Table of Contents

Full displacement model

$$\mathrm{NL} \underset{k_2\mathrm{L}}{\overset{k_1\mathrm{C}}{\rightleftharpoons}} \mathrm{NC}$$

Where:
N = N36MCA
L = C34DNP
C = C34

At t = 0 N_{Total} = NL

$$\frac{d[\mathrm{NL}]}{dt} = -k_1[\mathrm{C}][\mathrm{NL}] + k_2[\mathrm{L}][\mathrm{NC}]$$

$$let\ [\mathrm{NC}] = \mathrm{N}_{\mathrm{Total}} - [\mathrm{NL}]$$

$$\frac{d[\mathrm{NL}]}{dt} = -k_1[\mathrm{C}][\mathrm{NL}] + k_2[\mathrm{L}]\mathrm{N}_{\mathrm{Total}} - k_2[\mathrm{L}][\mathrm{NL}]$$

$$\frac{d[\mathrm{NL}]}{dt} = -\left(k_1[\mathrm{C}] + k_2[\mathrm{L}]\right)[\mathrm{NL}] + k_2[\mathrm{L}]\mathrm{N}_{\mathrm{Total}}$$

$$\int \frac{d[\mathrm{NL}]}{-\left(k_1[\mathrm{C}] + k_2[\mathrm{L}]\right)[\mathrm{NL}] + k_2[\mathrm{L}]\mathrm{N}_{\mathrm{Total}}} = \int dt$$

$$-\frac{1}{k_1[\mathrm{C}] + k_2[\mathrm{L}]} \ln \frac{\left(k_1[\mathrm{C}] + k_2[\mathrm{L}]\right)[\mathrm{NL}] - k_2[\mathrm{L}]\mathrm{N}_{\mathrm{Total}}}{k_1[\mathrm{C}]\mathrm{N}_{\mathrm{Total}}} = t \quad (\text{at t} = 0\ [\mathrm{NL}] = \mathrm{N}_{\mathrm{Total}})$$

$$let\ k_{obs} = k_1[\mathrm{C}] + k_2[\mathrm{L}]$$

$$\left(k_1[\mathrm{C}] + k_2[\mathrm{L}]\right)[\mathrm{NL}] - k_2[\mathrm{L}]\mathrm{N}_{\mathrm{Total}} = k_1[\mathrm{C}]\mathrm{N}_{\mathrm{Total}} e^{-k_{obs}t}$$

$$[\mathrm{NL}] = \frac{\left(k_1[\mathrm{C}]e^{-k_{obs}t} + k_2[\mathrm{L}]\right)\mathrm{N}_{\mathrm{Total}}}{k_1[\mathrm{C}] + k_2[\mathrm{L}]}$$

$$let\ [\mathrm{NL}] = \mathrm{N}_{\mathrm{Total}} - [\mathrm{NC}]$$

$$[\mathrm{NL}] = \frac{\left(k_1[\mathrm{C}]\mathrm{e}^{-k_{obs}t} + k_2[\mathrm{L}]\right)\mathrm{N}_{\mathrm{Total}}}{k_1[\mathrm{C}] + k_2[\mathrm{L}]}$$

$$\mathrm{N}_{\mathrm{Total}} - [\mathrm{NC}] = \frac{\left(k_1[\mathrm{C}]\mathrm{e}^{-k_{obs}t} + k_2[\mathrm{L}]\right)\mathrm{N}_{\mathrm{Total}}}{k_1[\mathrm{C}] + k_2[\mathrm{L}]}$$

$$[\mathrm{NC}] = \left(1 - \frac{\left(k_1[\mathrm{C}]\mathrm{e}^{-k_{obs}t} + k_2[\mathrm{L}]\right)}{k_1[\mathrm{C}] + k_2[\mathrm{L}]}\right)\mathrm{N}_{\mathrm{Total}}$$

$$[\mathrm{NC}] = \left(\frac{k_1[\mathrm{C}] + k_2[\mathrm{L}] - k_1[\mathrm{C}]\mathrm{e}^{-k_{obs}t} - k_2[\mathrm{L}]}{k_1[\mathrm{C}] + k_2[\mathrm{L}]}\right)\mathrm{N}_{\mathrm{Total}}$$

$$[\mathrm{NC}] = \frac{k_1[\mathrm{C}]}{k_1[\mathrm{C}] + k_2[\mathrm{L}]}\mathrm{N}_{\mathrm{Total}}\left(1 - \mathrm{e}^{-k_{obs}t}\right)$$

$$at\ t = 0 \quad [\mathrm{NC}] = 0$$

$$at\ t = \infty \quad [\mathrm{NC}] = \frac{\mathrm{N}_{\mathrm{Total}}[\mathrm{C}]}{[\mathrm{C}] + \frac{k_2}{k_1}[\mathrm{L}]}$$

This is our original thermodynamic equation f = Fmax[C]/([C]+α[L])

General Procedures for Organic Synthesis

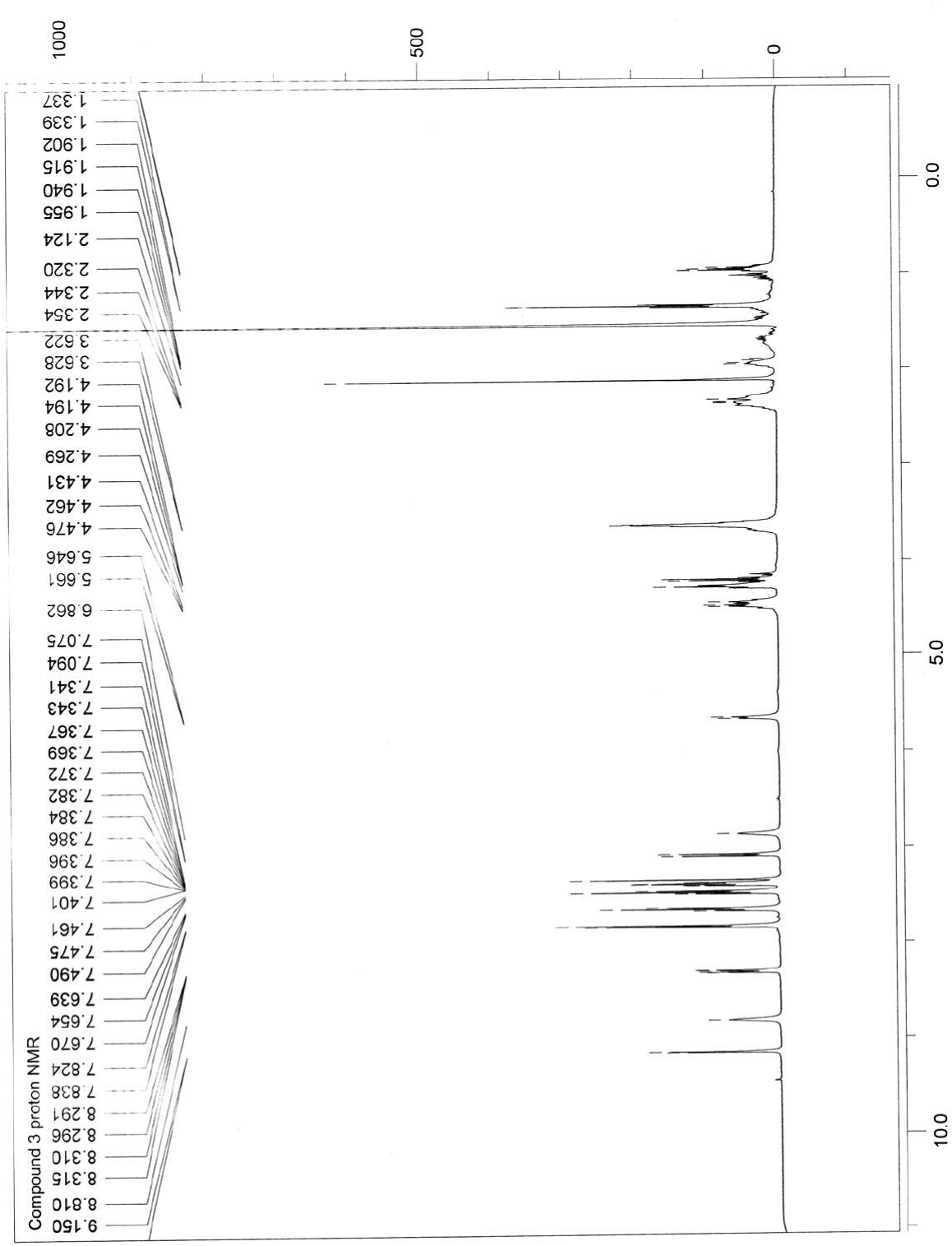
The following abbreviations were used to explain the multiplicities in NMR spectra: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad. All reactions were monitored by thin-layer chromatography (TLC) performed on 0.25 mm silicagel plates, with fractions being visualized by UV light. Reagent grade solvents for chromatography were obtained from commercial sources unless specified otherwise. Reagents were purchased at the highest commercial quality and used without further purification. All reactions were carried out under an argon atmosphere, unless otherwise noted. Reported yields were determined after purification for a homogenous material.

General Procedures for Peptide Synthesis

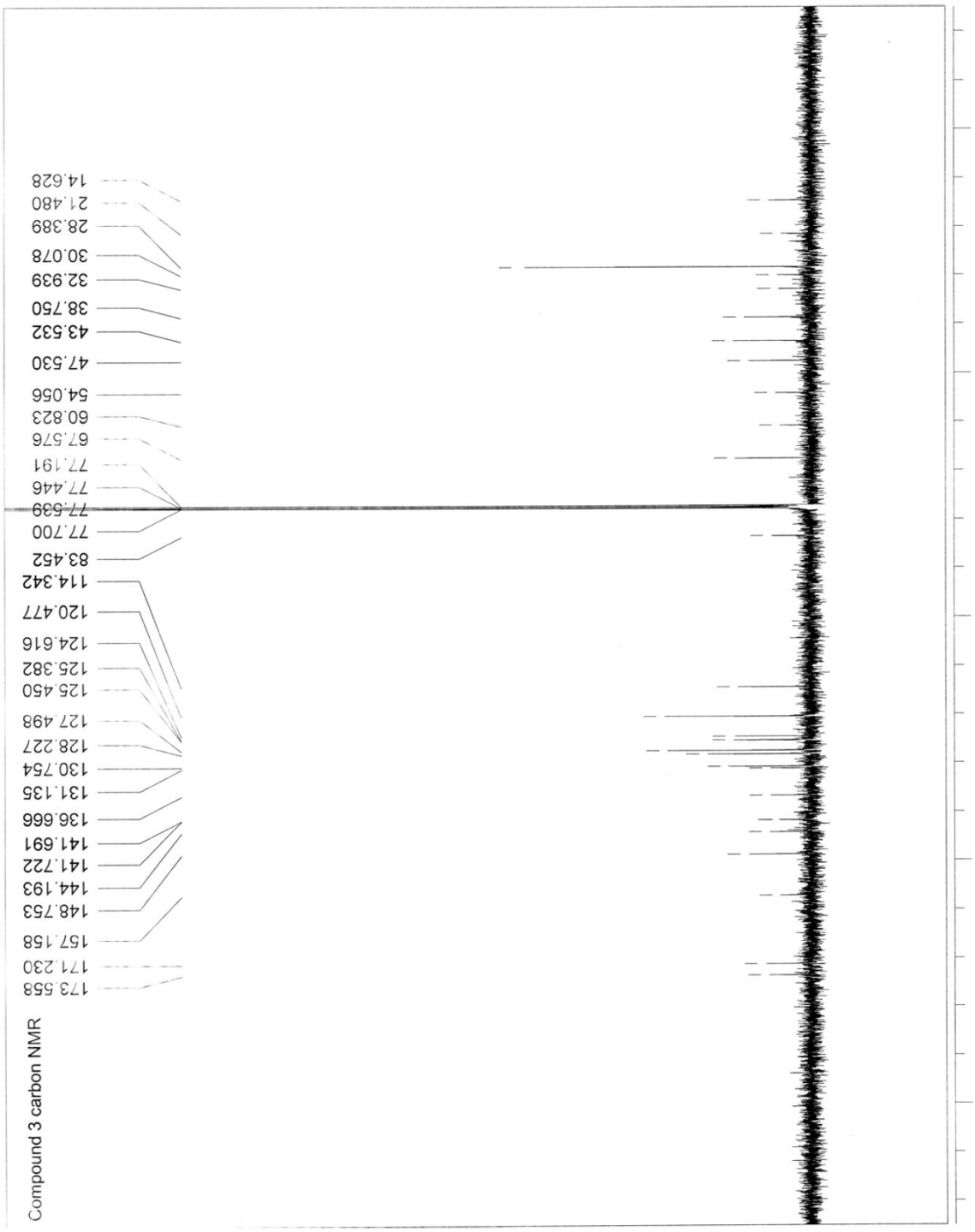
Acrylamide, *N,N,N,N*-tetramethylethylenediamine (TEMED), ammonium persulfate (APS) , Fmoc amino acids, the Rink Amide Linker, and Aminomethylpolystyrene resin (PL-AMS) at a loading of 2.6 mmol NH_2/g (75-150 *i*M) were obtained from commercial sources. Side chain protections were as follows: Lys (Mtt), Arg (Pbf); Asp (^{t}Bu), Asn (Trt), Glu (^{t}Bu), Gln (Trt), Lys (Mtt), Trp (Boc), Thr (^{t}Bu), Ser (^{t}Bu). All other amino acids were incorporated without side chain protection. RP-HPLC was performed using binary gradients of solvents A and B, where A is 0.1% TFA in water and B is 0.09% TFA in acetonitrile. Analytical RP-HPLC was performed at a flow rate of 1 mL/min, with detection at 214 nm during a linear gradient of 30-70% B over 30 min. Preparative RP-HPLC was performed at a flow rate of 10 mL/min, with detection at 220 nm during a linear gradient of 40-60%B over 40 min. In all cases, fractions were analyzed offline

using amass spectrometer and judged for purity after a consistent summing of 50 scans in multichannel analysis (MCA) mode. For preparative purification purposes, fractions that contained no consistent charged species which accounted for more than 10% of the total ion intensity were designated “pure” and pooled; the homogeneity of this pool was verified by analytical RP-HPLC and was >90%. All purification and synthetic manipulation steps were performed at ambient temperature unless otherwise indicated.

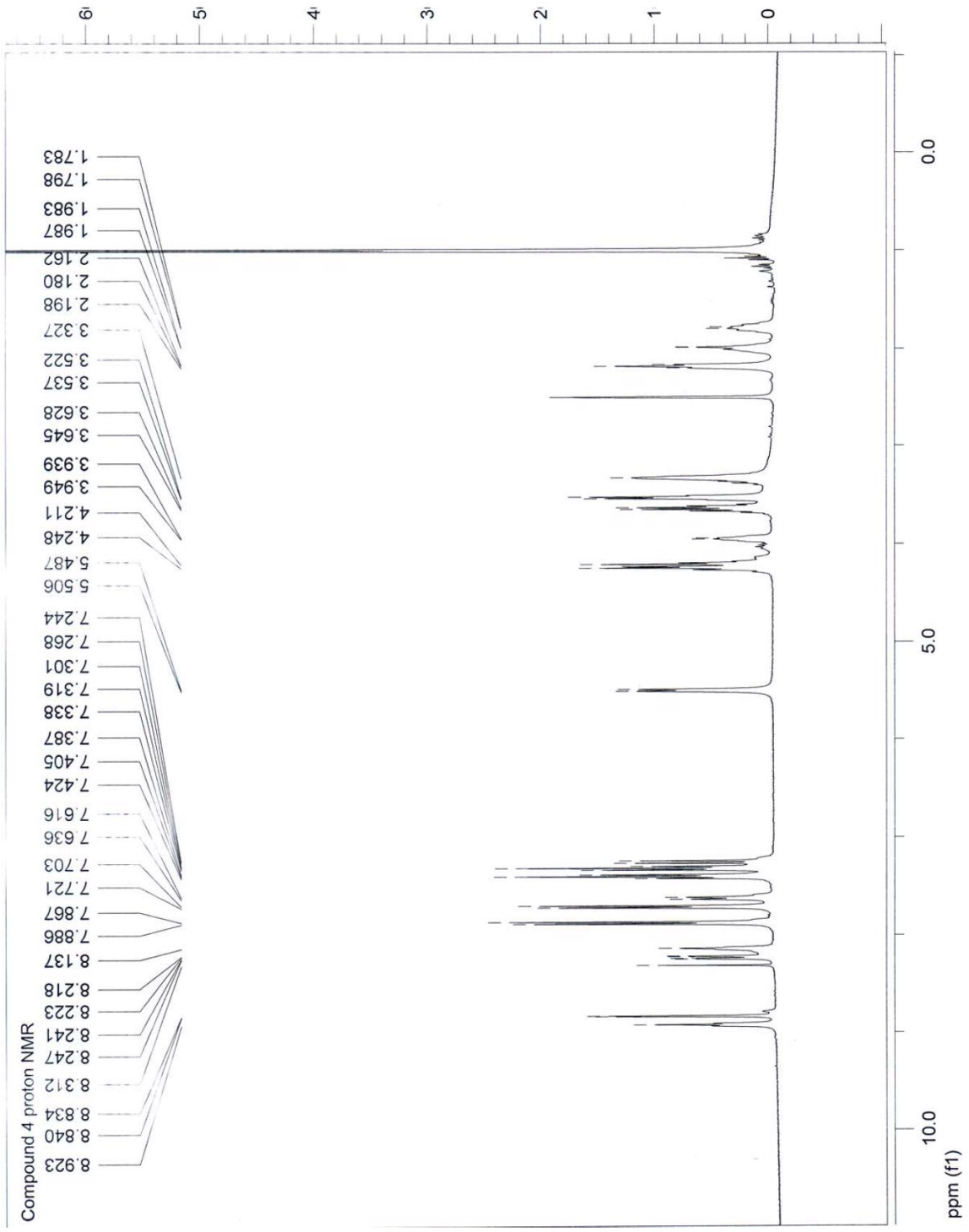
[1]HNMR of *tert*-butyl 2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-5-(2-(2,4-dinitrophenylamino)ethylamino)-5-oxopentanoate



^{13}CNMR of *tert*-butyl 2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-5-(2-(2,4-dinitrophenylamino)ethylamino)-5-oxopentanoate



^{1}H NMR of Compound 3



^{13}C NMR of Compound 3