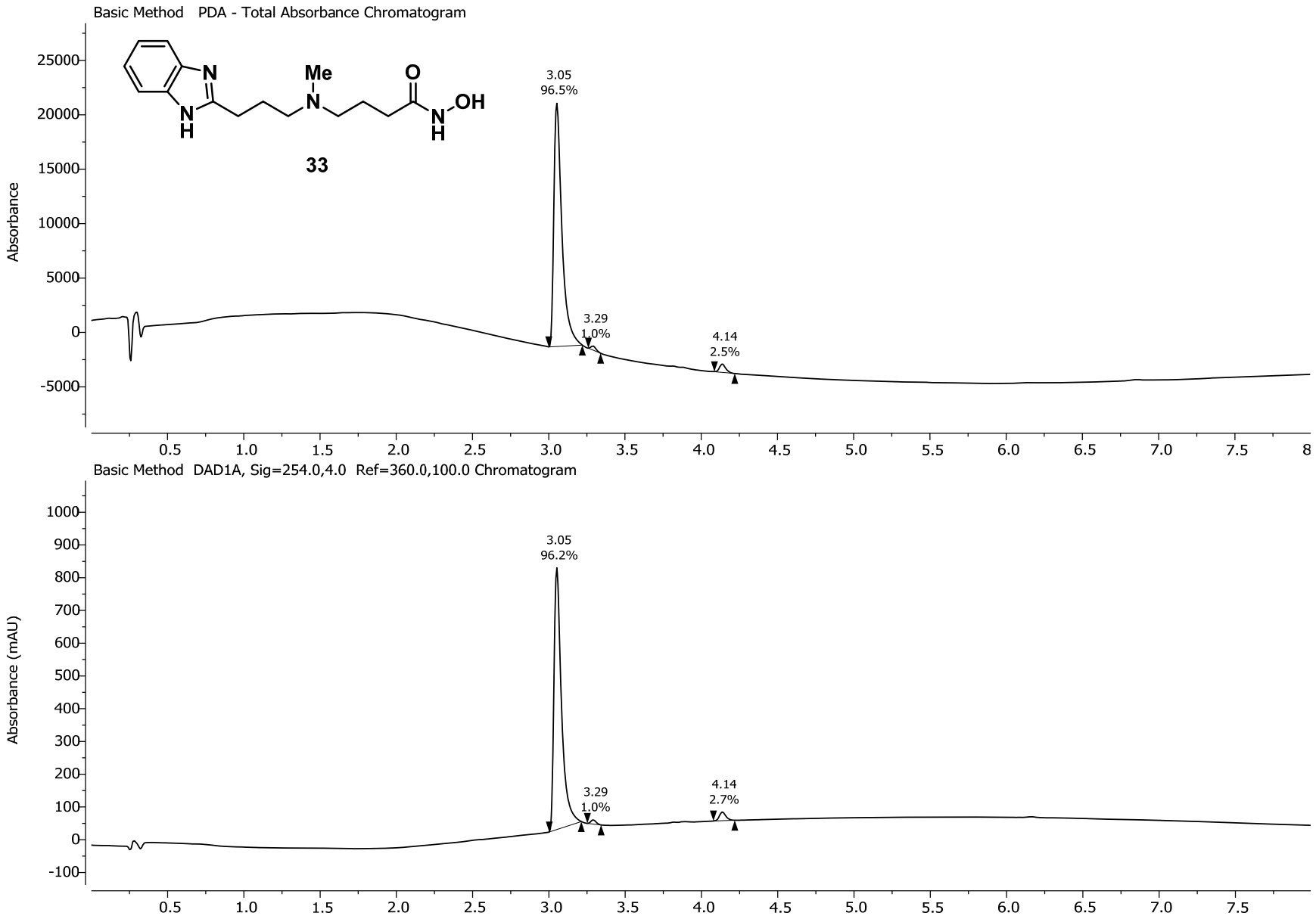

1H NMR, 600.14 MHz, 298.0 K
in DMSO
12.00
11.49
10.80
9.27
9.01
8.86
7.47
7.46
7.46
7.45
7.12
7.11
7.11
7.10
7.10
7.09
7.08
7.08
2.94
2.93
2.92
2.78
2.76
2.75
2.50 DMSO-d6
2.48
2.34
2.33
2.32
2.18
2.07
2.02
2.01
1.99
1.96
1.95
1.94
1.65
1.64
1.63
1.62
1.60
Me
O
H
N
N
N
OH
N
H
32
MeCN
1.52
0.67
1.98
f1 (ppm)
2.05
2.00
2.00
2.01
3.00
0.22
1.76
2.00
f1 (ppm)

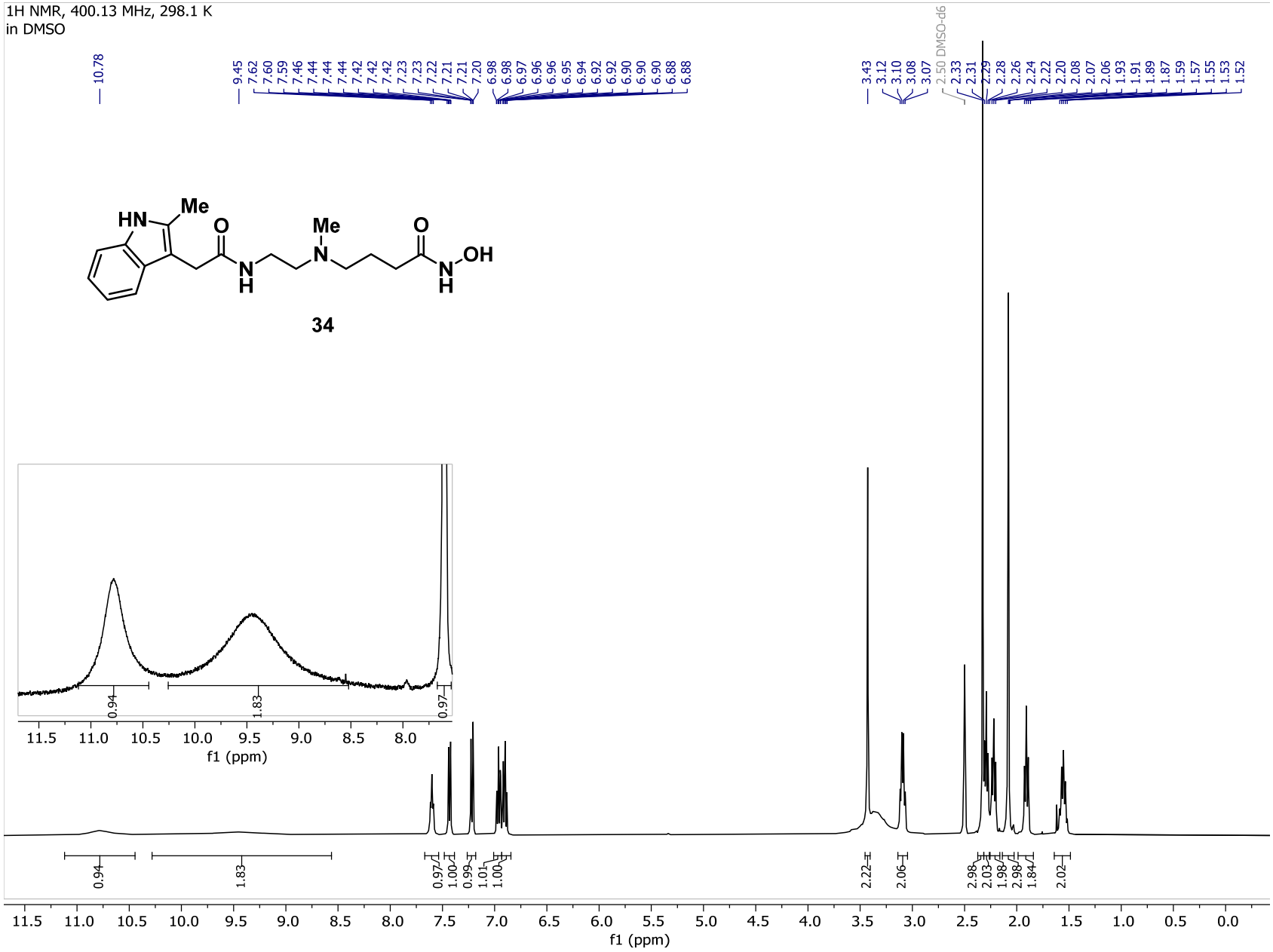

13C NMR, 150.92 MHz, 298.0 K
in DMSO
169.19
153.90
121.08
55.95
55.30
41.57
30.11
26.56
22.83
Me
O
H
N
N
N
N
OH
H
32
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-1
f1 (ppm)

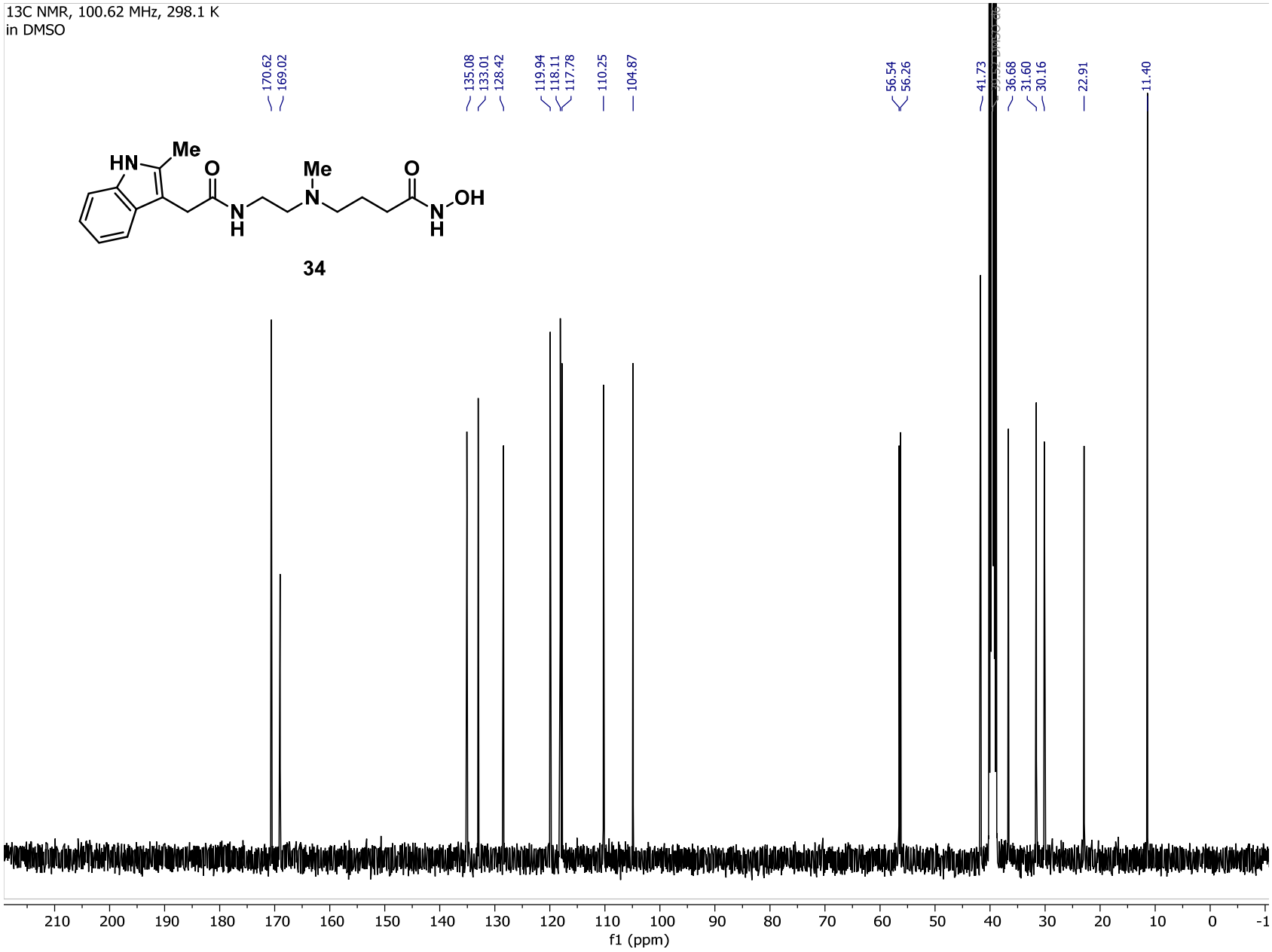

Basic Method PDA - Total Absorbance Chromatogram
Absorbance
25000
20000
15000
10000
5000
0
-5000
Me
O
H
N
N
N
OH
N
H
32
2.85
96.1%
3.26
2.7%
6.91
1.3%
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
Basic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
Absorbance (mAU)
900
800
700
600
500
400
300
200
100
0
-100
2.85
96.7%
3.25
2.4%
6.23
0.9%
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5

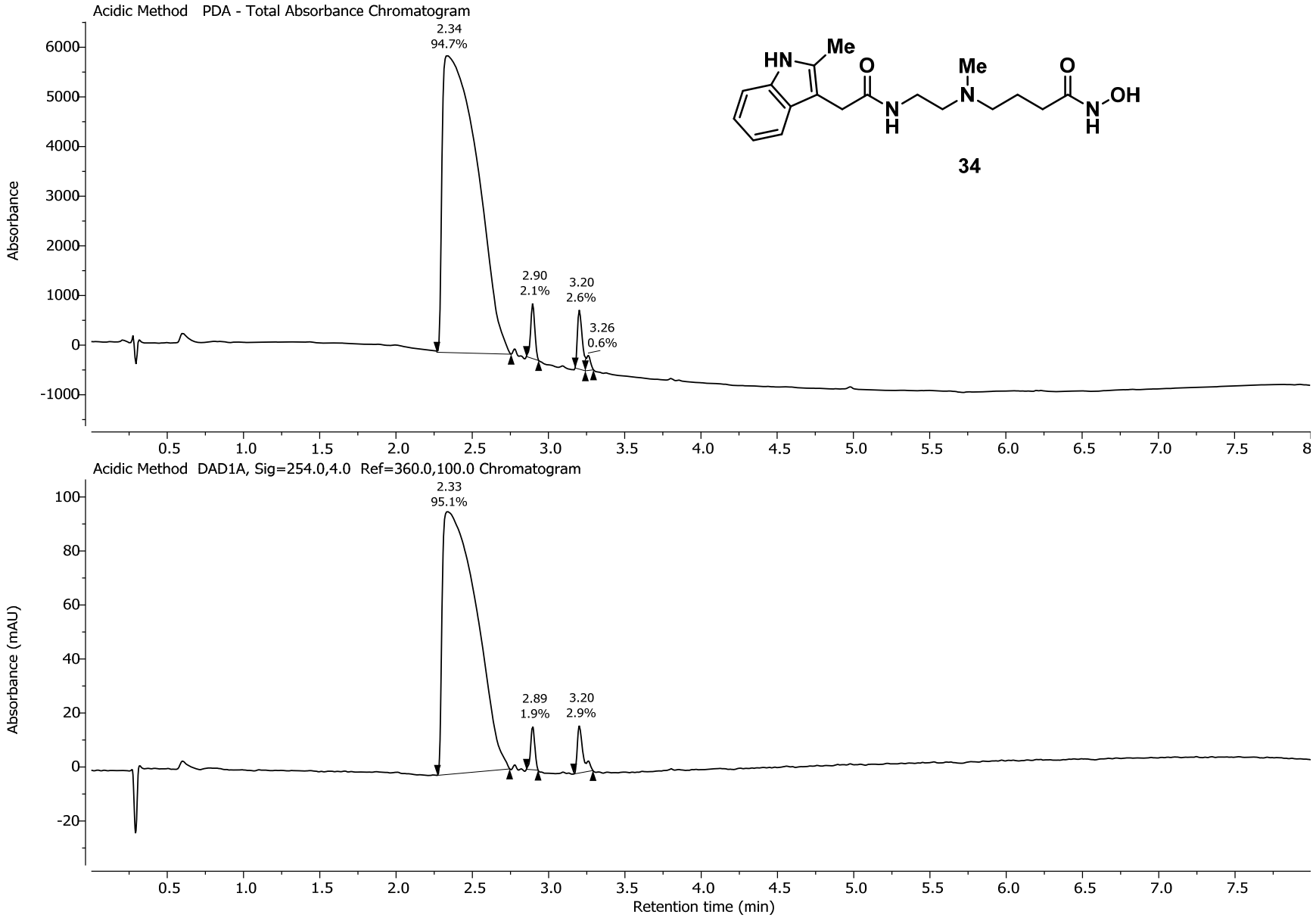

1H NMR, 600.13 MHz, 298.0 K
in DMSO
12.14
10.47
8.99
8.88
7.46
7.45
7.45
7.11
7.11
7.10
7.10
7.09
7.09
2.83
2.81
2.80
2.50 DMSO-d6
2.34
2.33
2.32
2.26
2.25
2.24
2.12
1.99
1.98
1.97
1.90
1.89
1.88
1.87
1.86
1.85
1.64
1.63
1.62
1.60
1.59
N
Me
O
N
N
OH
H
H
33
0.71
0.82
0.71
1.89
13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5
f1 (ppm)
0.69
0.82
0.71
1.89
1.99
2.00
2.08
1.97
3.00
1.82
2.00
2.02
13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

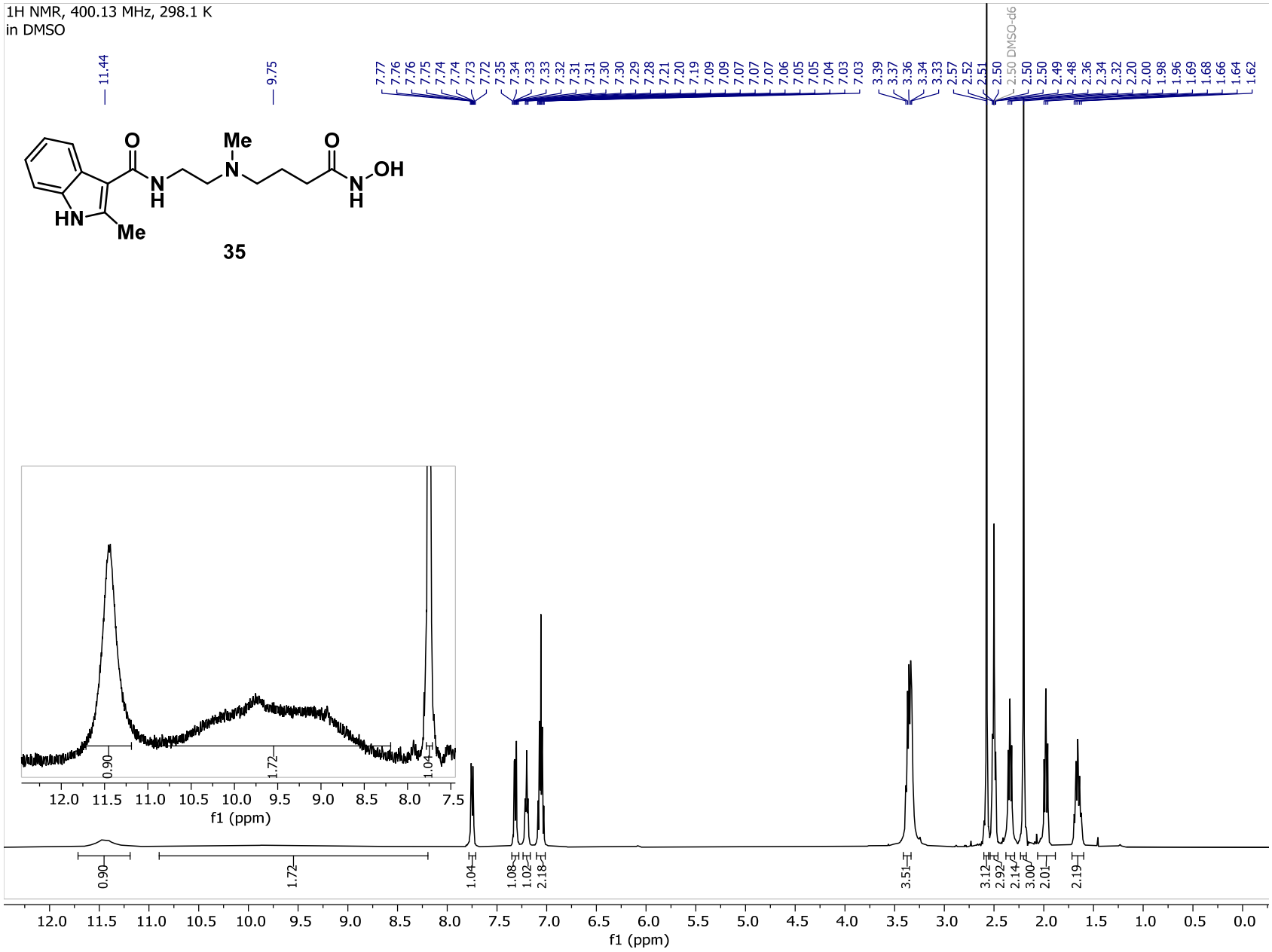

13C NMR, 100.62 MHz, 298.1 K
in DMSO, 37006 scans
169.18
155.20
142.25
134.87
121.08
117.32
111.66
56.57
56.36
41.74
39.52 DMSO-d6
30.16
26.44
25.40
22.87
N
Me
O
N
OH
N
H
N
H
33
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

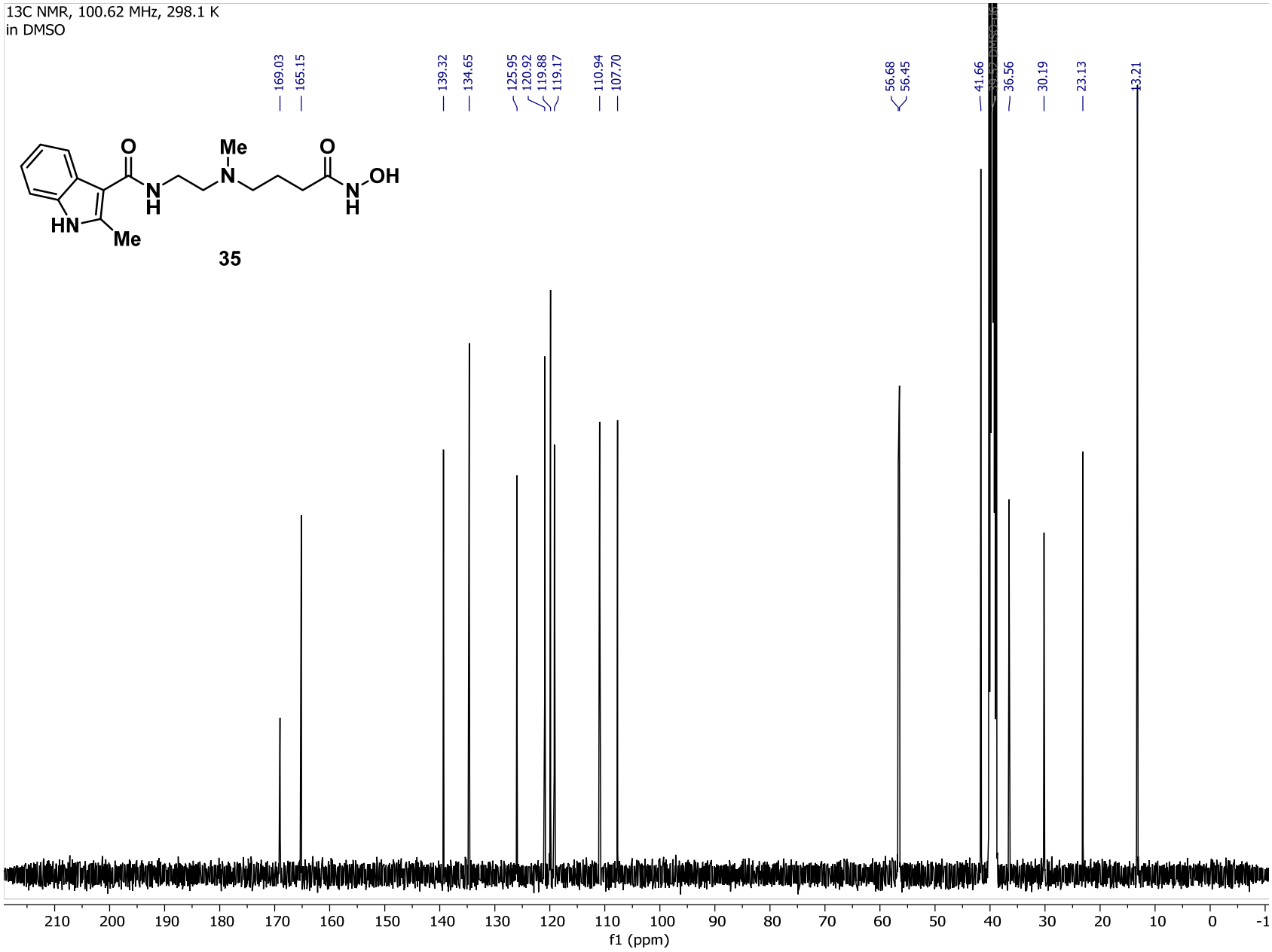

Basic Method PDA - Total Absorbance Chromatogram
Absorbance
25000
20000
15000
10000
5000
0
-5000
N
H
N
Me
N
O
N
H
OH
33
3.05
96.5%
3.29
1.0%
4.14
2.5%
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
Basic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
Absorbance (mAU)
1000
900
800
700
600
500
400
300
200
100
0
-100
3.05
96.2%
3.29
1.0%
4.14
2.7%
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5

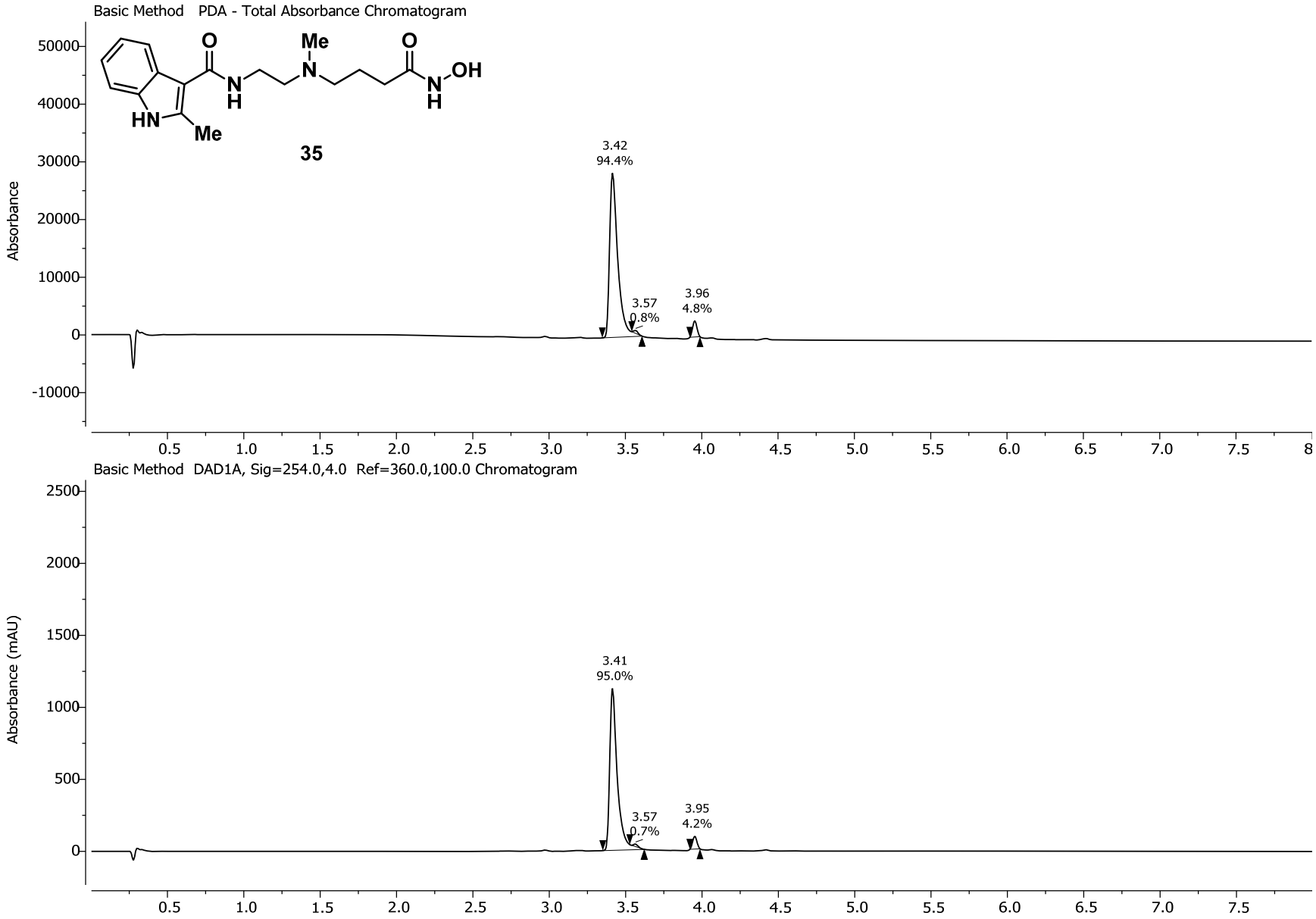

1H NMR, 400.13 MHz, 298.1 K
in DMSO
10.78
9.45
7.62
7.60
7.59
7.46
7.44
7.44
7.44
7.42
7.42
7.42
7.23
7.23
7.22
7.21
7.21
7.20
6.98
6.98
6.97
6.96
6.96
6.95
6.94
6.92
6.92
6.90
6.90
6.90
6.88
6.88
3.43
3.12
3.10
3.08
3.07
2.50 DMSO-d6
2.33
2.31
2.29
2.28
2.26
2.24
2.22
2.20
2.08
2.07
2.06
1.93
1.91
1.89
1.87
1.59
1.57
1.55
1.53
1.52
HN
Me
O
Me
N
O
N
H
N
H
OH
34
0.94
1.83
0.97
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0
f1 (ppm)
0.94
1.83
0.97
1.00
0.99
1.01
1.00
2.22
2.06
2.98
2.03
1.98
2.98
1.84
2.02
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


13C NMR, 100.62 MHz, 298.1 K
in DMSO
170.62
169.02
135.08
133.01
128.42
119.94
118.11
117.78
110.25
104.87
56.54
56.26
41.73
36.68
31.60
30.16
22.91
11.40
HN
Me
O
Me
N
O
N
H
N
H
OH
34
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-1
f1 (ppm)

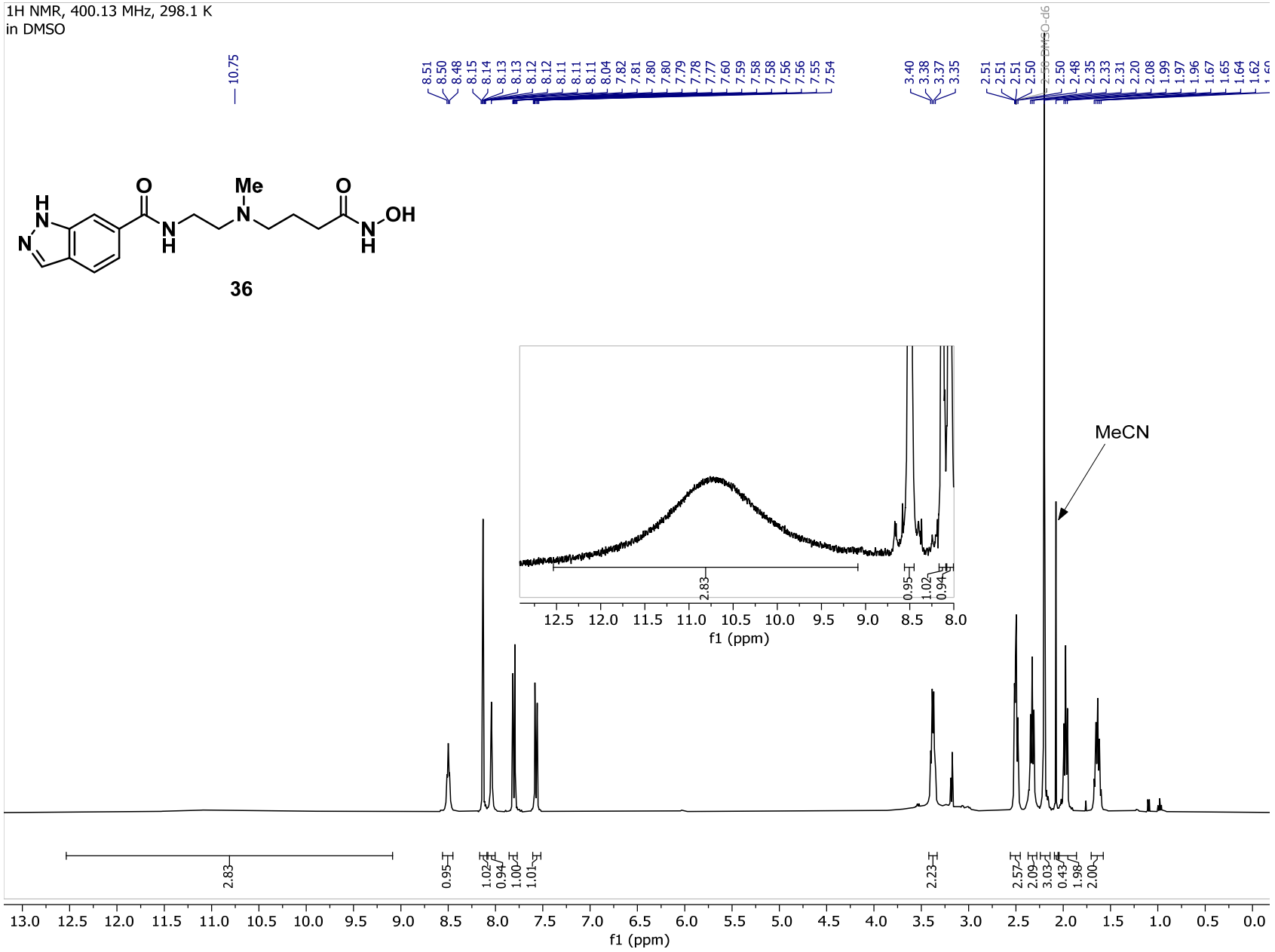

Acidic Method PDA - Total Absorbance Chromatogram
2.34
94.7%
2.90
2.1%
3.20
2.6%
3.26
0.6%
Absorbance
6000
5000
4000
3000
2000
1000
0
-1000
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8
HN
Me
O
Me
N
O
N
H
N
H
OH
34
Acidic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
2.33
95.1%
2.89
1.9%
3.20
2.9%
Absorbance (mAU)
100
80
60
40
20
0
-20
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
Retention time (min)

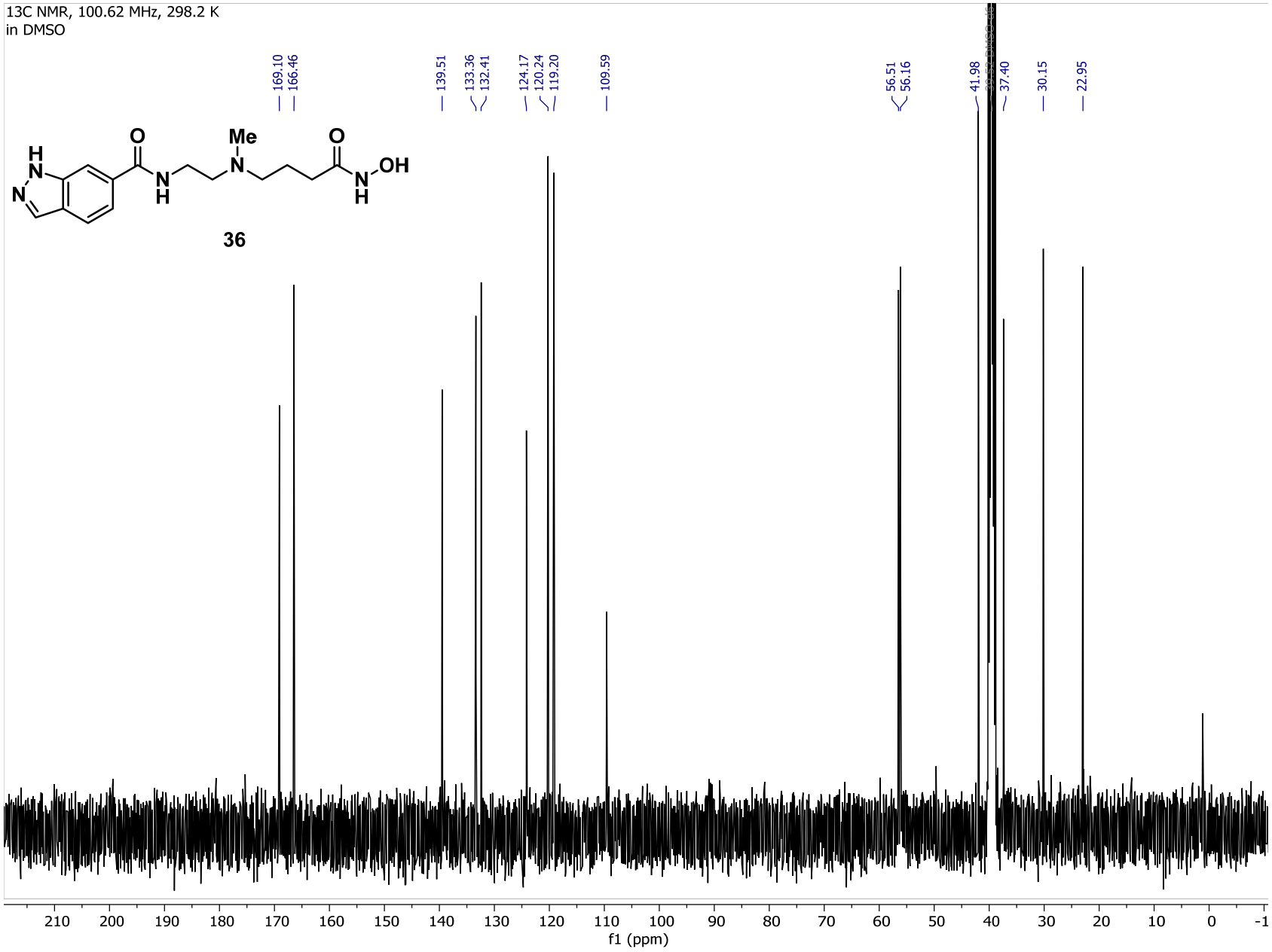

1H NMR, 400.13 MHz, 298.1 K
in DMSO
11.44
9.75
2.50 DMSO-d6
35
f1 (ppm)

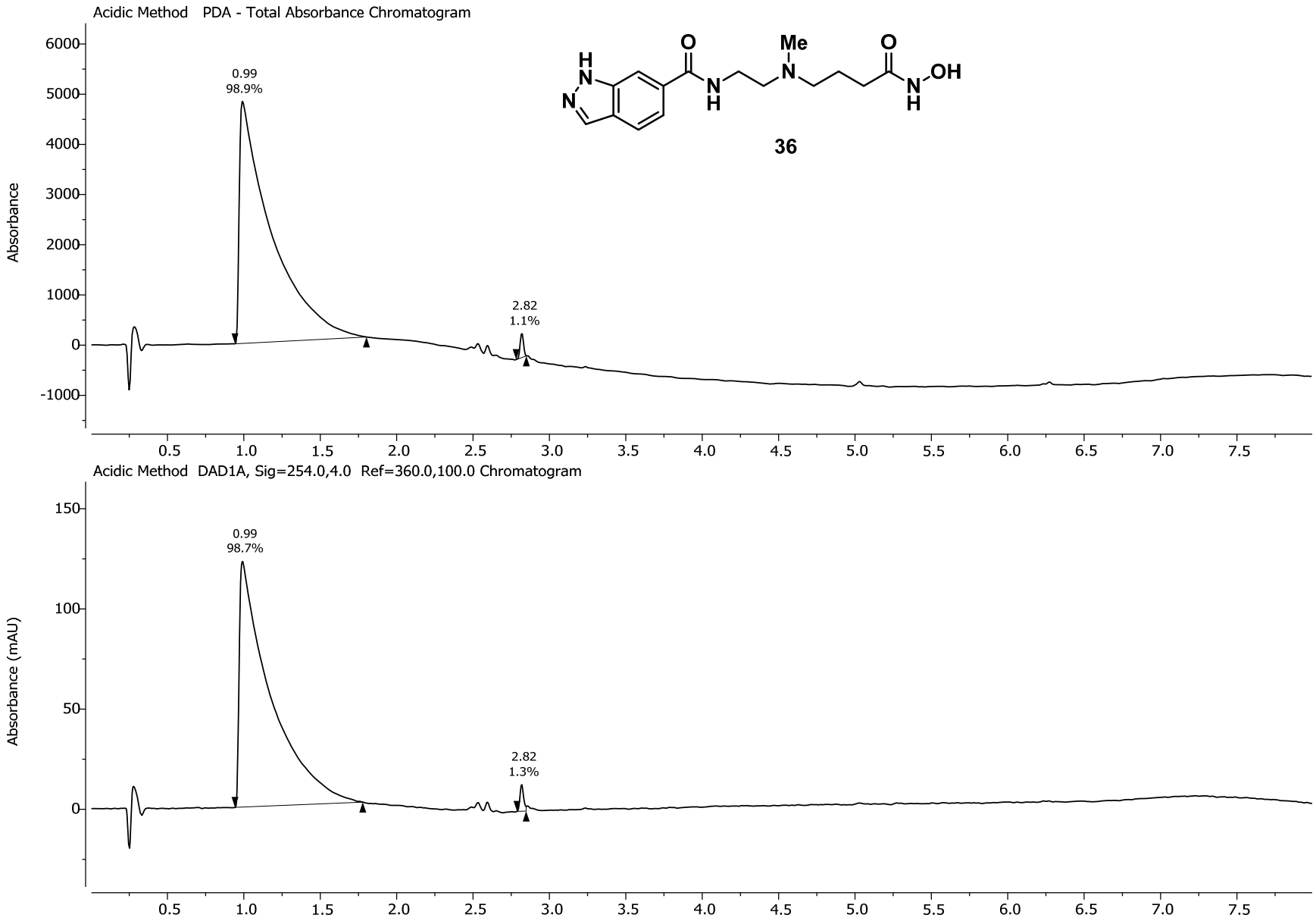

13C NMR, 100.62 MHz, 298.1 K
in DMSO
169.03
165.15
139.32
134.65
125.95
120.92
119.88
119.17
110.94
107.70
56.68
56.45
41.66
36.56
30.19
23.13
13.21
O
Me
O
N
N
OH
H
H
HN
Me
35
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-1
f1 (ppm)

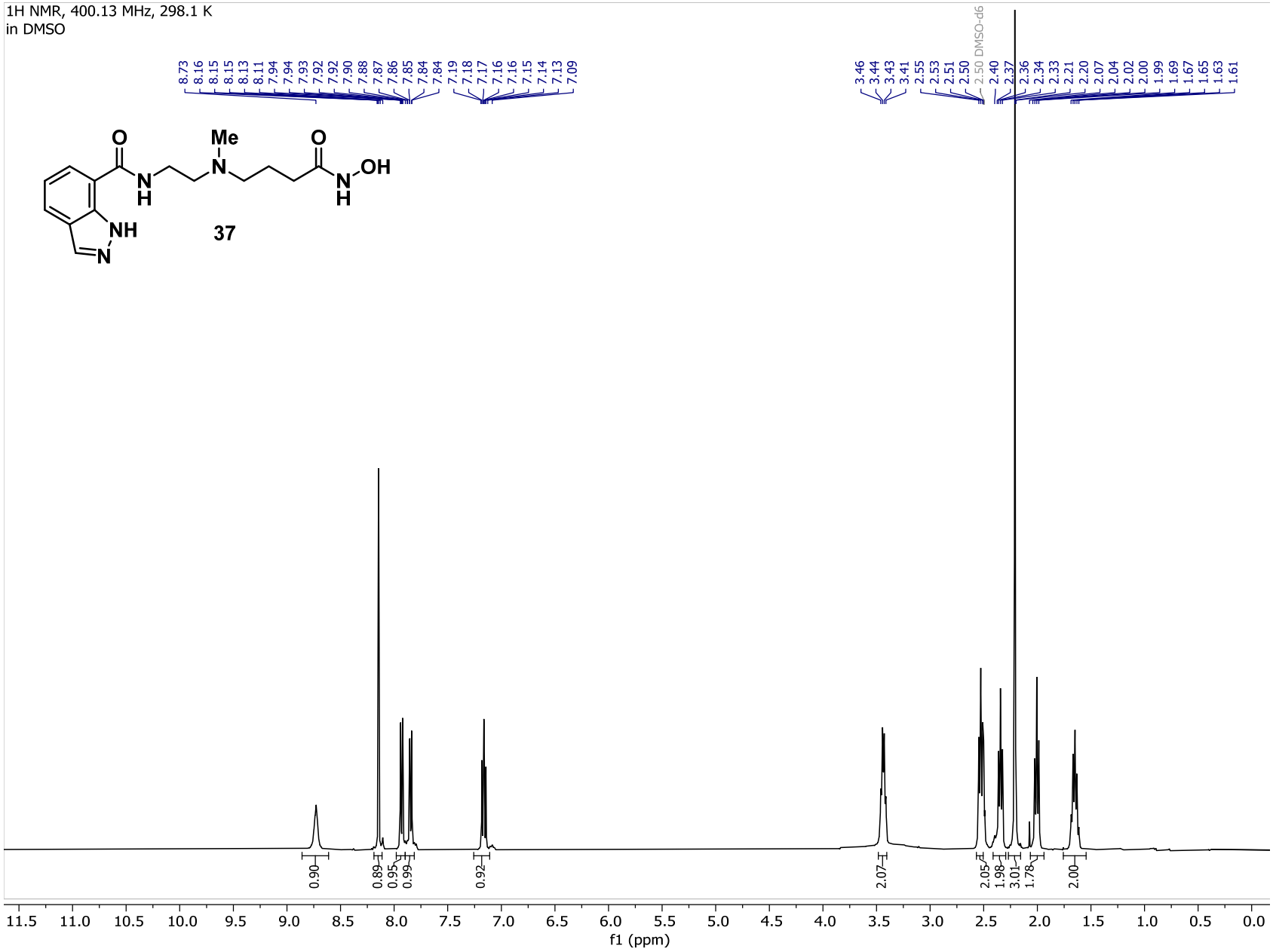

Basic Method PDA - Total Absorbance Chromatogram
Absorbance
50000
40000
30000
20000
10000
0
-10000
3.42
94.4%
3.57
0.8%
3.96
4.8%
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8
Basic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
Absorbance (mAU)
2500
2000
1500
1000
500
0
3.41
95.0%
3.57
0.7%
3.95
4.2%
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5

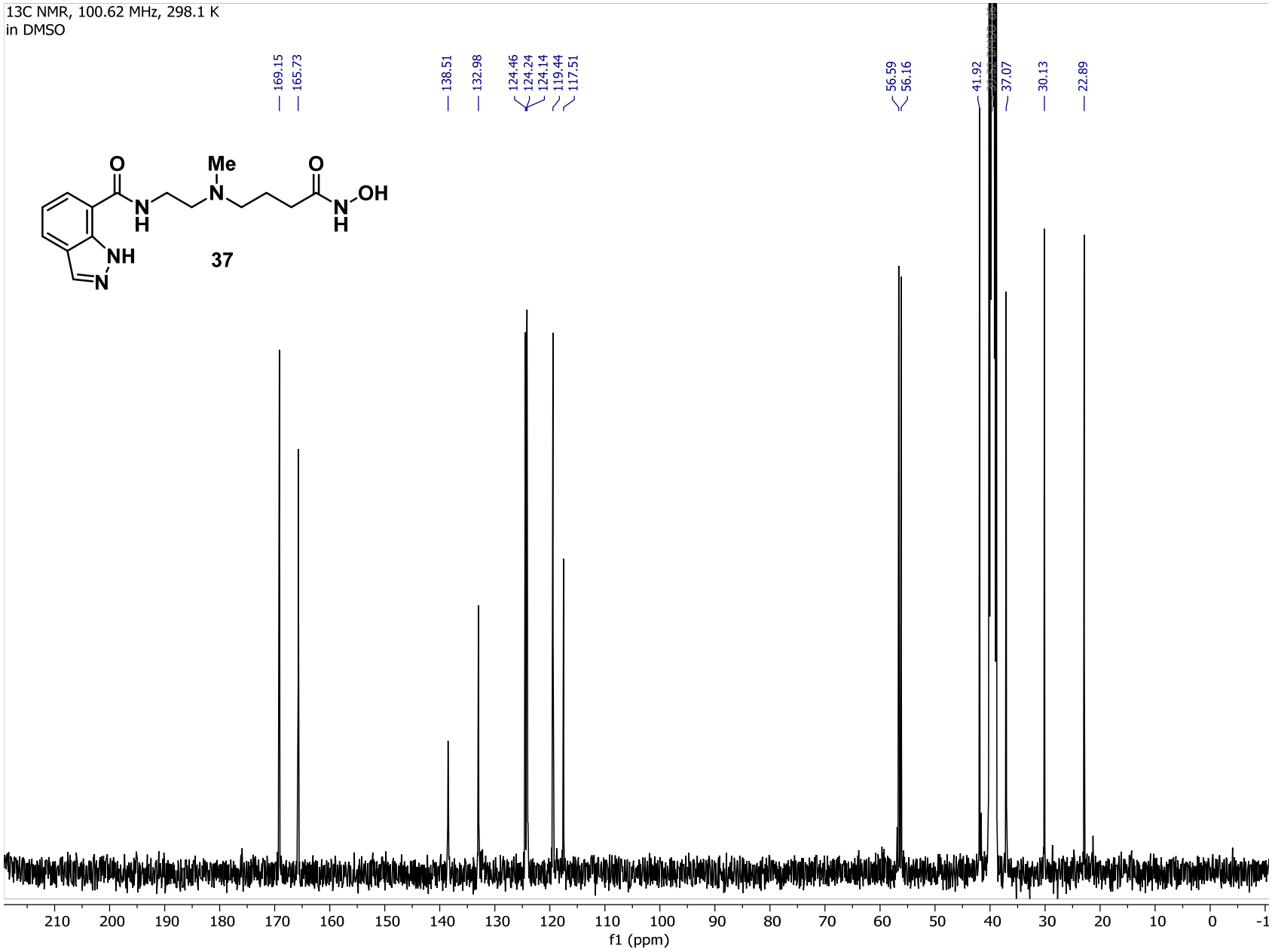
35

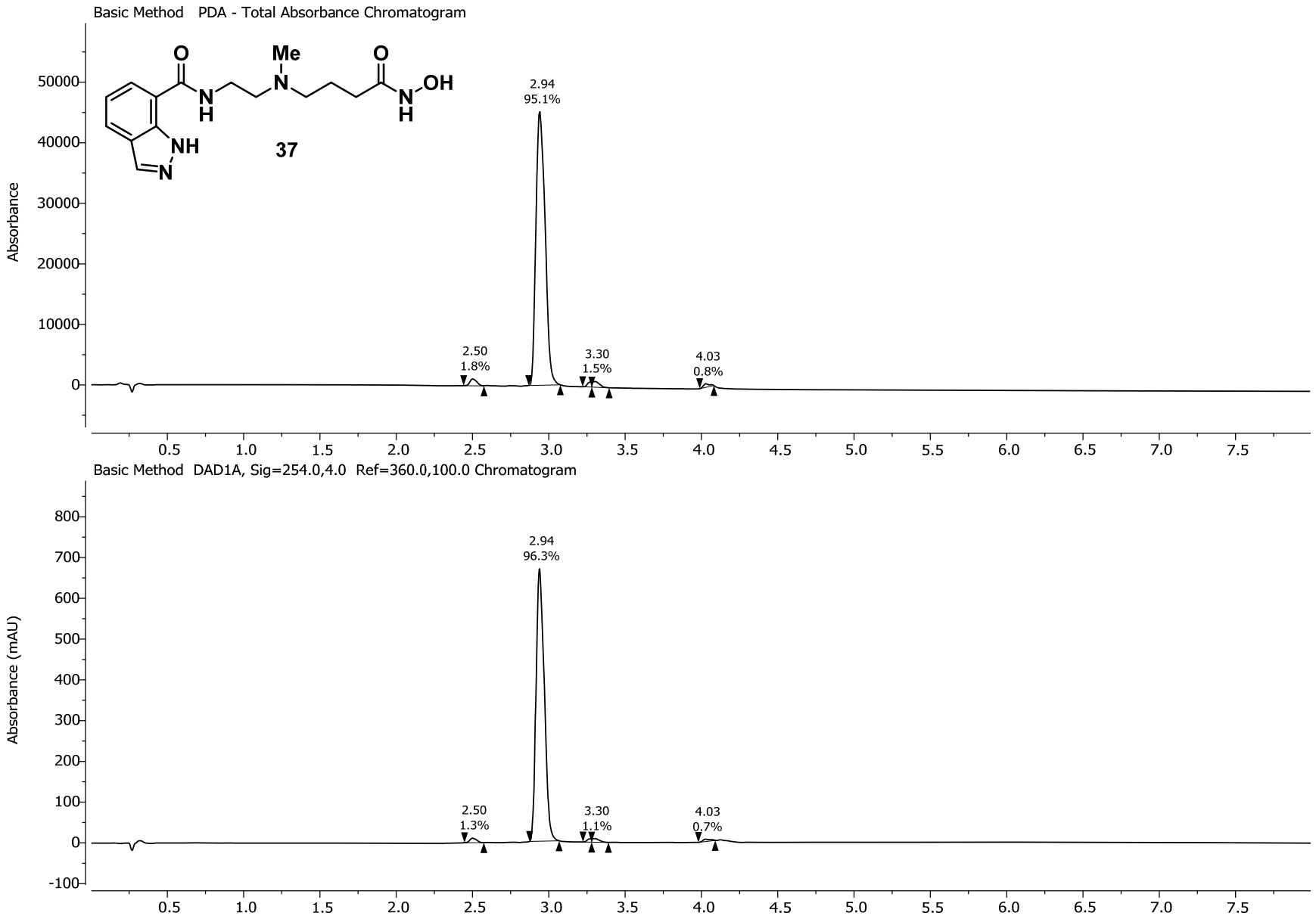

1H NMR, 400.13 MHz, 298.1 K
in DMSO
10.75
8.51 8.50 8.48 8.15 8.14 8.13 8.13 8.12 8.12 8.11 8.11 8.11 8.04 7.82 7.81 7.80 7.80 7.79 7.78 7.77 7.60 7.59 7.58 7.58 7.56 7.56 7.55 7.54
3.40 3.38 3.37 3.35
2.51 2.51 2.51 2.50 2.50 2.48 2.35 2.33 2.31 2.20 2.08 1.99 1.97 1.96 1.67 1.65 1.64 1.62
MeCN
36
2.83 0.95 1.02 0.94 1.00 1.01 2.23 2.57 2.09 3.03 0.43 1.98 2.00
f1 (ppm)

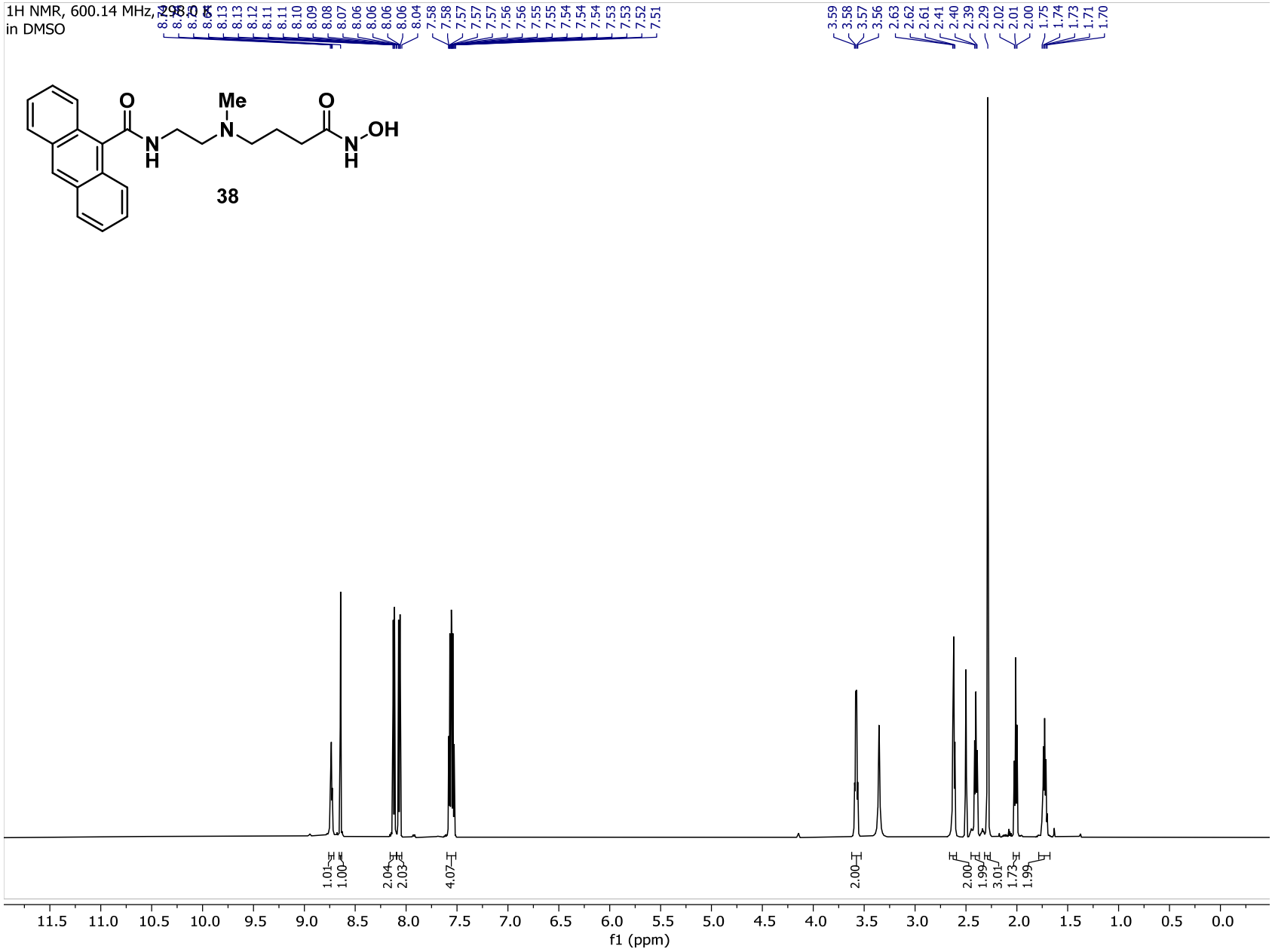

13C NMR, 100.62 MHz, 298.2 K
in DMSO
169.10
166.46
139.51
133.36
132.41
124.17
120.24
119.20
109.59
56.51
56.16
41.98
37.40
30.15
22.95
36
f1 (ppm)

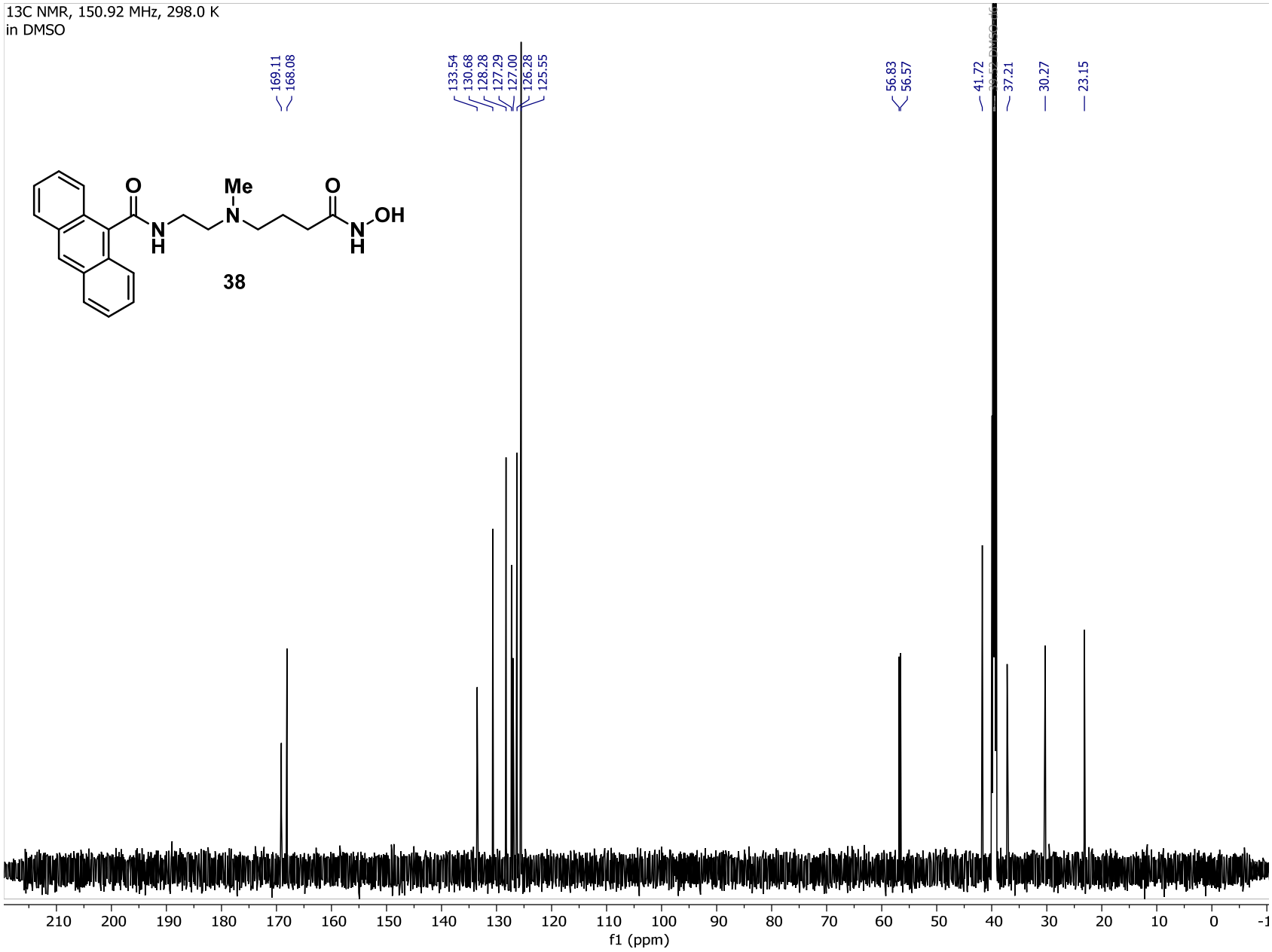

Acidic Method PDA - Total Absorbance Chromatogram
Absorbance
0.99
98.9%
2.82
1.1%
Me
O
OH
36
Acidic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
Absorbance (mAU)
0.99
98.7%
2.82
1.3%

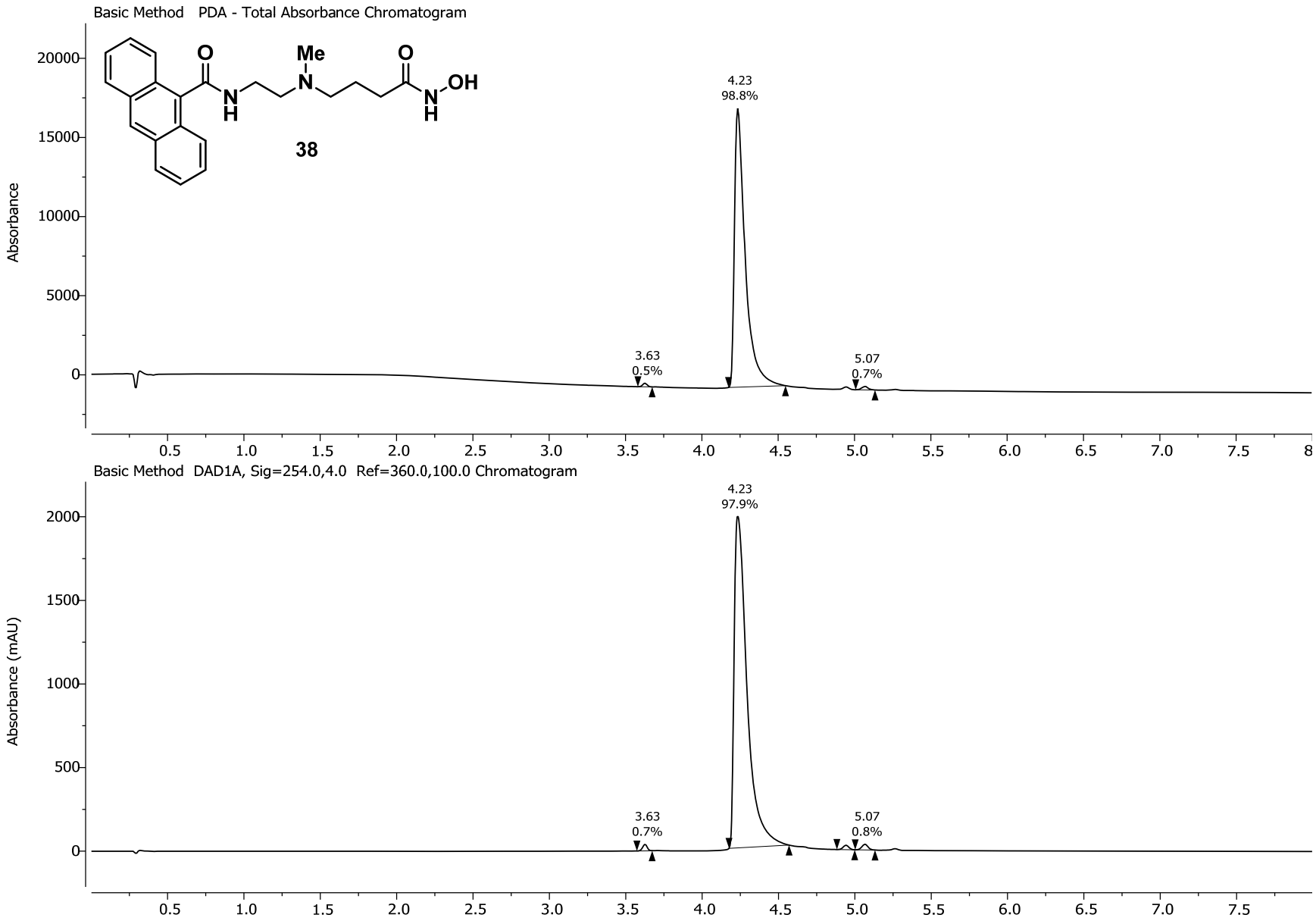

1H NMR, 400.13 MHz, 298.1 K
in DMSO
8.73
8.16
8.15
8.15
8.13
8.11
7.94
7.94
7.93
7.92
7.92
7.90
7.88
7.87
7.86
7.85
7.84
7.84
7.19
7.18
7.17
7.16
7.16
7.15
7.14
7.13
7.09
3.46
3.44
3.43
3.41
2.55
2.53
2.51
2.50
2.50 DMSO-d6
2.40
2.37
2.36
2.34
2.33
2.21
2.20
2.07
2.04
2.02
2.00
1.99
1.69
1.67
1.65
1.63
1.61
O
Me
O
N
N
N
OH
H
H
NH
N
37
0.90
0.89
0.95
0.99
0.92
2.07
2.05
1.98
3.01
1.78
2.00
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

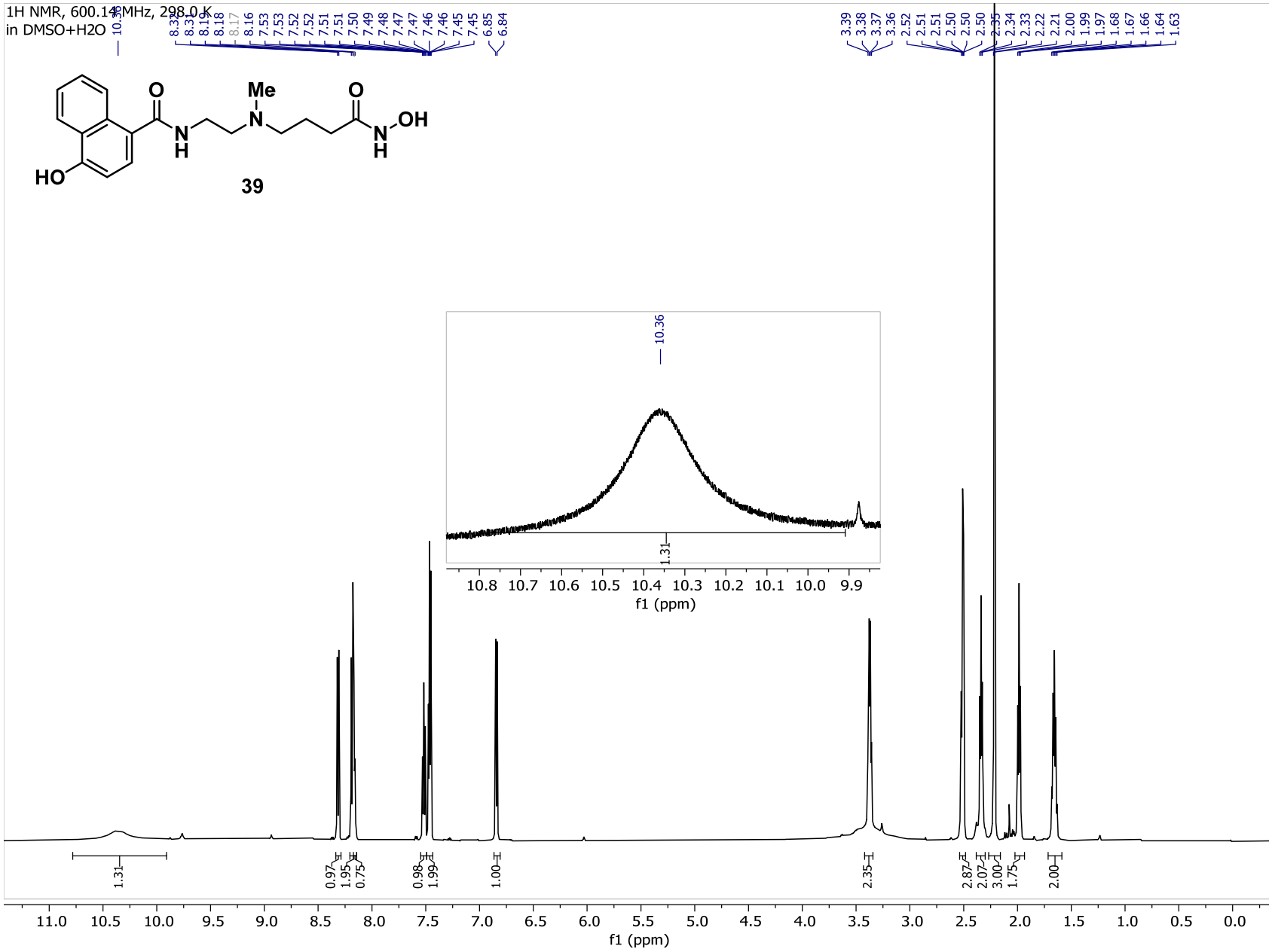

13C NMR, 100.62 MHz, 298.1 K
in DMSO
169.15
165.73
138.51
132.98
124.46
124.24
124.14
119.44
117.51
56.59
56.16
41.92
37.07
30.13
22.89
O
Me
O
N
H
N
N
H
OH
NH
N
37
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-1
f1 (ppm)

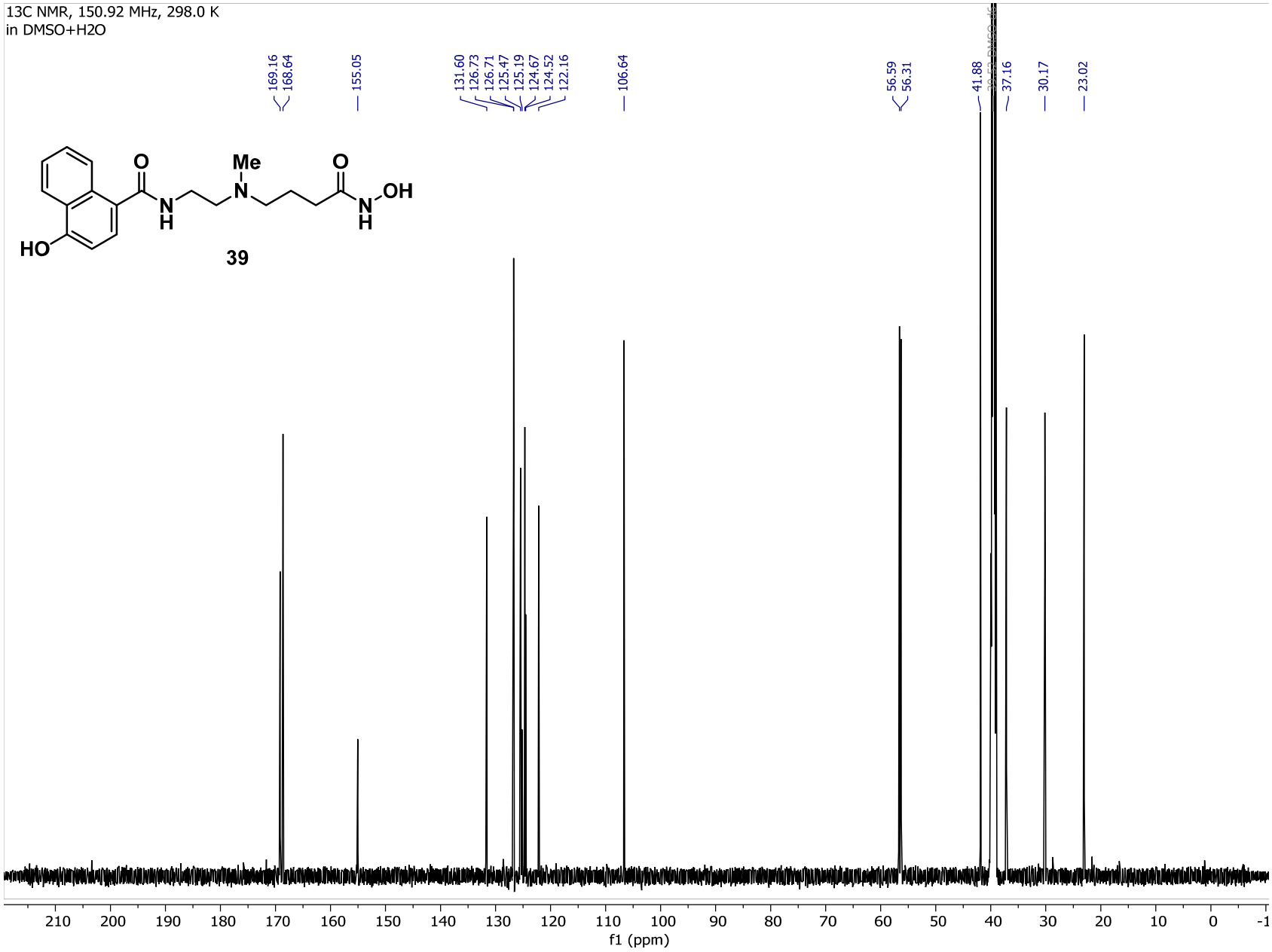

Basic Method PDA - Total Absorbance Chromatogram
Absorbance
2.94
95.1%
2.50
1.8%
3.30
1.5%
4.03
0.8%
37
Basic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
Absorbance (mAU)
2.94
96.3%
2.50
1.3%
3.30
1.1%
4.03
0.7%

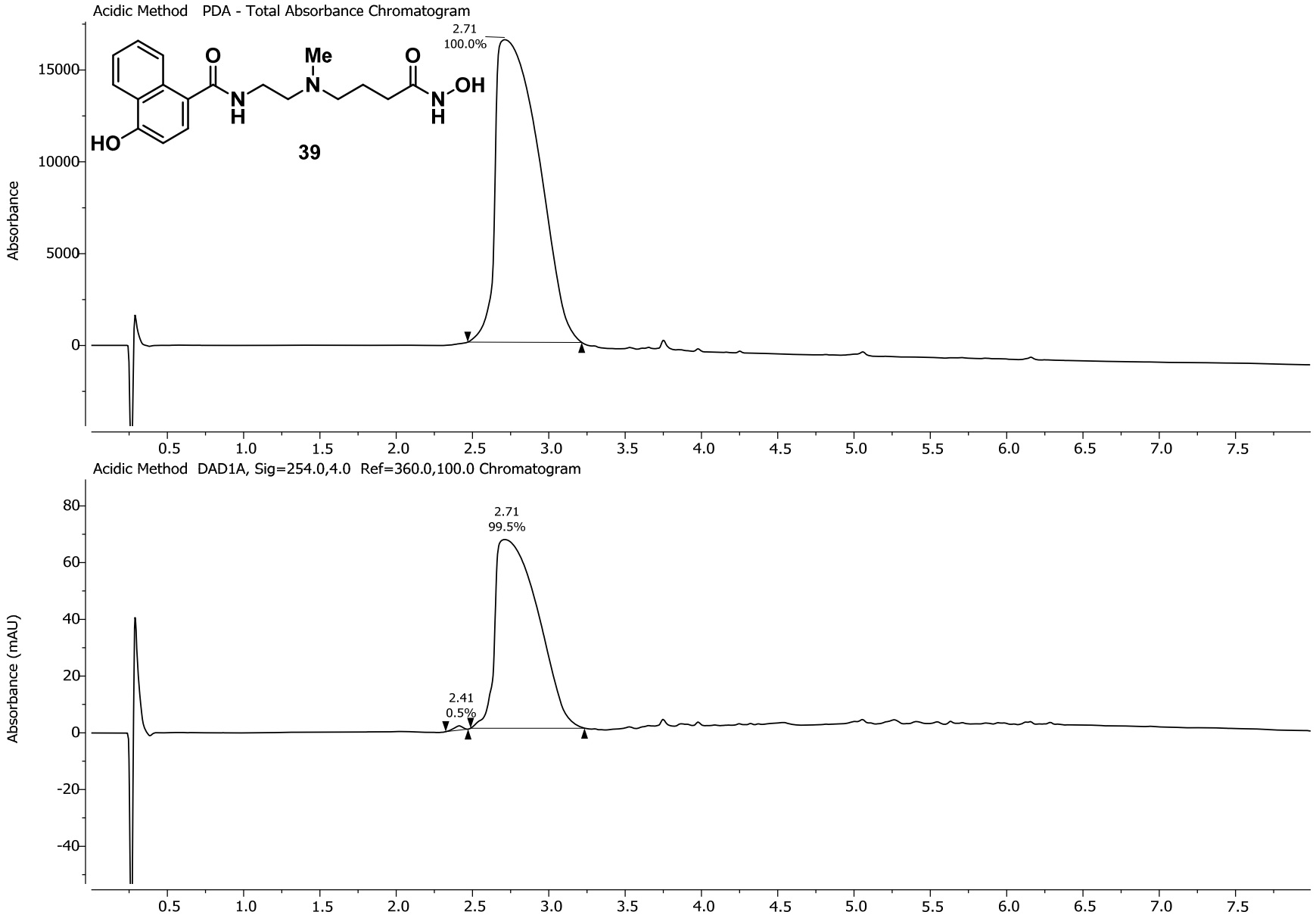

1H NMR, 600.14 MHz, 298.0 K
in DMSO
8.14 8.13 8.13 8.13 8.12 8.11 8.11 8.10 8.09 8.08 8.07 8.06 8.06 8.06 8.06 8.04
7.58 7.58 7.57 7.57 7.57 7.56 7.56 7.55 7.55 7.54 7.54 7.54 7.53 7.53 7.52 7.51
3.59 3.58 3.57 3.56
2.63 2.62 2.61 2.41 2.40 2.39 2.29
2.02 2.01 2.00
1.75 1.74 1.73 1.71 1.70
O
Me
O
N
N
N
OH
H
H
38
1.01 1.00 2.04 2.03 4.07 2.00 2.00 1.99 3.01 1.73 1.99
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

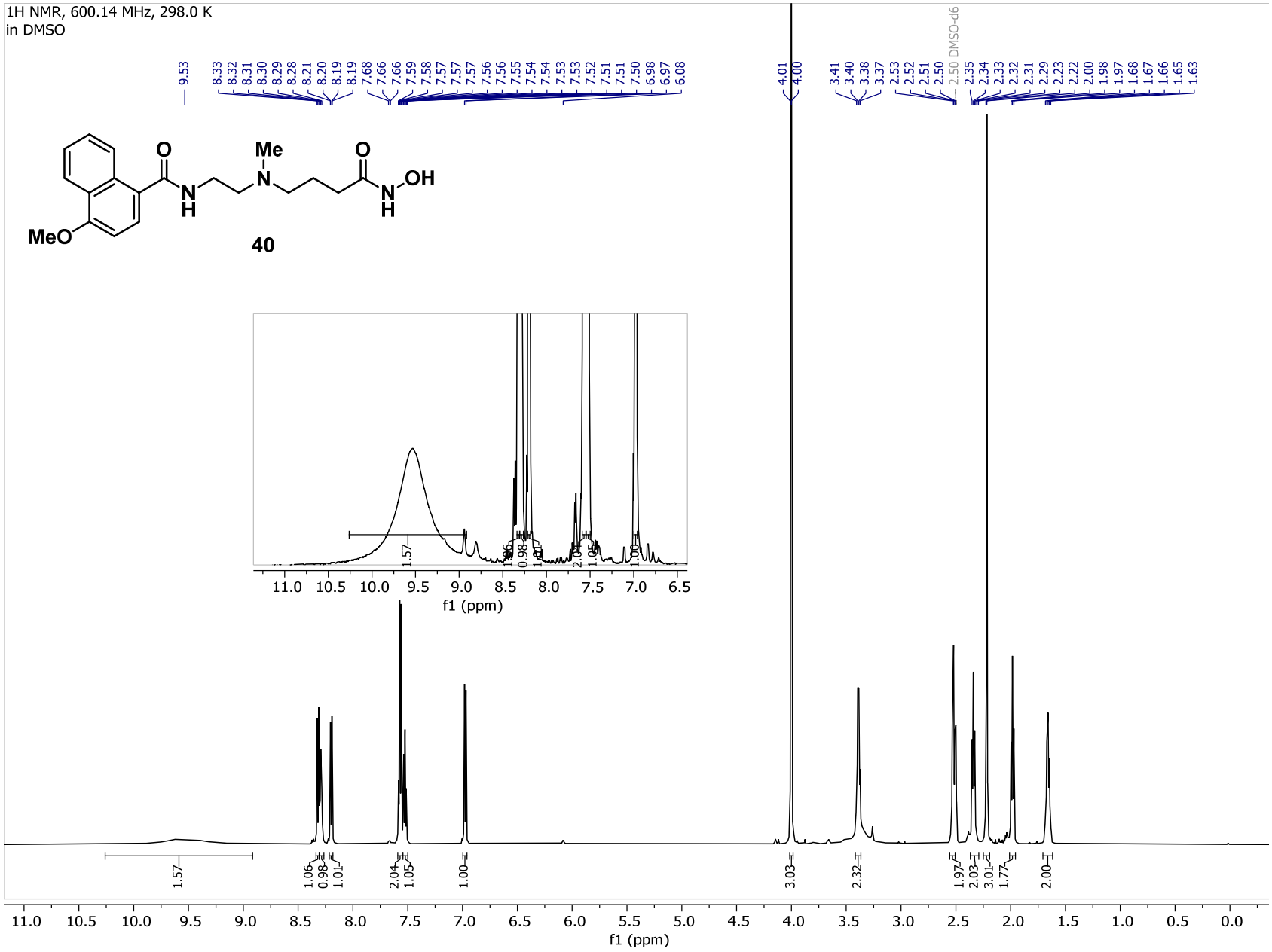

13C NMR, 150.92 MHz, 298.0 K
in DMSO
169.11
168.08
133.54
130.68
128.28
127.29
127.00
126.28
125.55
56.83
56.57
41.72
37.21
30.27
23.15
O
Me
O
N
H
N
N
H
OH
38
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-1
f1 (ppm)

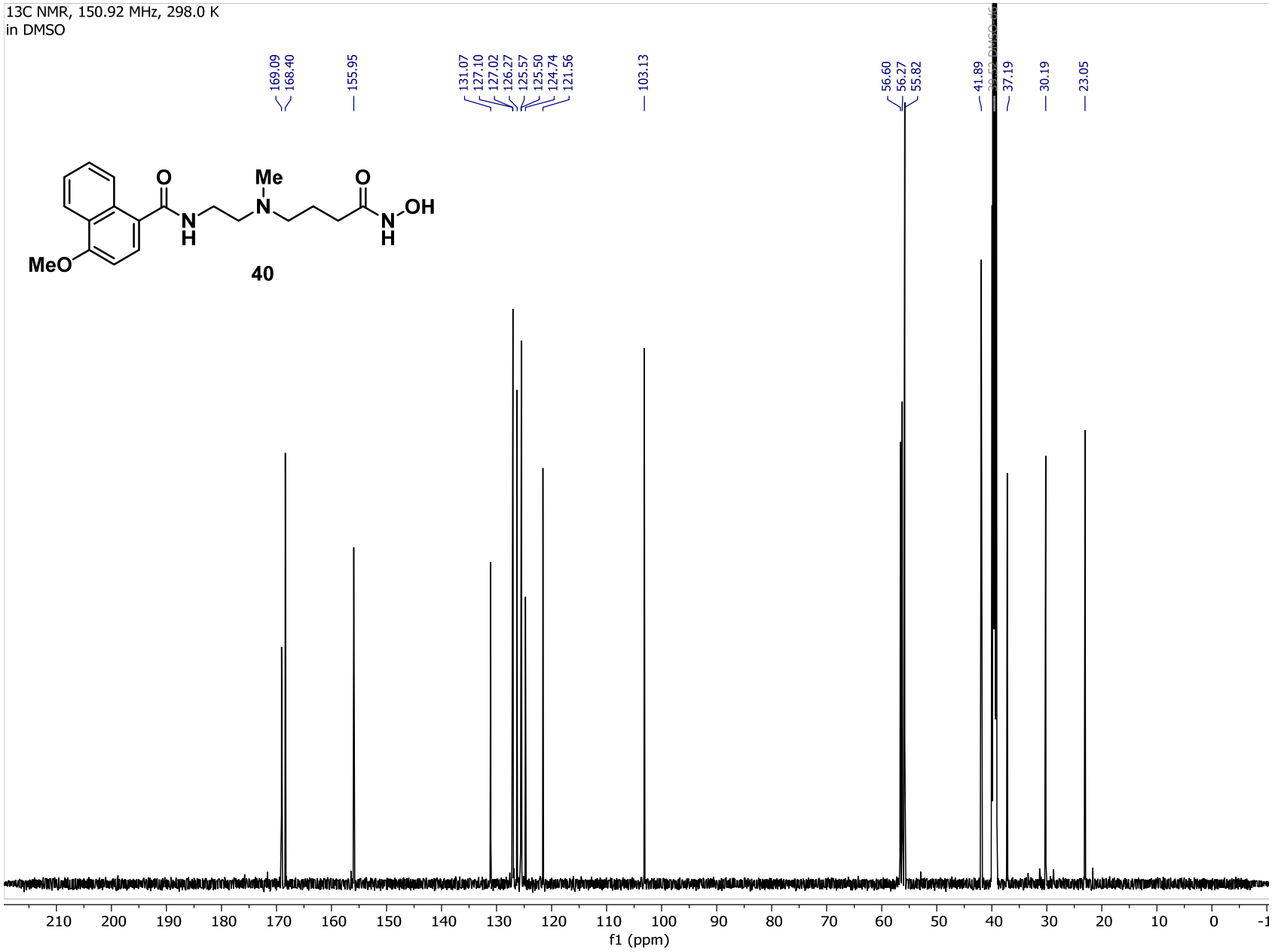

Basic Method PDA - Total Absorbance Chromatogram
Absorbance
20000
15000
10000
5000
0
O
Me
O
N
H
N
N
H
OH
38
4.23
98.8%
3.63
0.5%
5.07
0.7%
0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8
Basic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
Absorbance (mAU)
2000
1500
1000
500
0
4.23
97.9%
3.63
0.7%
5.07
0.8%
0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5

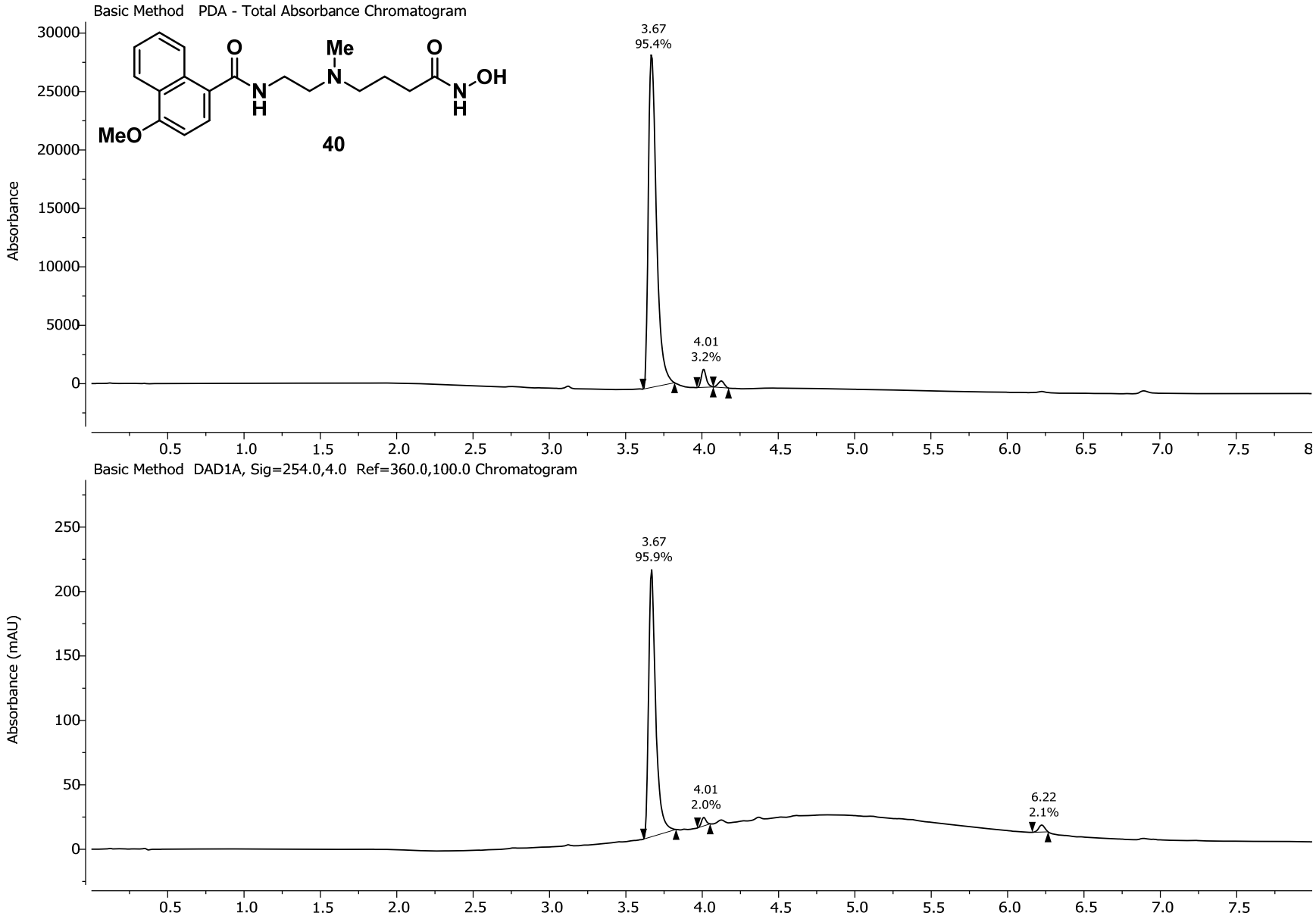

1H NMR, 600.13MHz, 298.0 K
in DMSO+H2O
10.36
8.32 8.31 8.19 8.18 8.17 8.16 7.53 7.53 7.52 7.52 7.51 7.51 7.50 7.49 7.48 7.47 7.47 7.46 7.46 7.45 7.45 6.85 6.84
3.39 3.38 3.37 3.36 2.52 2.51 2.51 2.50 2.50 2.50 2.35 2.34 2.33 2.22 2.21 2.00 1.99 1.97 1.68 1.67 1.66 1.64 1.63
O
Me
O
N
N
OH
H
H
HO
39
10.36
1.31
10.8 10.7 10.6 10.5 10.4 10.3 10.2 10.1 10.0 9.9
f1 (ppm)
1.31
0.97
1.95
0.75
0.98
1.99
1.00
2.35
2.87
2.07
3.00
1.75
2.00
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

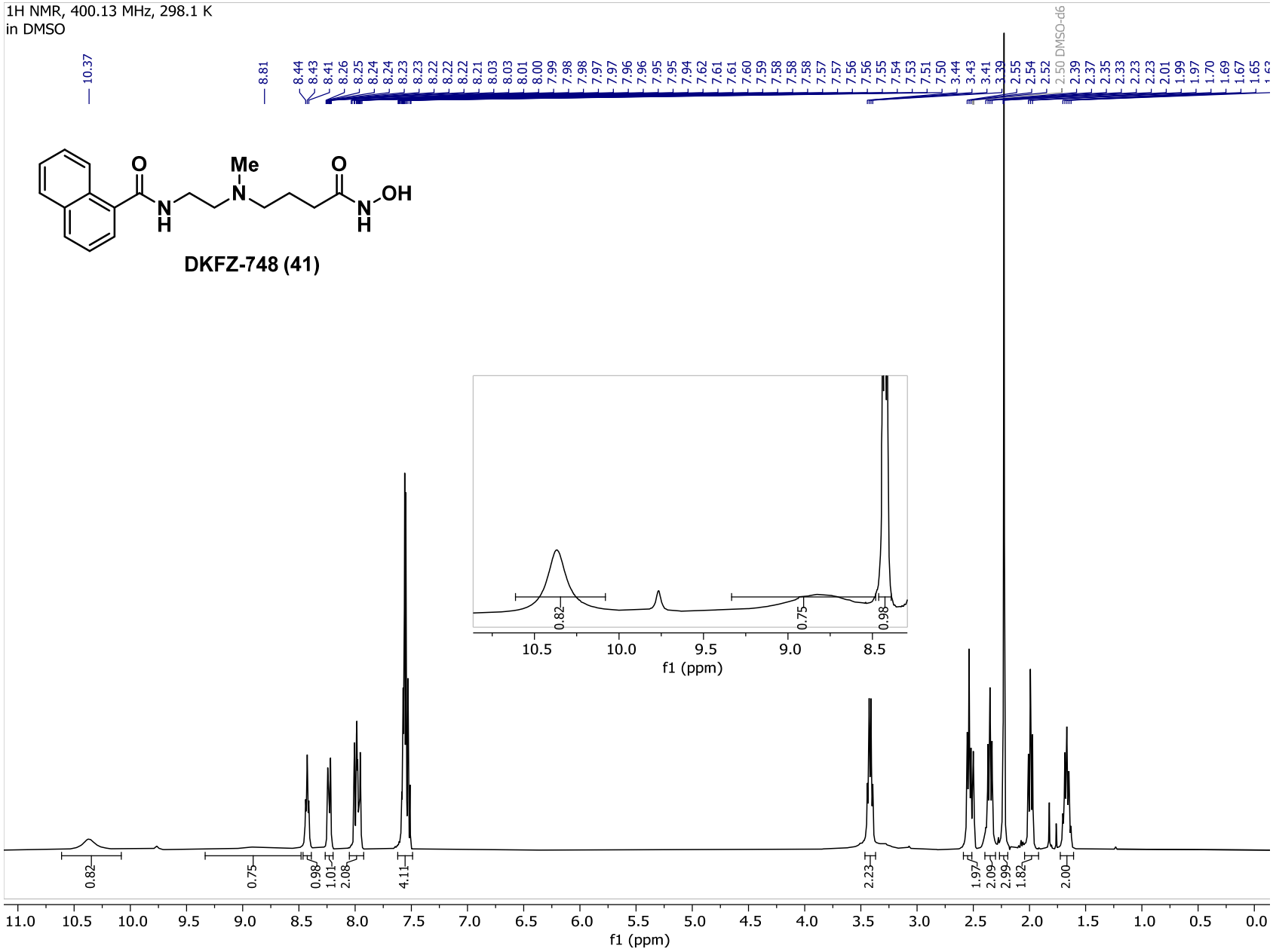

13C NMR, 150.92 MHz, 298.0 K
in DMSO+H2O
169.16
168.64
155.05
131.60
126.73
126.71
125.47
125.19
124.67
124.52
122.16
106.64
56.59
56.31
41.88
37.16
30.17
23.02
O
Me
O
N
N
OH
H
H
HO
39
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -1
f1 (ppm)

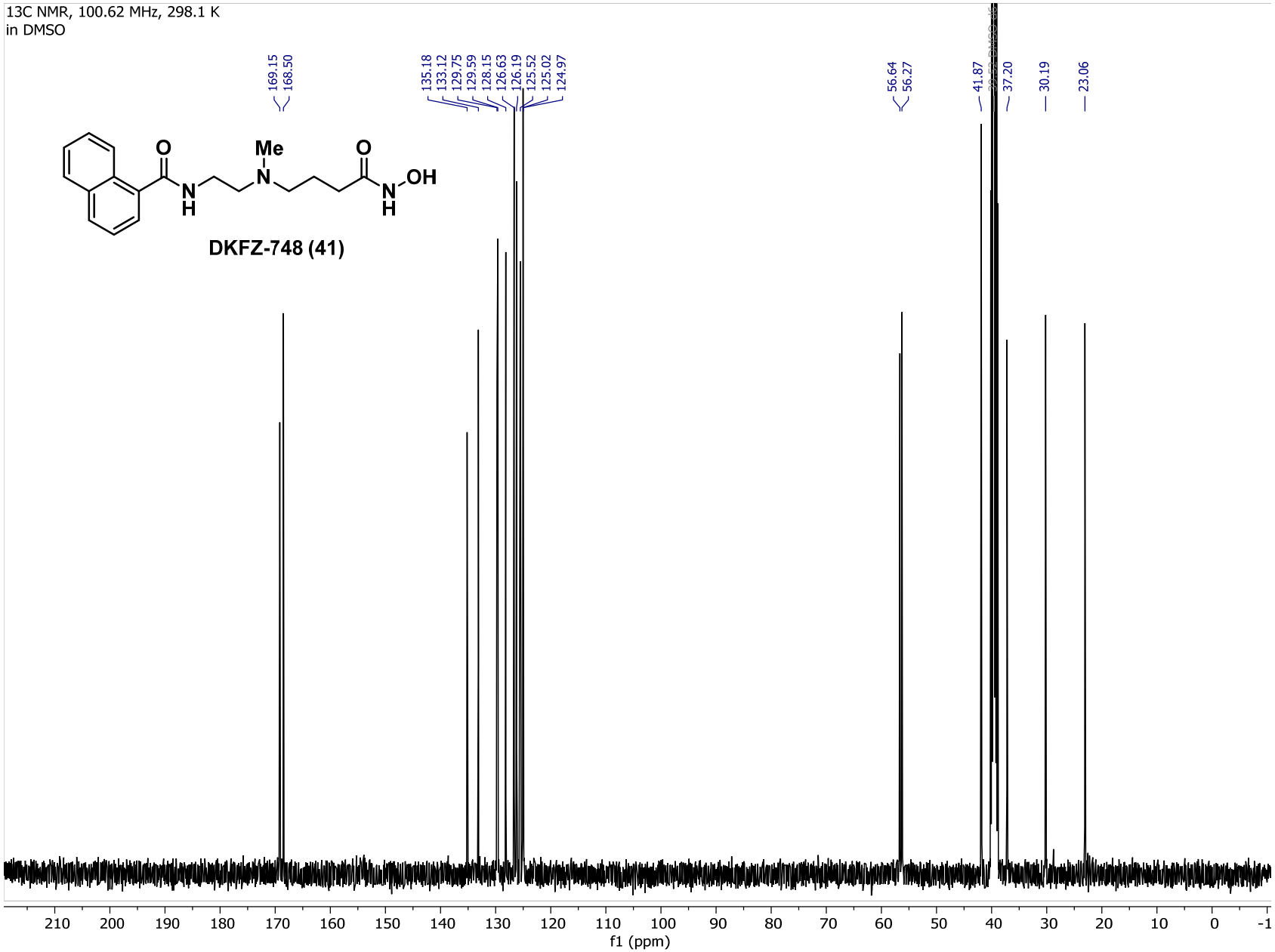

Acidic Method PDA - Total Absorbance Chromatogram
2.71
100.0%
39
Absorbance
Acidic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
2.71
99.5%
2.41
0.5%
Absorbance (mAU)

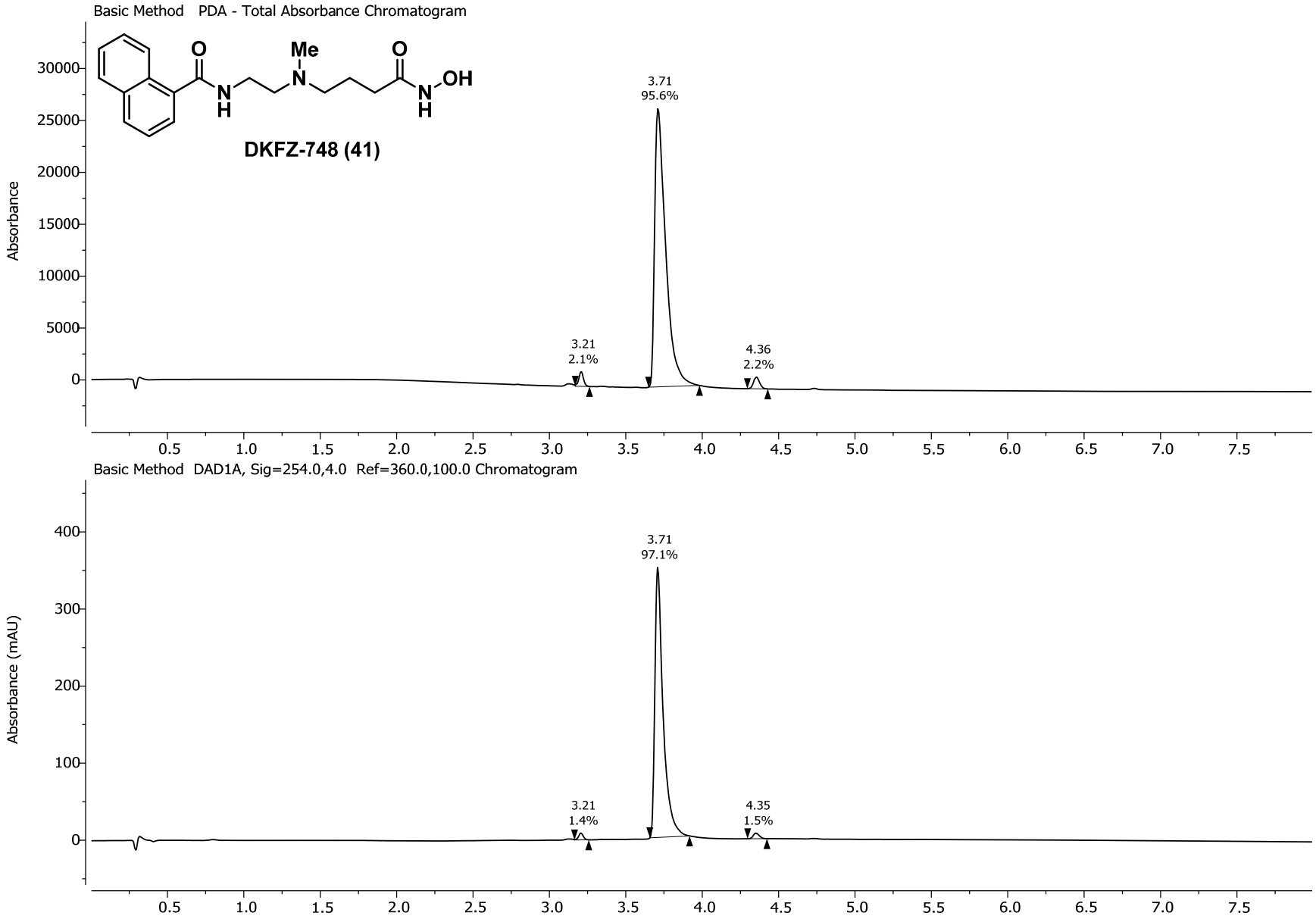

1H NMR, 600.14 MHz, 298.0 K
in DMSO
40
f1 (ppm)

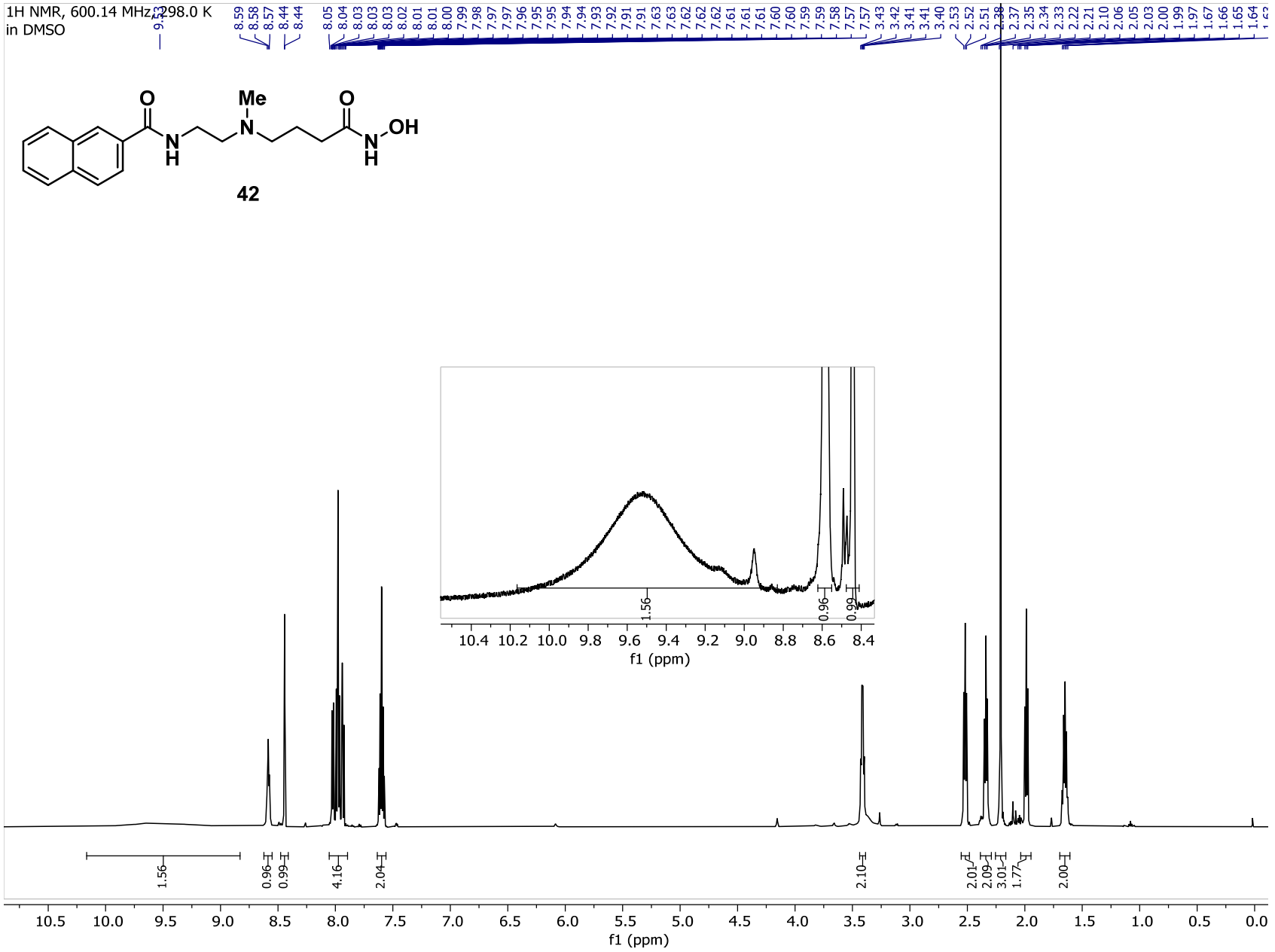

13C NMR, 150.92 MHz, 298.0 K
in DMSO
169.09
168.40
155.95
131.07
127.10
127.02
126.27
125.57
125.50
124.74
121.56
103.13
56.60
56.27
55.82
41.89
39.52 DMSO
37.19
30.19
23.05
O
Me
O
N
N
OH
H
H
MeO
40
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -1
f1 (ppm)

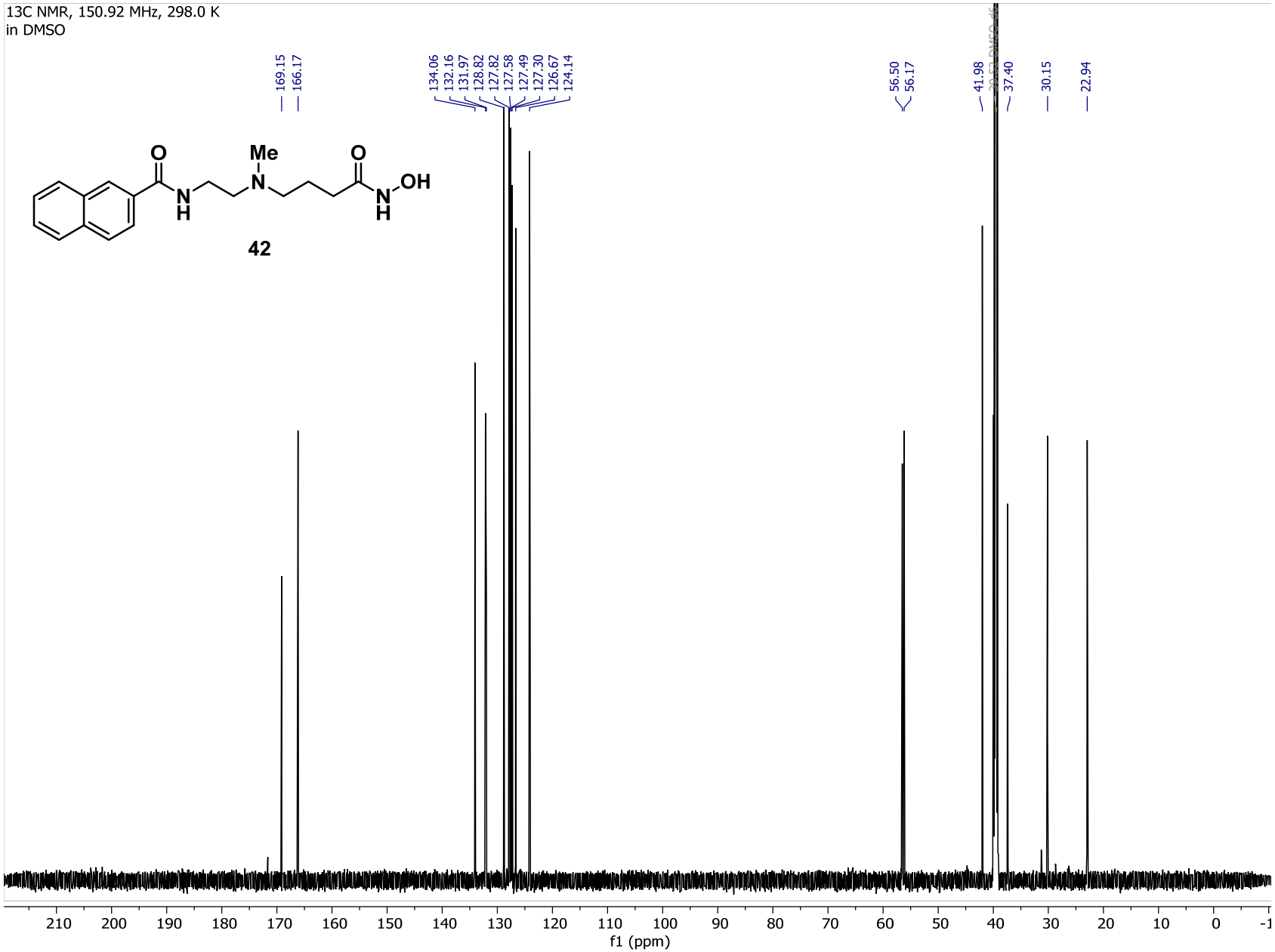

Basic Method PDA - Total Absorbance Chromatogram
Absorbance
3.67
95.4%
4.01
3.2%
40
Basic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
Absorbance (mAU)
3.67
95.9%
4.01
2.0%
6.22
2.1%

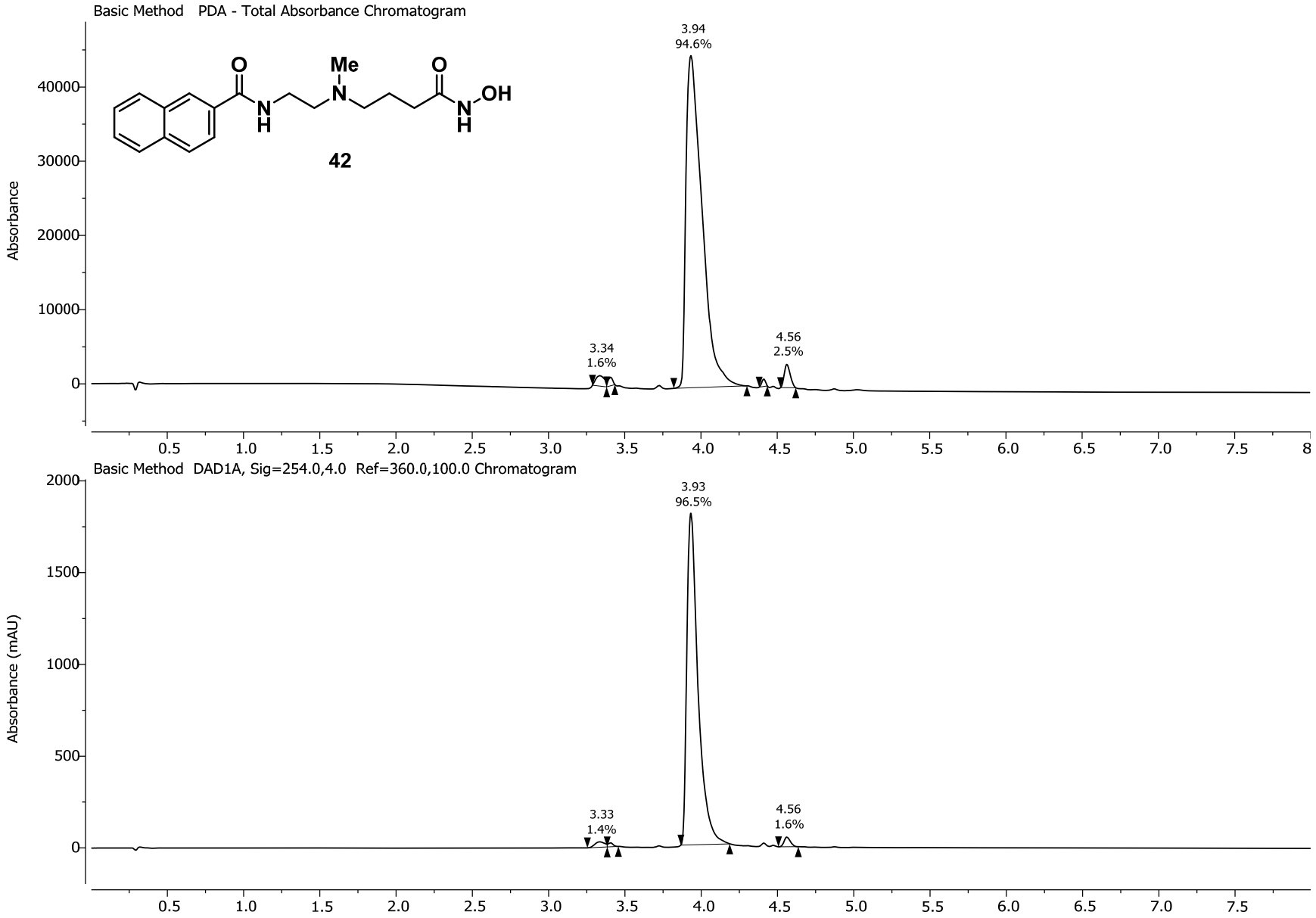

1H NMR, 400.13 MHz, 298.1 K
in DMSO
DKFZ-748 (41)
f1 (ppm)

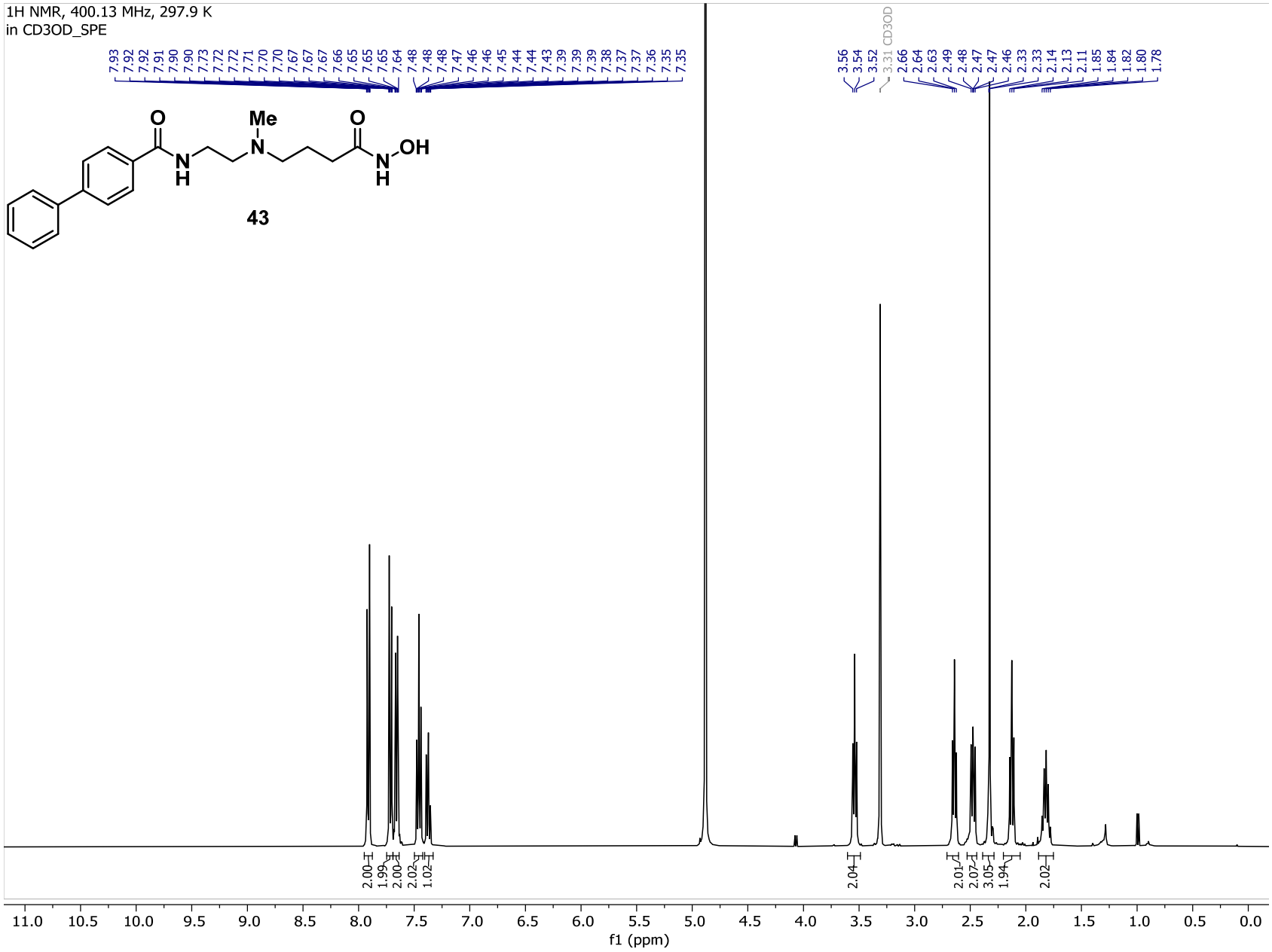

13C NMR, 100.62 MHz, 298.1 K
in DMSO
169.15
168.50
135.18
133.12
129.75
129.59
128.15
126.63
126.19
125.52
125.02
124.97
56.64
56.27
41.87
37.20
30.19
23.06
O
Me
O
N
H
N
N
H
OH
DKFZ-748 (41)
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-1
f1 (ppm)

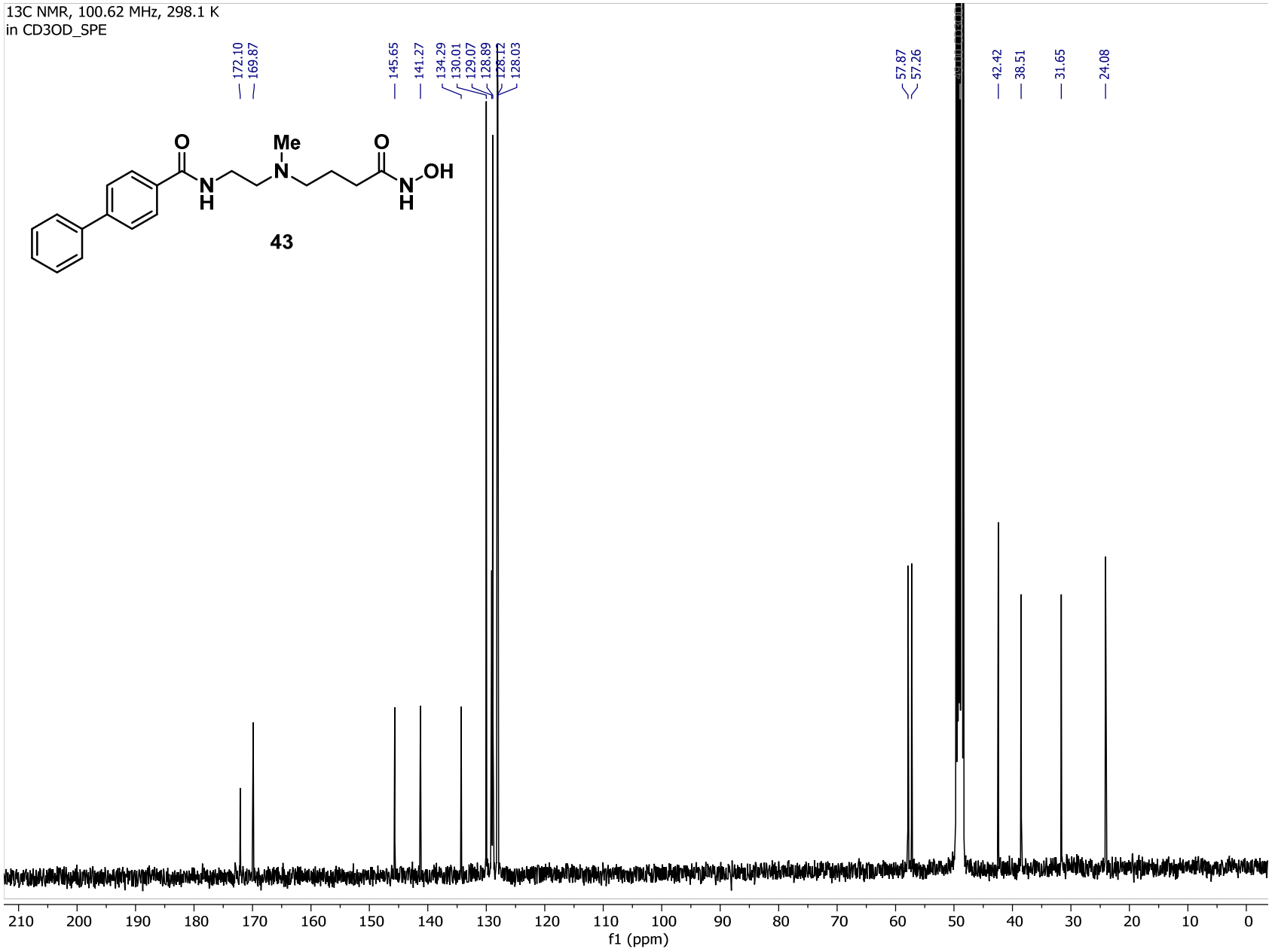

Basic Method PDA - Total Absorbance Chromatogram
DKFZ-748 (41)
Absorbance
3.21
2.1%
3.71
95.6%
4.36
2.2%
Basic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
Absorbance (mAU)
3.21
1.4%
3.71
97.1%
4.35
1.5%

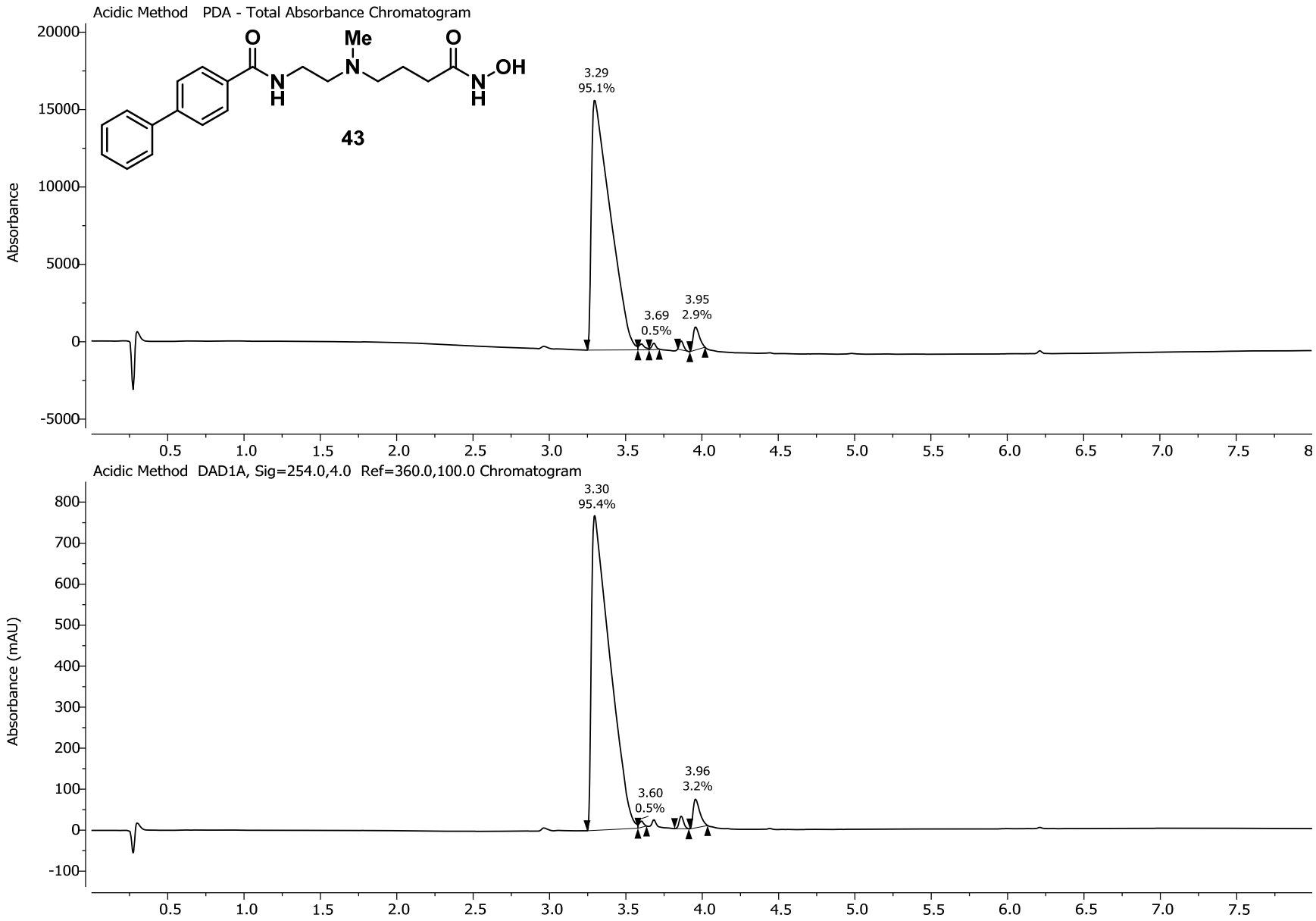

1H NMR, 600.14 MHz, 298.0 K
in DMSO
O
Me
O
N
N
N
OH
H
H
42
1.56
0.96
0.99
4.16
2.04
2.10
2.01
2.09
3.01
1.77
2.00
f1 (ppm)

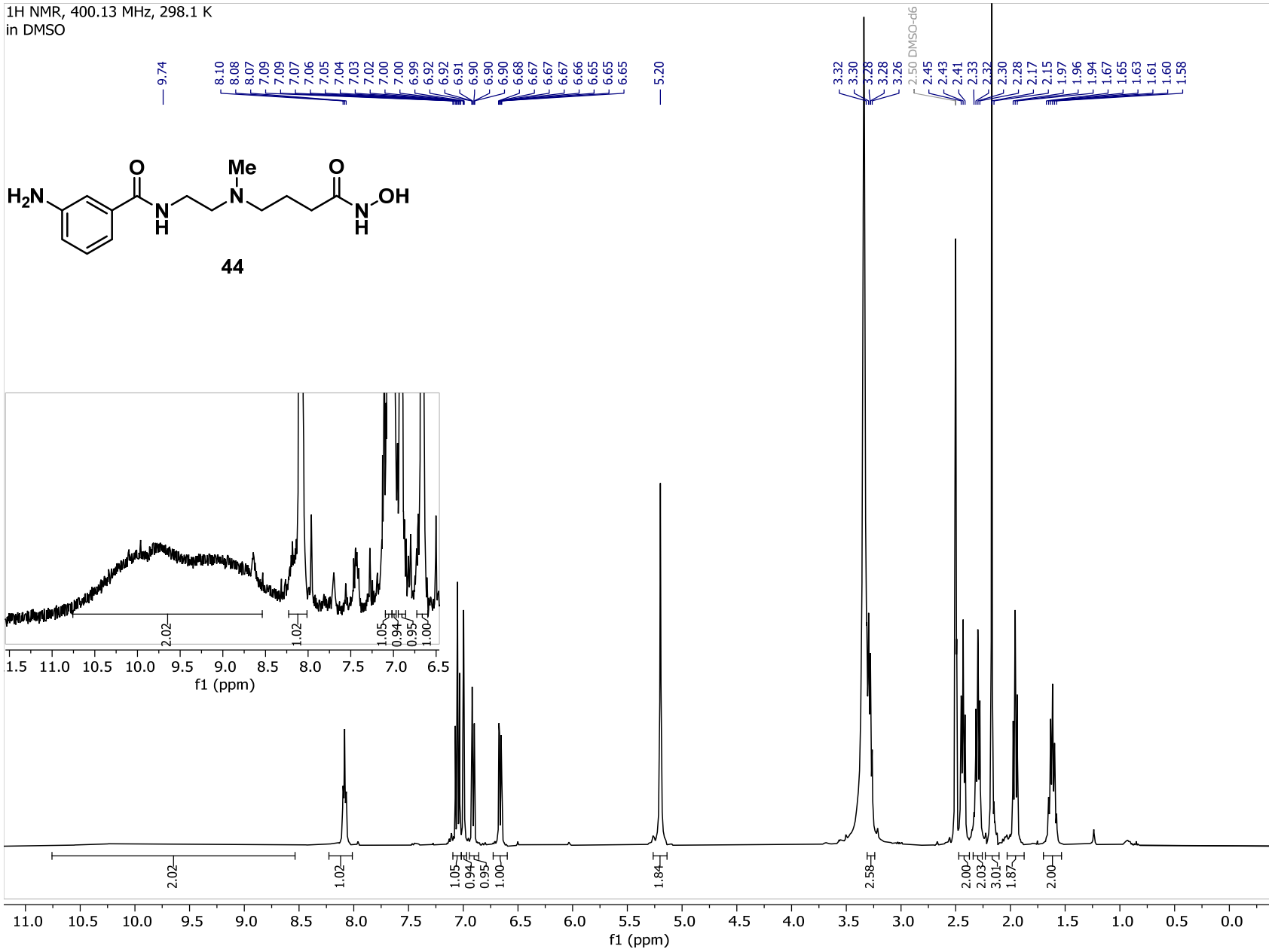

13C NMR, 150.92 MHz, 298.0 K
in DMSO
169.15
166.17
134.06
132.16
131.97
128.82
127.82
127.58
127.49
127.30
126.67
124.14
56.50
56.17
41.98
37.40
30.15
22.94
O
Me
O
N
N
OH
H
H
42
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-1
f1 (ppm)

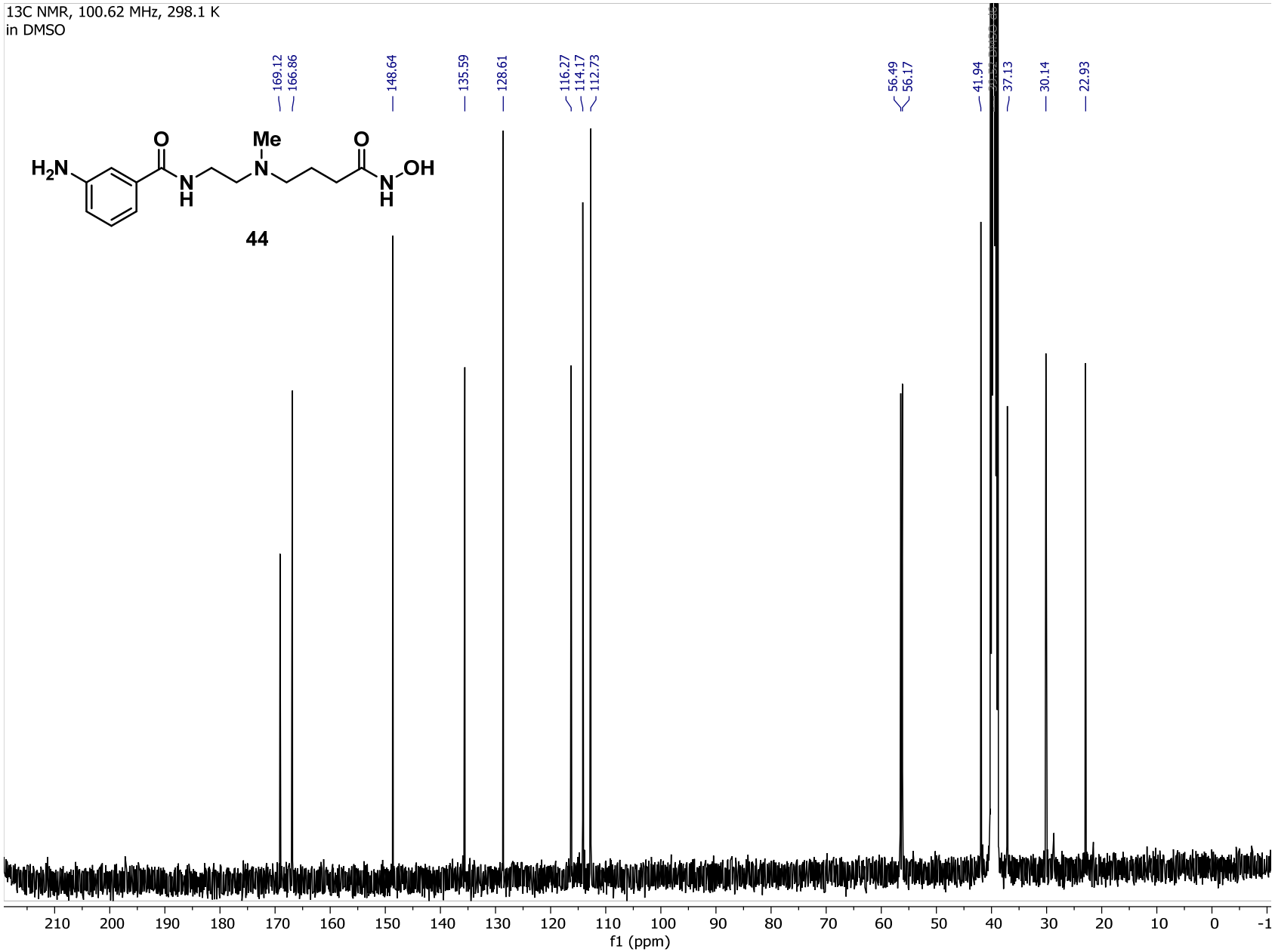

Basic Method PDA - Total Absorbance Chromatogram
Absorbance
3.94
94.6%
3.34
1.6%
4.56
2.5%
42
Basic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
Absorbance (mAU)
3.93
96.5%
3.33
1.4%
4.56
1.6%

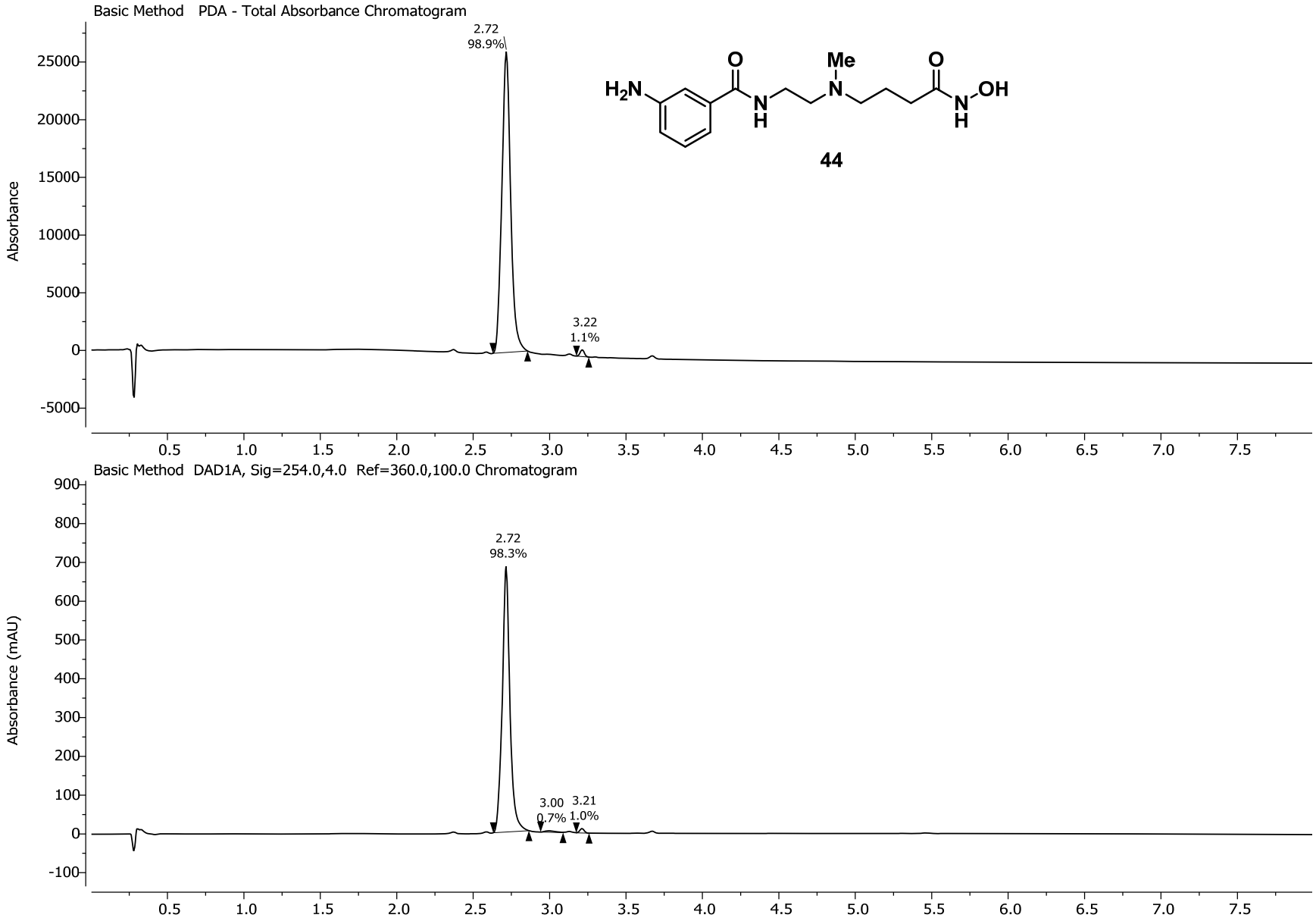

1H NMR, 400.13 MHz, 297.9 K
in CD3OD_SPE
7.93 7.92 7.92 7.91 7.90 7.90 7.73 7.72 7.72 7.71 7.70 7.70 7.67 7.67 7.67 7.66 7.65 7.65 7.65 7.64 7.48 7.48 7.48 7.47 7.46 7.46 7.45 7.44 7.44 7.43 7.39 7.39 7.39 7.38 7.37 7.37 7.36 7.35 7.35
3.56 3.54 3.52 3.31 CD3OD 2.66 2.64 2.63 2.49 2.48 2.47 2.47 2.46 2.33 2.33 2.14 2.13 2.11 1.85 1.84 1.82 1.80 1.78
O
Me
O
N
H
N
N
H
OH
43
2.00 1.99 2.00 2.02 1.02 2.04 2.01 2.07 3.05 1.94 2.02
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

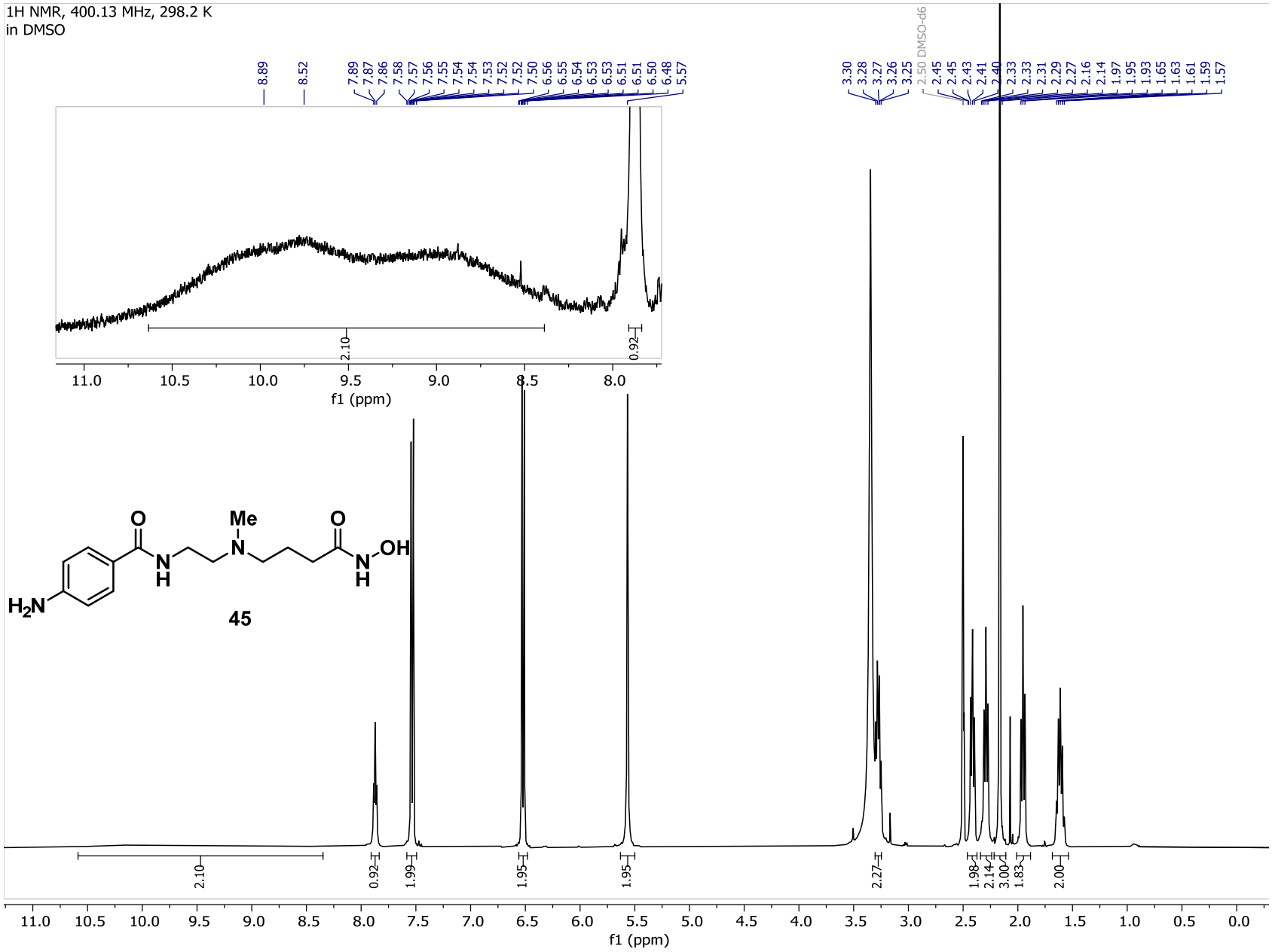

13C NMR, 100.62 MHz, 298.1 K
in CD3OD_SPE
172.10
169.87
145.65
141.27
134.29
130.01
129.07
128.89
128.12
128.03
57.87
57.26
42.42
38.51
31.65
24.08
O
Me
O
N
H
N
N
H
OH
43
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

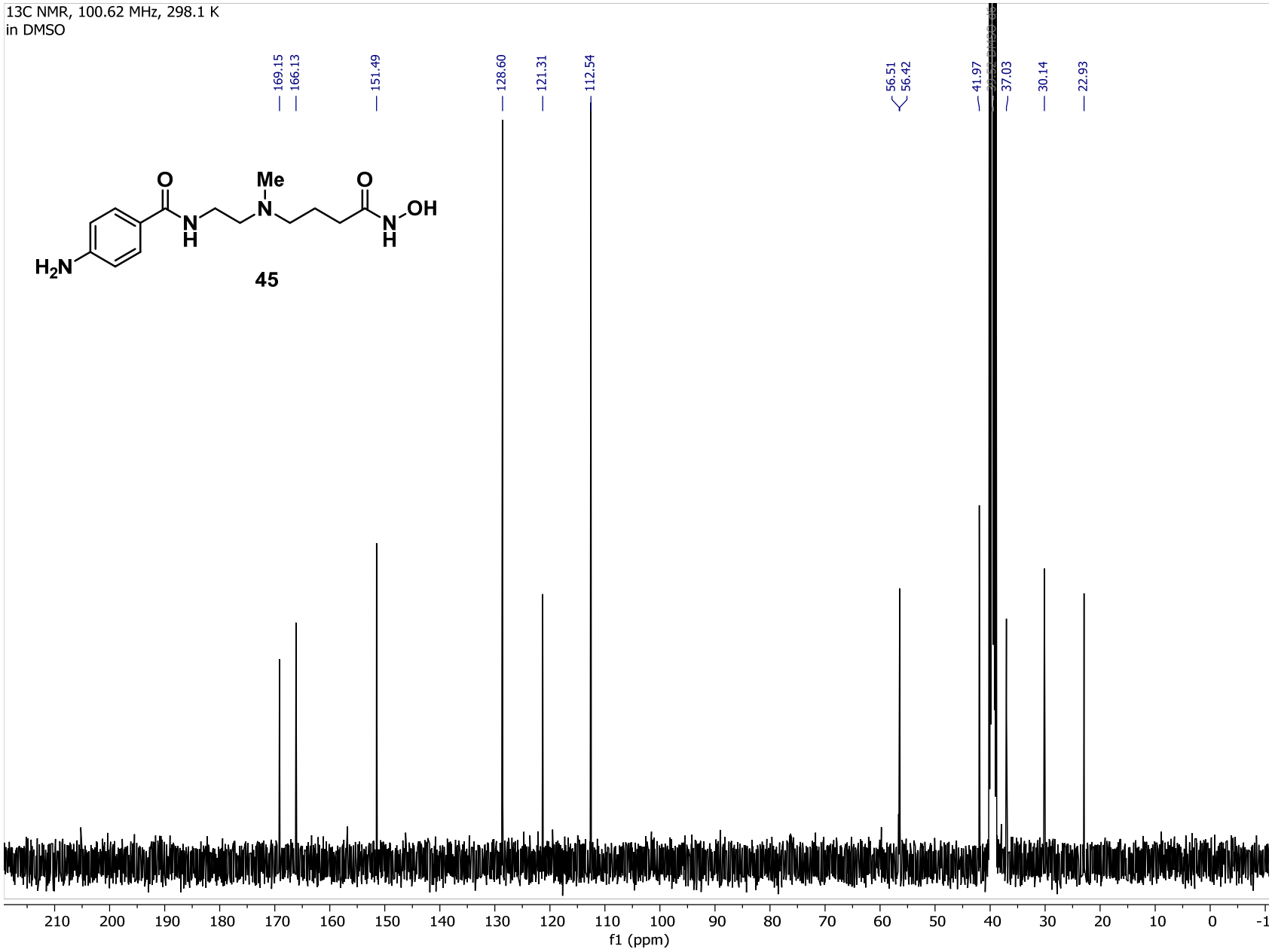

Acidic Method PDA - Total Absorbance Chromatogram
Absorbance
20000
15000
10000
5000
0
-5000
O
Me
O
N
H
N
N
H
OH
43
3.29
95.1%
3.69
0.5%
3.95
2.9%
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
Acidic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
Absorbance (mAU)
800
700
600
500
400
300
200
100
0
-100
3.30
95.4%
3.60
0.5%
3.96
3.2%
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5

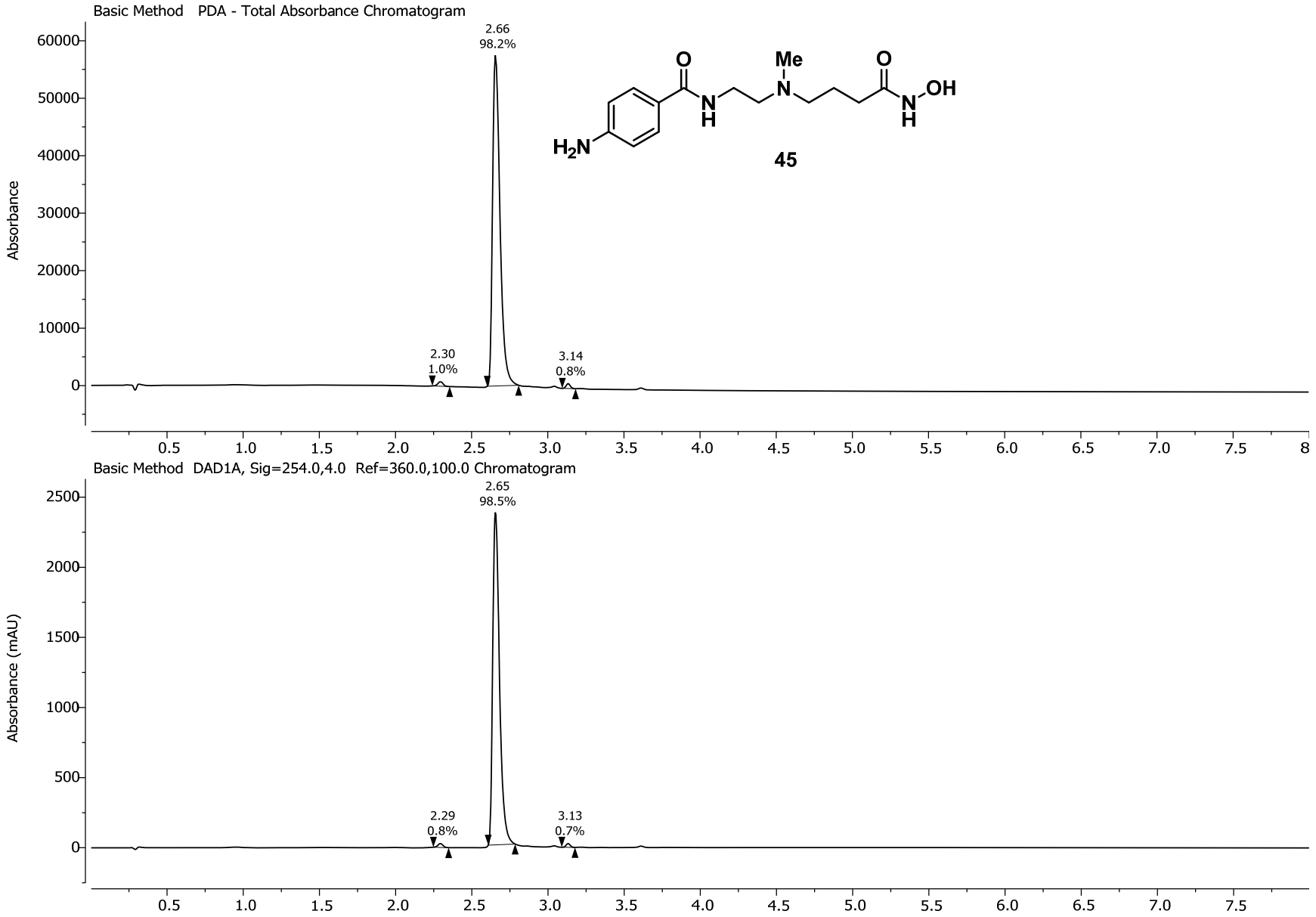

1H NMR, 400.13 MHz, 298.1 K
in DMSO
9.74
8.10 8.08 8.07 7.09 7.09 7.07 7.06 7.05 7.04 7.03 7.02 7.00 7.00 6.99 6.92 6.92 6.91 6.90 6.90 6.90 6.68 6.67 6.67 6.67 6.66 6.65 6.65 6.65
5.20
3.32 3.30 3.28 3.28 3.26
2.50 DMSO-d6
2.45 2.43 2.41 2.33 2.32 2.30 2.28 2.17 2.15 1.97 1.96 1.94 1.67 1.65 1.63 1.61 1.60 1.58
O
Me
O
H2N
N
H
N
N
H
OH
44
2.02 1.02 1.05 0.94 0.95 1.00
1.84
2.58
2.00 2.03 3.01 1.87 2.00
f1 (ppm)

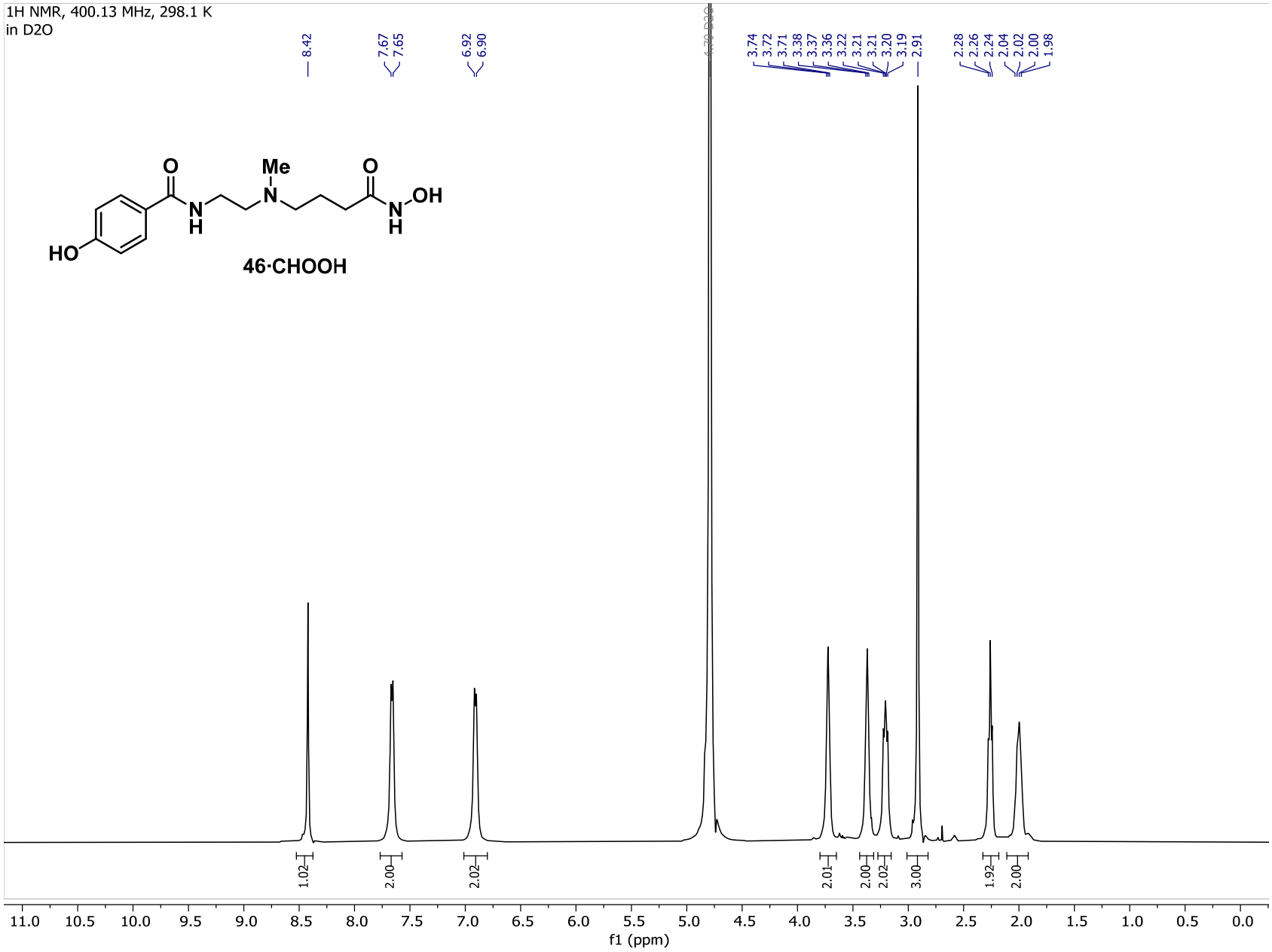

13C NMR, 100.62 MHz, 298.1 K
in DMSO
169.12
166.86
148.64
135.59
128.61
116.27
114.17
112.73
56.49
56.17
41.94
37.13
30.14
22.93
O
Me
O
H2N
N
N
OH
H
H
44
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-1
f1 (ppm)

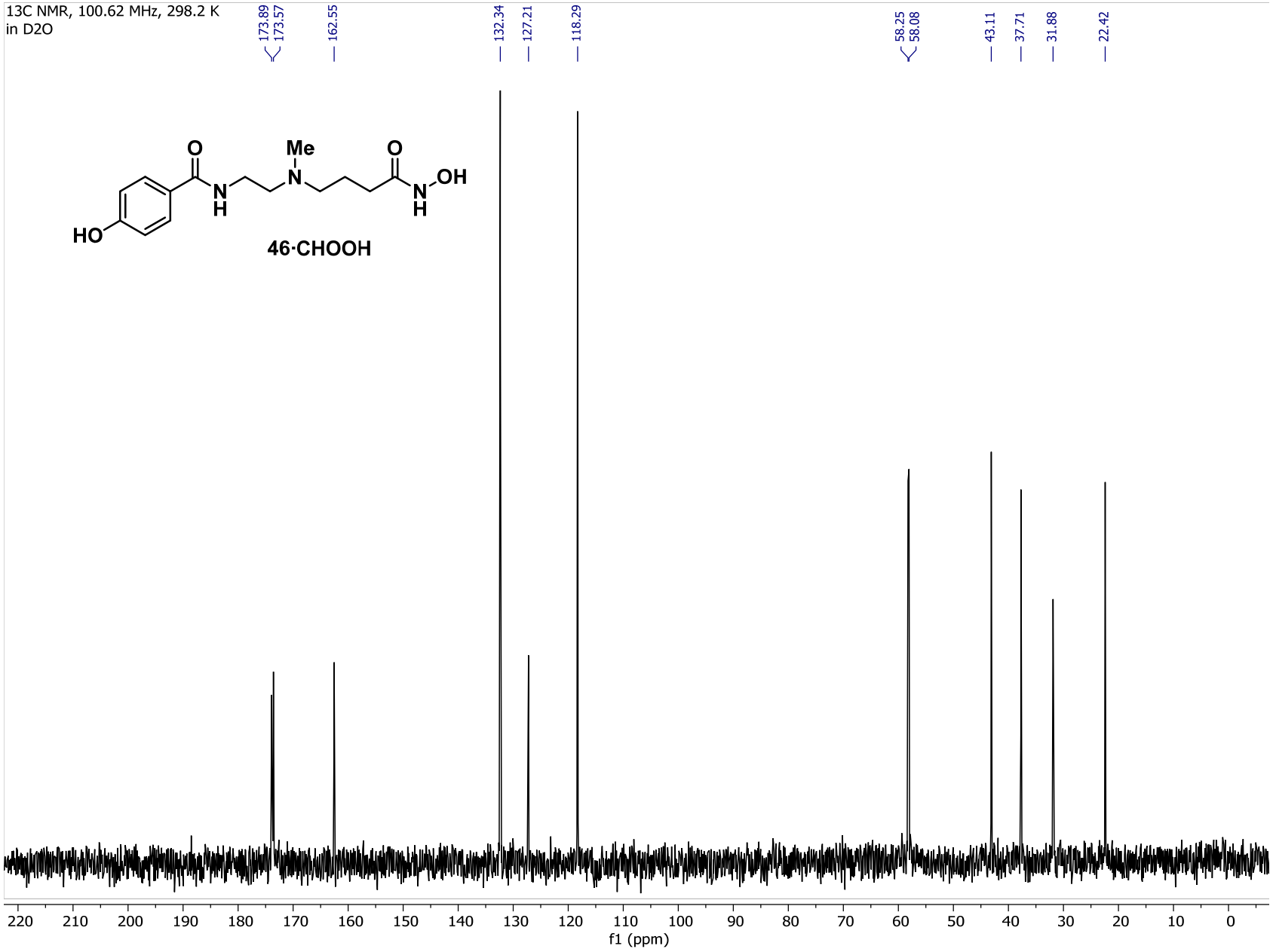

Basic Method PDA - Total Absorbance Chromatogram
2.72
98.9%
3.22
1.1%
Absorbance
25000
20000
15000
10000
5000
0
-5000
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
H2N
O
N
H
Me
N
O
N
H
OH
44
Basic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
2.72
98.3%
3.00
0.7%
3.21
1.0%
Absorbance (mAU)
900
800
700
600
500
400
300
200
100
0
-100
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5

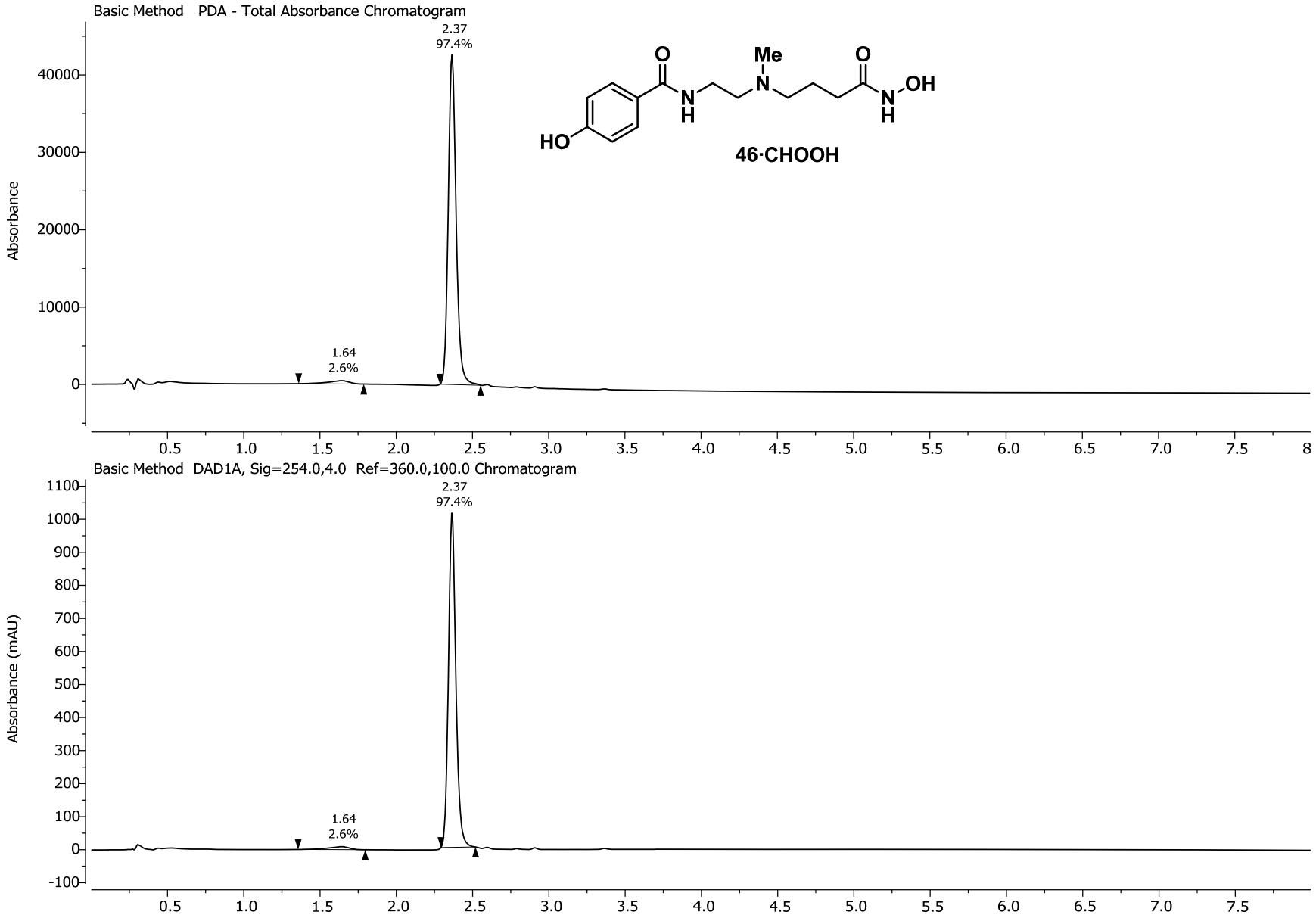

1H NMR, 400.13 MHz, 298.2 K
in DMSO
8.89
8.52
7.89
7.87
7.86
7.58
7.57
7.56
7.55
7.54
7.54
7.53
7.52
7.52
7.50
6.56
6.55
6.54
6.53
6.53
6.51
6.51
6.50
6.48
5.57
3.30
3.28
3.27
3.26
3.25
2.50 DMSO-d6
2.45
2.45
2.43
2.41
2.40
2.33
2.33
2.31
2.29
2.27
2.16
2.14
1.97
1.95
1.93
1.65
1.63
1.61
1.59
1.57
2.10
0.92
f1 (ppm)
O
Me
O
N
H
N
N
H
OH
H2N
45
2.10
0.92
1.99
1.95
1.95
2.27
1.98
2.14
3.00
1.83
2.00
f1 (ppm)

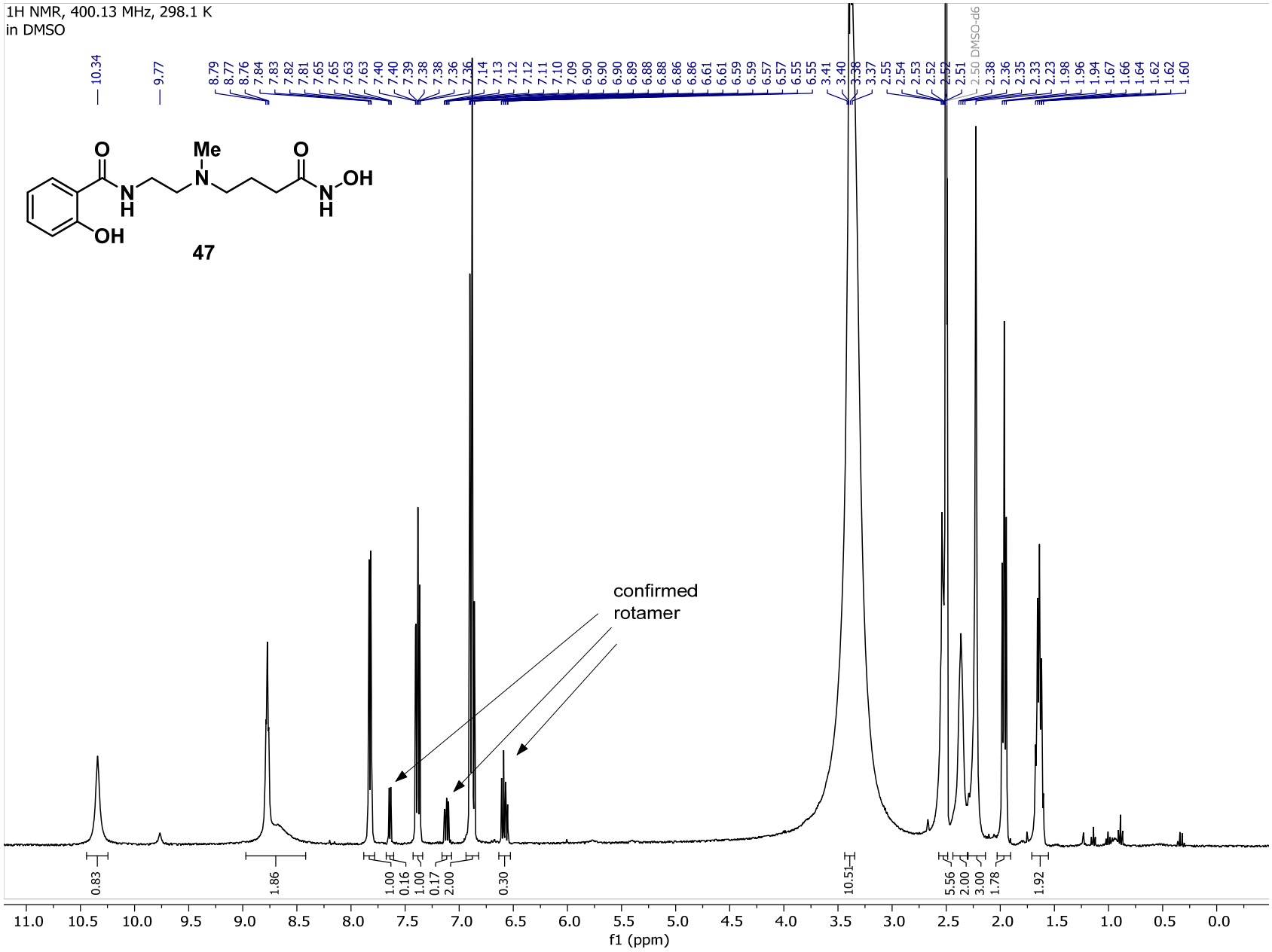

13C NMR, 100.62 MHz, 298.1 K
in DMSO
169.15
166.13
151.49
128.60
121.31
112.54
56.51
56.42
41.97
37.03
30.14
22.93
O
Me
O
N
H
N
N
H
OH
H2N
45
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-1
f1 (ppm)

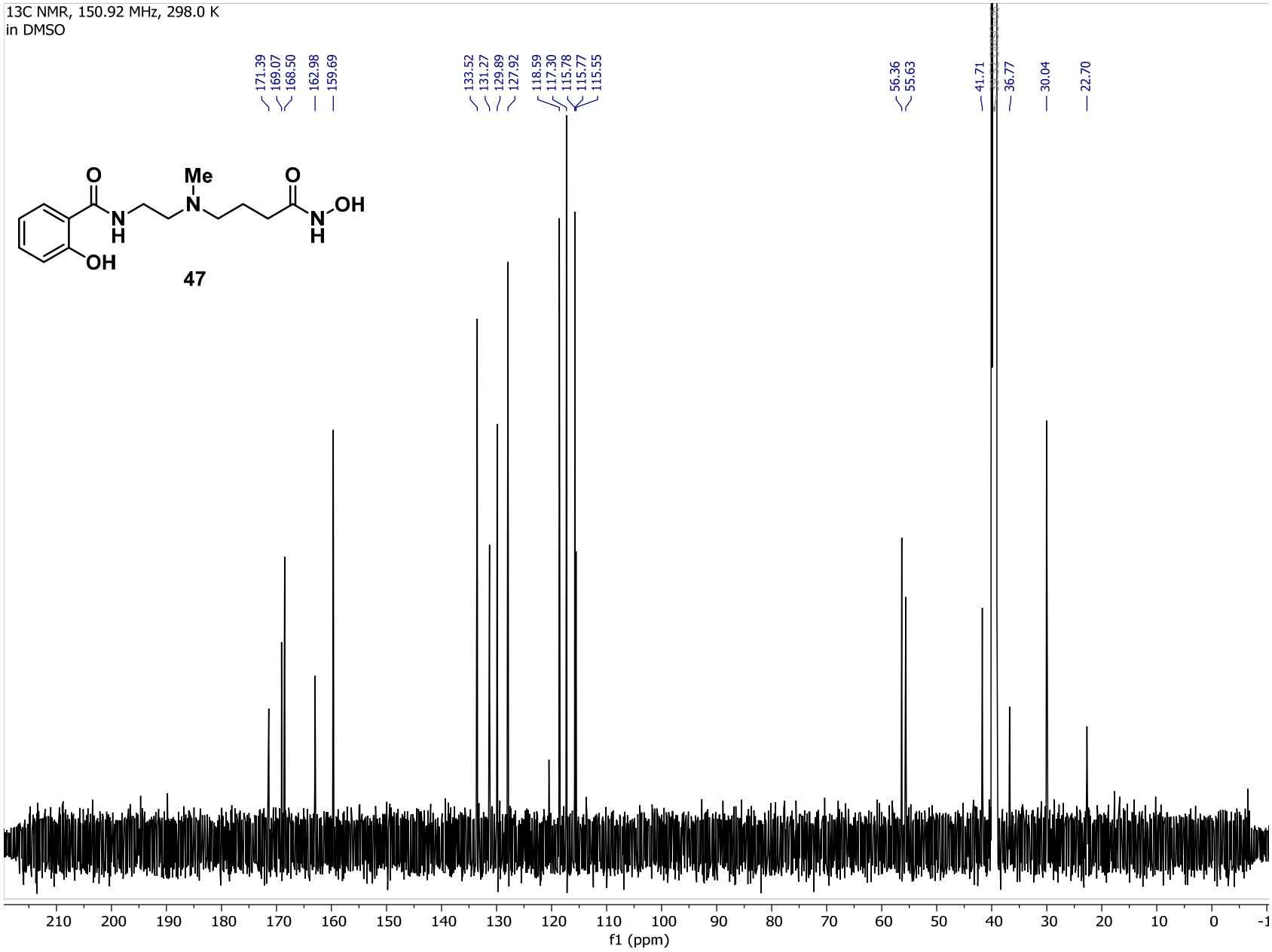

Basic Method PDA - Total Absorbance Chromatogram
Absorbance
2.66
98.2%
2.30
1.0%
3.14
0.8%
45
Basic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
Absorbance (mAU)
2.65
98.5%
2.29
0.8%
3.13
0.7%

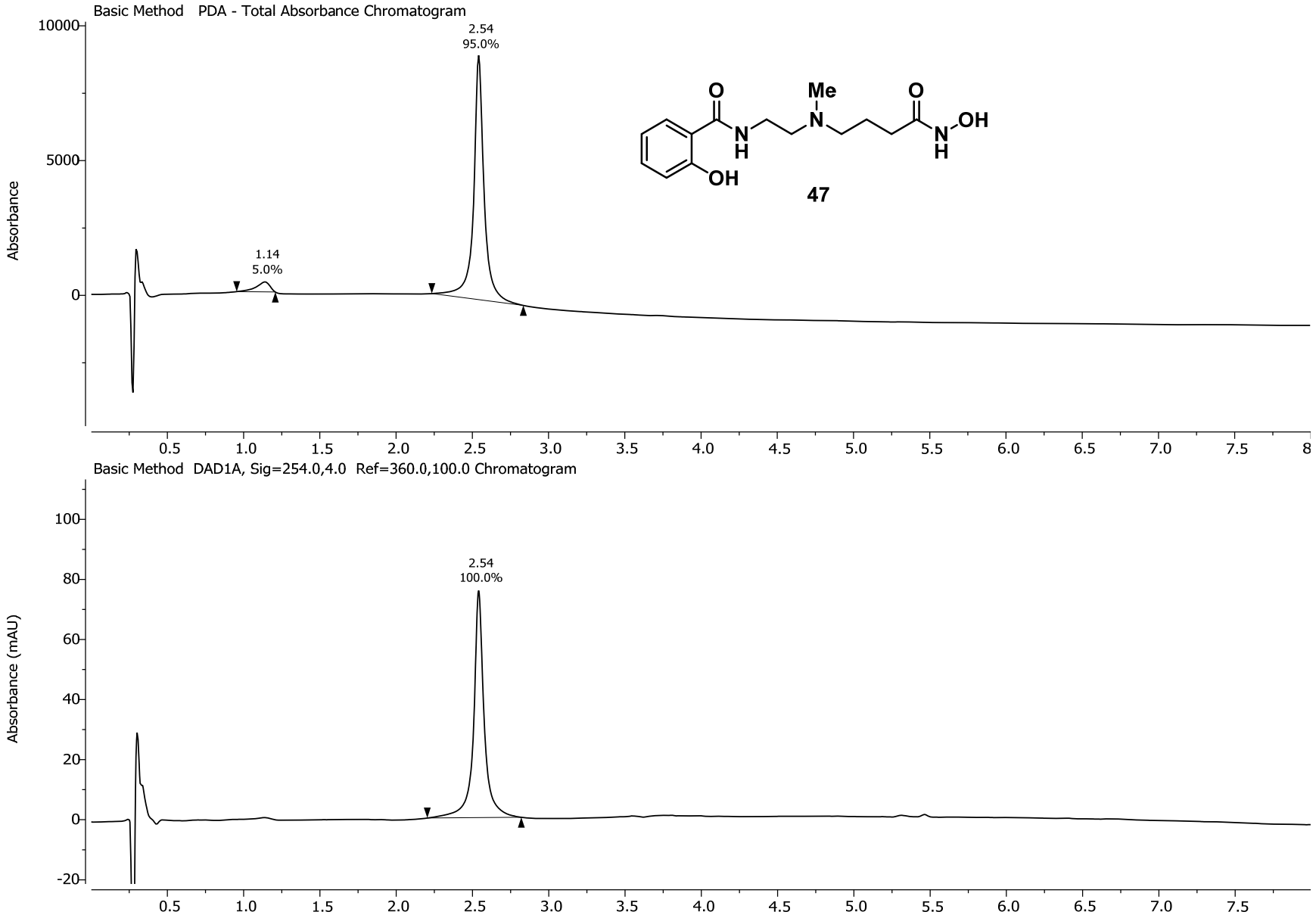

1H NMR, 400.13 MHz, 298.1 K
in D2O
8.42
7.67
7.65
6.92
6.90
4.79
3.74
3.72
3.71
3.38
3.37
3.36
3.22
3.21
3.21
3.20
3.19
2.91
2.28
2.26
2.24
2.04
2.02
2.00
1.98
O
Me
O
N
N
OH
H
H
HO
46·CHOOH
1.02
2.00
2.02
2.01
2.00
2.02
3.00
1.92
2.00
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

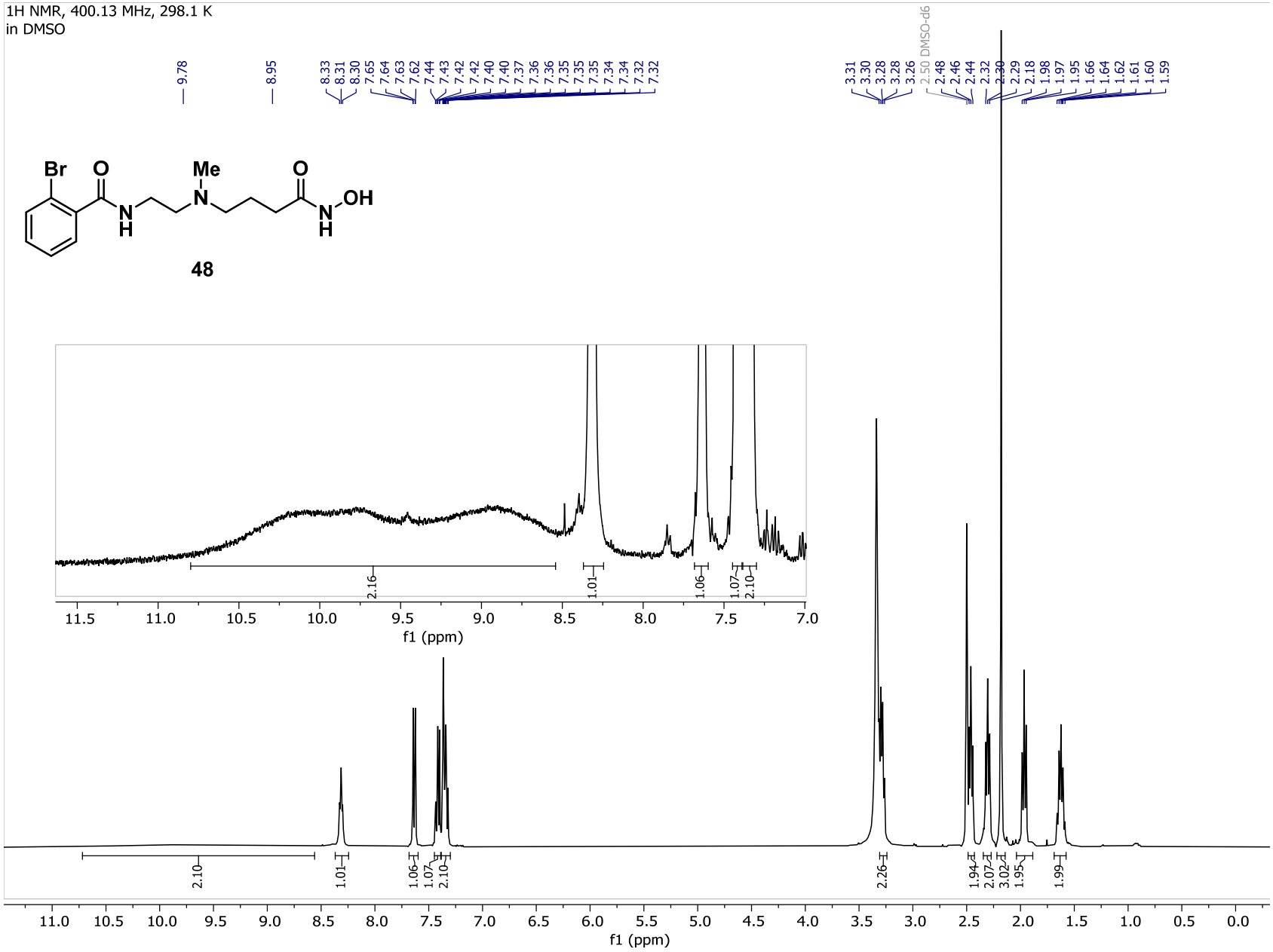

13C NMR, 100.62 MHz, 298.2 K
in D2O
173.89
173.57
162.55
132.34
127.21
118.29
58.25
58.08
43.11
37.71
31.88
22.42
O
Me
O
N
N
N
OH
H
H
HO
46·CHOOH
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

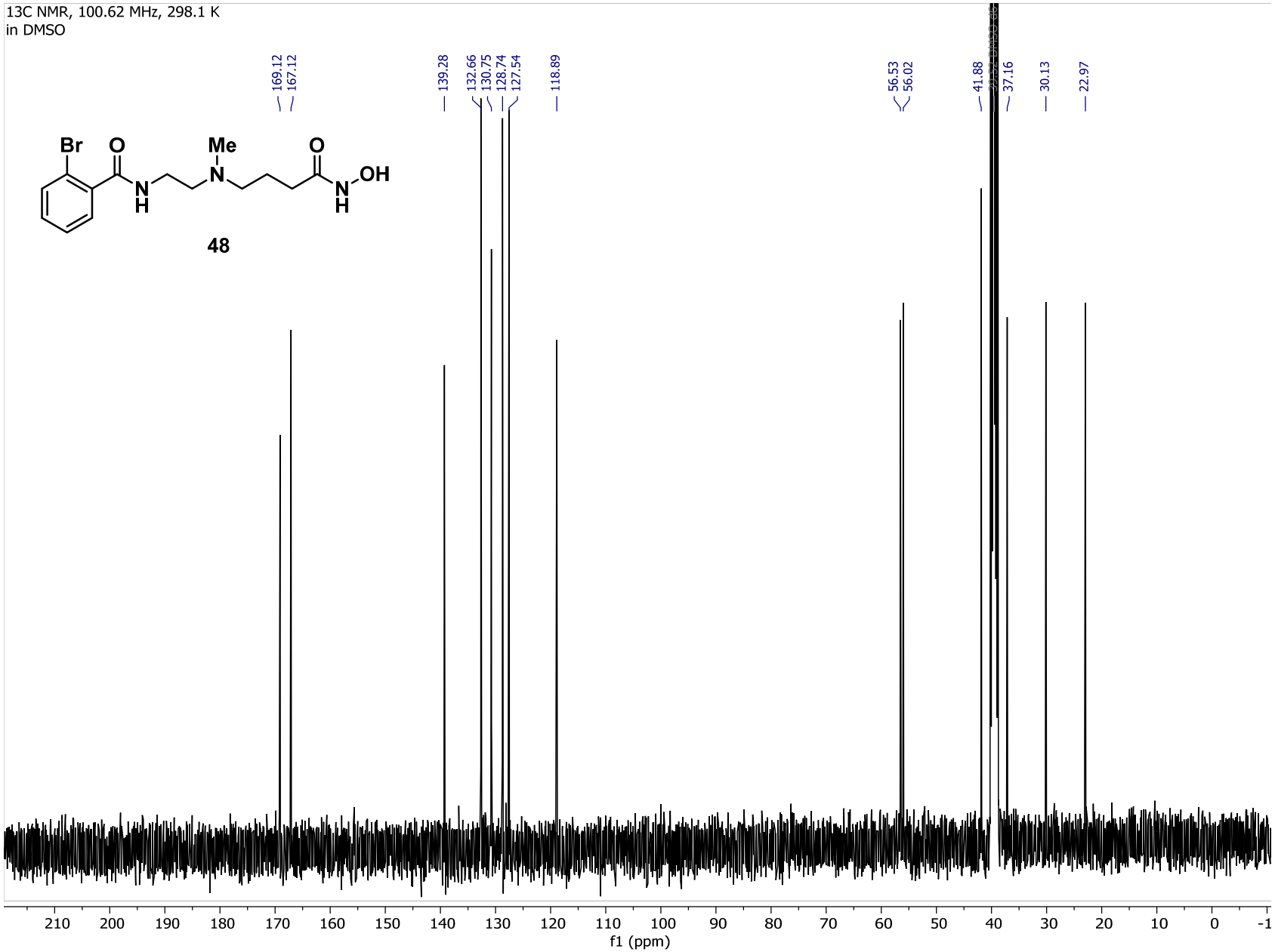

Basic Method PDA - Total Absorbance Chromatogram
2.37
97.4%
1.64
2.6%
Absorbance
40000
30000
20000
10000
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8
O
Me
O
N
N
OH
H
H
HO
46·CHOOH
Basic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
2.37
97.4%
1.64
2.6%
Absorbance (mAU)
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5

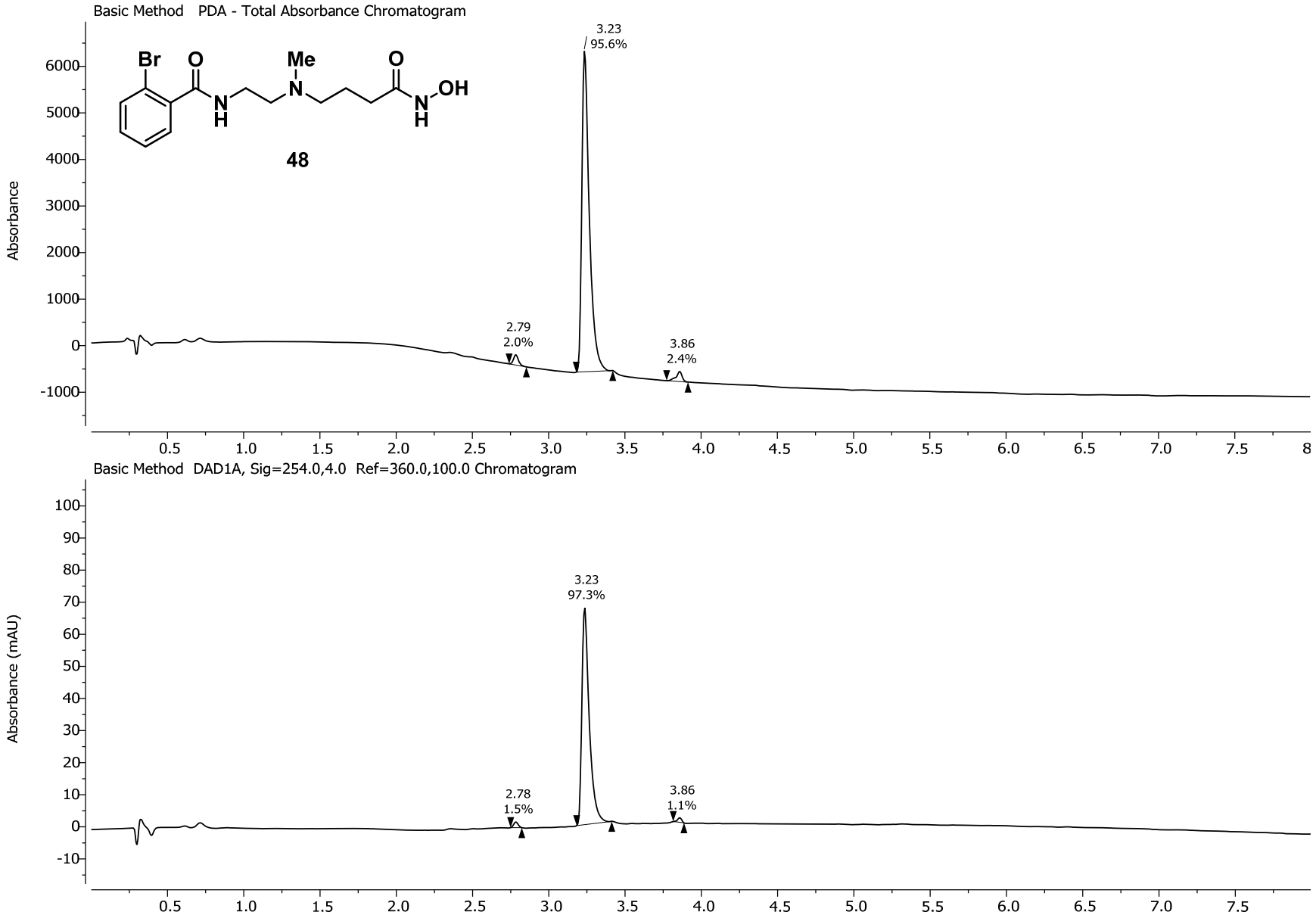

1H NMR, 400.13 MHz, 298.1 K
in DMSO
47
confirmed
rotamer
f1 (ppm)

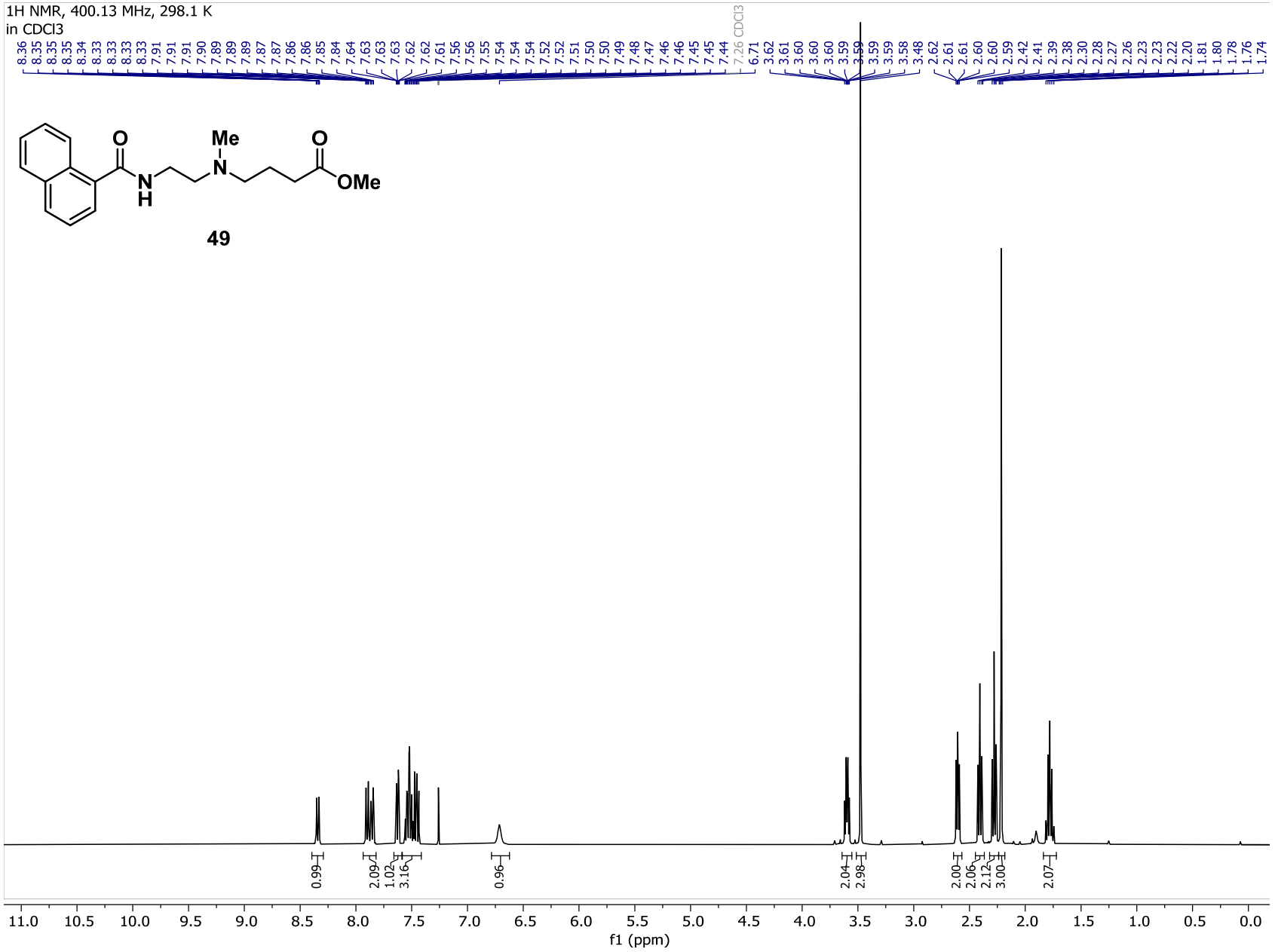

13C NMR, 150.92 MHz, 298.0 K
in DMSO
171.39
169.07
168.50
162.98
159.69
133.52
131.27
129.89
127.92
118.59
117.30
115.78
115.77
115.55
56.36
55.63
41.71
36.77
30.04
22.70
O
Me
O
N
N
OH
H
H
OH
47
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-1
f1 (ppm)

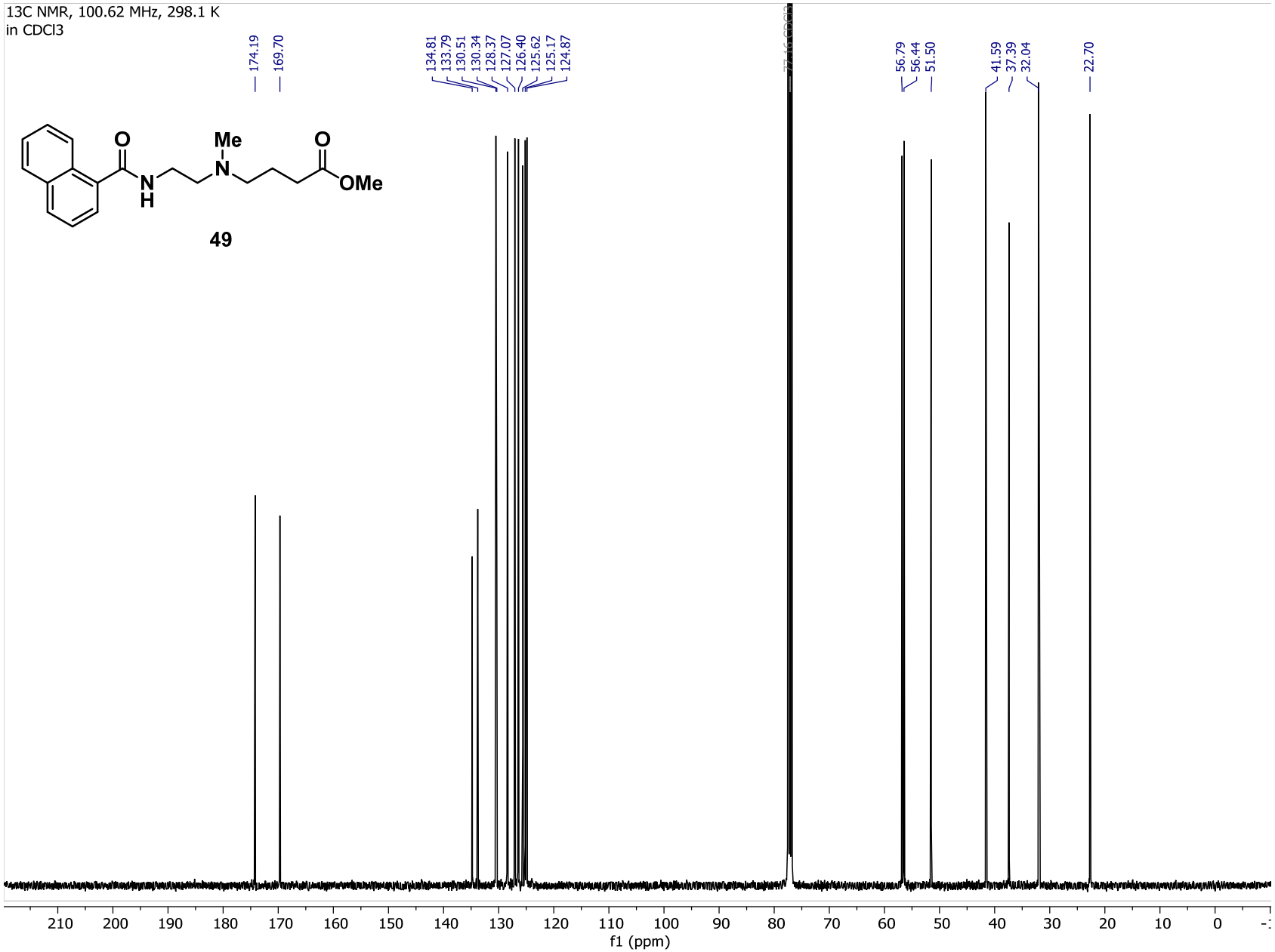

Basic Method PDA - Total Absorbance Chromatogram
Absorbance
10000
5000
0
2.54
95.0%
1.14
5.0%
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8
O
Me
O
N
H
N
N
H
OH
OH
47
Basic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
Absorbance (mAU)
100
80
60
40
20
0
-20
2.54
100.0%
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5

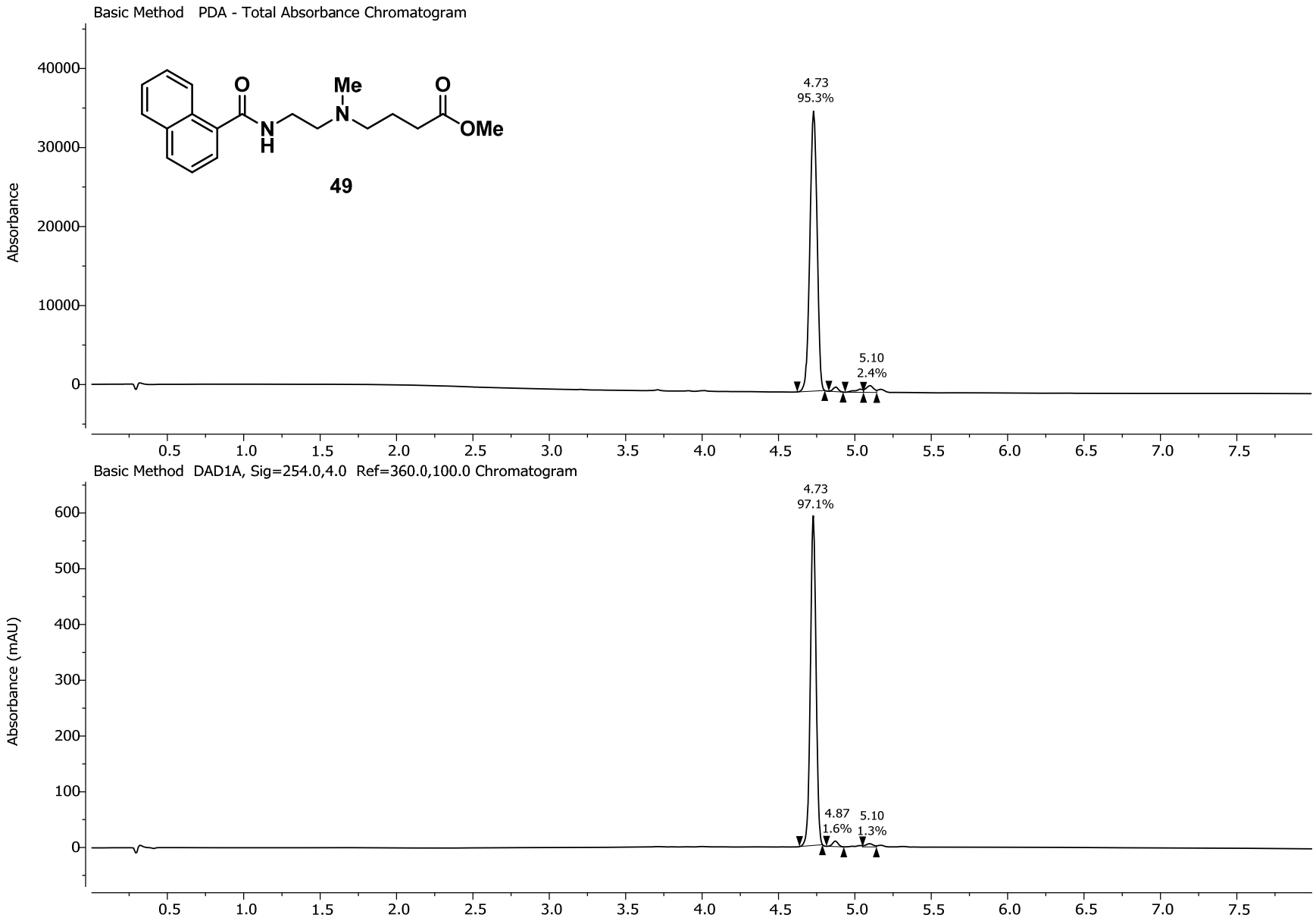

1H NMR, 400.13 MHz, 298.1 K
in DMSO
9.78
8.95
8.33
8.31
8.30
7.65
7.64
7.63
7.62
7.44
7.43
7.42
7.42
7.40
7.40
7.37
7.36
7.36
7.35
7.35
7.35
7.34
7.34
7.32
7.32
3.31
3.30
3.28
3.26
2.50 DMSO-d6
2.48
2.46
2.44
2.32
2.30
2.29
2.18
1.98
1.97
1.95
1.66
1.64
1.62
1.61
1.60
1.59
Br
O
Me
O
N
H
N
N
H
OH
48
2.16
1.01
1.06
1.07
2.10
f1 (ppm)
2.10
1.01
1.06
1.07
2.10
2.26
1.94
2.07
3.02
1.95
1.99
f1 (ppm)


13C NMR, 100.62 MHz, 298.1 K
in DMSO
169.12
167.12
139.28
132.66
130.75
128.74
127.54
118.89
56.53
56.02
41.88
37.16
30.13
22.97
Br
O
N
H
Me
N
O
N
H
OH
48
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-1
f1 (ppm)


Basic Method PDA - Total Absorbance Chromatogram
Br
O
Me
O
N
H
N
N
H
OH
48
3.23
95.6%
2.79
2.0%
3.86
2.4%
Absorbance
6000
5000
4000
3000
2000
1000
0
-1000
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
Basic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
3.23
97.3%
2.78
1.5%
3.86
1.1%
Absorbance (mAU)
100
90
80
70
60
50
40
30
20
10
0
-10
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5


1H NMR, 400.13 MHz, 298.1 K
in CDCl3
CDCl3
O
Me
O
N
N
H
OMe
49
0.99
2.09
1.02
3.16
0.96
2.04
2.98
2.00
2.06
2.12
3.00
2.07
f1 (ppm)


13C NMR, 100.62 MHz, 298.1 K
in CDCl3
174.19
169.70
134.81
133.79
130.51
130.34
128.37
127.07
126.40
125.62
125.17
124.87
77.16 CDCl3
56.79
56.44
51.50
41.59
37.39
32.04
22.70
O
Me
O
N
N
H
OMe
49
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


Basic Method PDA - Total Absorbance Chromatogram
Absorbance
40000
30000
20000
10000
0
O
Me
O
N
N
H
OMe
49
4.73
95.3%
5.10
2.4%
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
Basic Method DAD1A, Sig=254.0,4.0 Ref=360.0,100.0 Chromatogram
Absorbance (mAU)
600
500
400
300
200
100
0
4.73
97.1%
4.87
1.6%
5.10
1.3%
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5