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Citation: Zheng, Tengfei, Justin L. Bullock, and Elizabeth M. Nolan. "Siderophore-Mediated Cargo Delivery to the Cytoplasm of Escherichia coli and Pseudomonas aeruginosa: Syntheses of Monofunctionalized Enterobactin Scaffolds and Evaluation of Enterobactin–Cargo Conjugate Uptake." Journal of the American Chemical Society 134, no. 44 (November 7, 2012): 18388-18400.

As Published: http://dx.doi.org/10.1021/ja3077268

Publisher: American Chemical Society (ACS)

Persistent URL: http://hdl.handle.net/1721.1/83505

Version: Author's final manuscript: final author's manuscript post peer review, without publisher's formatting or copy editing

Siderophore-Mediated Cargo Delivery to the Cytoplasm of *Escherichia coli* and *Pseudomonas aeruginosa*: Syntheses of Monofunctionalized Enterobactin Scaffolds and Evaluation of Enterobactin-Cargo Conjugate Uptake

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Abstract

The design and syntheses of monofunctionalized enterobactin (Ent, L- and D-isomers) scaffolds where one catecholate moiety of enterobactin houses an alkene, aldehyde, or carboxylic acid moiety at the C5 position are described. These molecules are key precursors to a family of ten enterobactin-cargo conjugates presented in this work, which were designed to probe the extent to which the Gram-negative ferric enterobactin uptake and processing machinery recognizes, transports, and utilizes derivatized enterobactin scaffolds. A series of growth recovery assays employing enterobactin-deficient *E. coli* ATCC 33475 (*ent*-) revealed that six conjugates based on L-Ent having relatively small cargos promoted *E. coli* growth under iron-limiting conditions whereas negligible-to-no growth recovery was observed for four conjugates with relatively large cargos. No growth recovery was observed for the enterobactin receptor deficient strain of *E. coli* H1187 (*fepA-) or* the enterobactin esterase-deficient derivative of *E. coli* K-12 JW0576 *(fes*-), or when the D-isomer of enterobactin was employed. These results demonstrate that the *E. coli* ferric enterobactin transport machinery identifies and delivers select cargo-modified scaffolds to the *E. coli* cytoplasm. *Pseudomonas aeruginosa* PAO1 K648 (*pvd*-, *pch*-) exhibited greater promiscuity than that of *E. coli* for the uptake and utilization of the enterobactin-cargo conjugates, and growth promotion was observed for eight conjugates under iron-limiting conditions. Enterobactin may be utilized for delivering molecular cargos via its transport machinery to the cytoplasm of *E. coli* and *P. aeruginosa* thereby providing a means to overcome the Gram-negative outer membrane permeability barrier.

Introduction

Siderophores are low-molecular-weight high-affinity Fe(III) chelators that are biosynthesized and exported by bacteria, fungi, and plants during periods of nutrient limitation for acquiring this essential metal ion from the extracellular milieu.[1,2] Both naturally-occurring and synthetic siderophore mimics are useful for bioremediation,[3] iron chelation therapies,[4,5] antibiotic drug-delivery strategies,[6-14] Fe(III) detection,[15-18] protein identification,[19] and pathogen capture.[20, 21] These types of applications benefit from or require siderophores amenable to facile and site-specific synthetic modification. In this work, we expand the current toolkit of site-specifically modifiable siderophore scaffolds to include triscatecholate enterobactin, and we report that various synthetic enterobactin-cargo conjugates are actively transported to the cytoplasm of the Gram-negative bacterial species *Escherichia coli* and *Pseudomonas aeruginosa* by the enterobactin uptake machinery.

Enterobactin (Ent, **1**, Figure 1A) is a canonical siderophore biosynthesized by Gram-negative species of *Enterobacteriaceae* that include *Escherichia coli*, *Salmonella*, and *Klebsiella*.[22] Decades of exploration pertaining to enterobactin biosynthesis and coordination chemistry, in addition to investigations of the proteins involved in its cellular transport and processing, provide a detailed molecular and physiological understanding of how this chelate contributes to bacterial iron homeostasis and colonization.[22] The enterobactin synthetase is comprised of four proteins, EntBDEF, and is responsible for the production of enterobactin from L-serine and 2,3-dihydroxybenzoic acid (DHB).[23] Following biosynthesis, Ent is exported into the extracellular space where it scavenges Fe(III). Enterobactin coordinates Fe(III) by its three catecholate groups with $K_a \sim 10^{49}$ M^{-1}.[24] In *E. coli*, the outer membrane transporter FepA (and to a lesser extent Cir and Fiu) recognizes and binds ferric enterobactin with sub-nanomolar affinity,[25,26] and provides periplasmic entry where the siderophore forms a complex with the periplasmic binding protein FepB.[27] Subsequently, $[Fe(Ent)]^{3-}$ is transported into the cytosol, which requires the action of ExbBD, TonB, and FepCDG, the latter of which constitute the inner-membrane ATP-binding cassette (ABC) transporter system (Figure 1B).[28-32] Fes, the cytosolic

enterobactin esterase, catalyzes the hydrolysis of the $[Fe(Ent)]^{3-}$ macrolactone,[33] and the ferric reductase YgjH may subsequently assist in Fe(III) release such that the metal ion can be used metabolically.[34] Several pathogenic Gram-negative species harbor gene clusters (e.g. *iroA*, MccE492) responsible for post-assembly line modifications of the enterobactin scaffold to provide the salmochelins.[33,35-38] Salmochelins are a family of glucosylated enterobactin derivatives where the sugar moieties are attached to the C5 position of one or more catecholate rings (e.g. MGE **2** and DGE **3**, Figure 1A).[39]

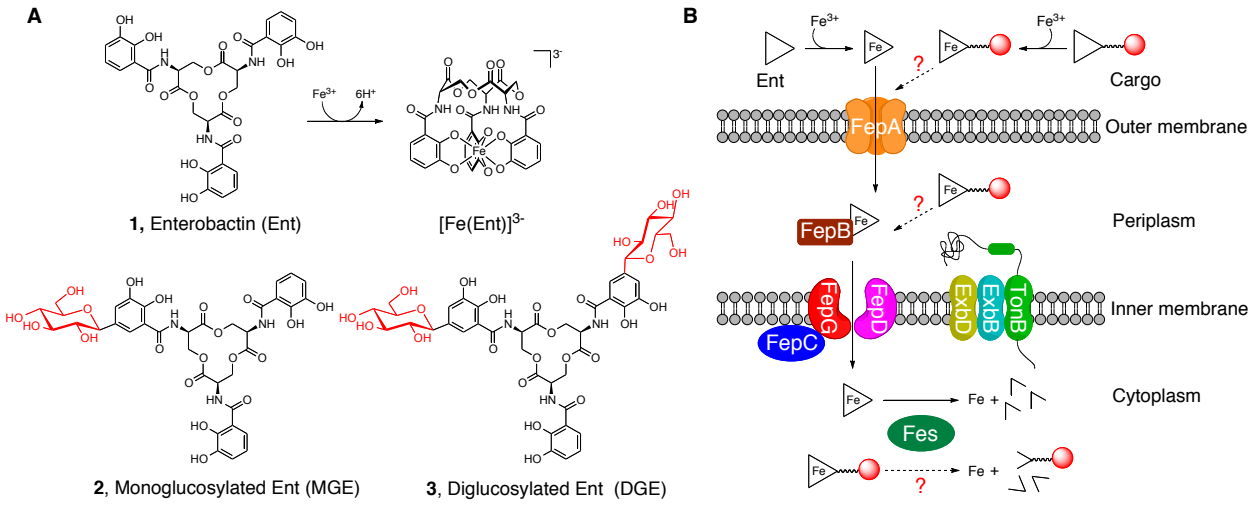


Figure 1. Siderophores and siderophore transport machinery relevant to this work. (A) Structures of enterobactin **1** and the salmochelins MGE **2** and DGE **3**. (B) Cartoon depiction of the enterobactin transport and processing machinery in *E. coli*.

Gram-negative bacteria have an outer membrane that serves as a permeability barrier and prevents cellular entry of many molecules, including antibiotics (e.g. vancomycin). Siderophore uptake machinery provides one route to overcome this permeability barrier,[6-14] and enterobactin and its transporter FepA have been identified as a desirable siderophore/receptor pair for cargo delivery to Gram-negative bacterial species.[13,37] FepA-mediated uptake of the ribosomal peptide antibiotics colicin B[40] and MccE492m,[41] in addition to bacteriophage,[42]

indicates that this receptor has the capacity to transport large molecules. Moreover, the catecholate siderophore transporters of *E. coli* (e.g. Fiu, Cir) recognize synthetic catechol-modified β-lactam antibiotics;[43-46] these serendipitous observations motivated early "Trojan horse" delivery strategies. Indeed, small-molecule antibiotics appended to siderophore-inspired di- and tricatecholate platforms have been evaluated for antibacterial activity with mixed results.[47-51] Most recently, amoxicillin and ampicillin, β-lactam antibiotics that act in the periplasm and target bacterial cell wall biosynthesis, were covalently linked to a tripodal catecholate platform and remarkably afforded ca. 10^2- to 10^3-fold enhanced activity against *P. aeruginosa* PAO1 compared to the free drug.[49]

The ability of FepABCDG and the TonB-ExbB-ExbD system of *E. coli*, as well as the enterobactin transport machinery of other bacterial species, to recognize and provide cytosolic transport of unnatural cargo appended to the native ligand remains unexplored. Enterobactin exhibits C_3 symmetry and houses no unique functional group for site-specific synthetic modification. Total syntheses of enterobactin,[52-56] hydrolytically stable enterobactin analogs,[57-60] and salmochelins[61] have been reported. To the best of our knowledge, no enterobactin scaffold housing a site-specific synthetic handle has been presented. Such scaffolds are a pre-requisite for employing enterobactin in a variety of paradigms that include cargo delivery, iron and siderophore detection, and bacterial capture.

Herein we present a family of ten enterobactin-cargo conjugates that are based on a monofunctionalized enterobactin scaffold. Inspired by the salmochelins, we have derivatized enterobactin at the C5 position of the catecholate, which provides a point for site-specific modification without compromising the Fe(III)-binding groups or the macrolactone (Figure 2). Moreover, we report that the ferric enterobactin uptake machineries of *Escherichia coli* and *Pseudomonas aeruginosa* PAO1 deliver enterobactin-derivatized cargo to the cytoplasm of both species under iron deficient conditions, and that cargo size is an important and species/strain-specific parameter to evaluate in enterobactin conjugate design.

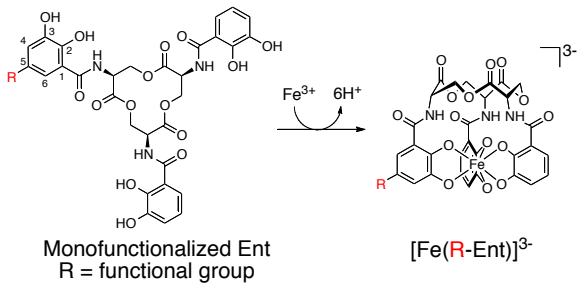


Figure 2. Enterobactin substituted at the C5 position.

Experimental

Reagents. Dimethylformamide (DMF) and dichloromethane (CH_2Cl_2) were dried over 4 Å molecular sieves or by using a VAC solvent purification system (Vacuum Atmospheres). Anhydrous dimethyl sulfoxide (DMSO) was purchased from Sigma-Aldrich and used as received. The triserine lactone **4** and its D-isomer **5** were synthesized according to a literature procedure.[55] 2,3-Bis(benzyloxy)benzoic acid **6**,[62] vancomycin-alkyne **7**,[63] and *tert*-butyl (2-oxo-2-(prop-2-yn-1-ylamino)ethyl)carbamate **8**,[63] were synthesized according to literature procedures. L-Ent **1** and its D-isomer **9** were synthesized as reported elsewhere.[55,56] *Tert*-butyl 3-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)propanoate **10** was purchased from BOC Sciences (Shirley, NY), 11-azido-3,6,9-trioxaundecan-1-amine **11** was purchased from Fluka, 6-((*tert*-butyoxycarbonyl)amino)hexanoic acid **12** was purchased from Advanced Chem Tech, and Fmoc-PEG-CO_2H **13** was purchased from Chem-Impex International, Inc. The syntheses of the PEG-derivatized cargos **14-18** are provided as Supporting Information. Methyl-5-allyl-3-methoxysalicylate **19** was obtained from Sigma Aldrich. All other chemicals were purchased from Sigma-Aldrich, Alfa Aesar, or TCI in the highest available purity and used as received.

General Synthetic Materials and Methods. EMD TLC silica gel 60 F_{254} plates were used for analytical thin-layer chromatography. EMD PLC silica gel 60 F_{254} plates of 1-mm thickness were used for preparative TLC. Zeoprep 60HYD silica gel (40-63 μm) obtained from Zeochem was used for flash chromatography. ^{1}H, ^{19}F, and ^{13}C NMR spectra were collected on a Varian

300 or 500 MHz spectrophotometer, which were operated at ambient probe temperature (283 K) and housed in the Department of Chemistry Instrumentation Facility. The ^{1}H and ^{13}C NMR spectra were referenced to internal standards and ^{19}F spectra were referenced to an external CF_3Cl standard. An Avatar FTIR instrument was used to acquire IR spectra. Optical absorption spectra were recorded on an Agilent 8453 diode array spectrophotometer (1-cm quartz cuvettes, Starna). General methods for high performance liquid chromatography and mass spectrometry, ^{1}H and ^{13}C NMR spectra, and IR spectroscopic data are provided as Supporting Information.

Methyl-5-allyl-2,3-dihydroxybenzoate (20). Methyl-5-allyl-3-methoxysalicylate (**19**, 2.22 g, 10.0 mmol) and anhydrous *N*,*N*-diisopropylethylamine (DIPEA, 1.94 g, 15.0 mmol) were dissolved in 125 mL of dry CH_2Cl_2 and stirred at rt for five min. The solution was cooled to −78 °C in an acetone/dry ice bath, and boron tribromide (BBr_3, 1M solution in CH_2Cl_2, 30 mL, 30 mmol) was added slowly over ca. 10 min via a syringe to afford a yellow solution. The reaction was stirred at −78 °C for 1 h, warmed to −30 °C over the course of 1 h, and subsequently warmed to rt and stirred for another 4.5 h. Water (200 mL) was added slowly to quench the reaction, and the organic phase was washed with saturated aqueous potassium bicarbonate (K_2CO_3, 3 x 100 mL). The organic phase was dried over sodium sulfate (Na_2SO_4), and the solvent was removed under reduced pressure to afford a brown oil. Flash chromatography on silica gel with a solvent gradient (100% hexanes to 20% EtOAc/hexanes) gave the product as a white solid (1.09 g, 53%). TLC R_f =0.5 (silica, CH_2Cl_2); mp = 55-56 ^{o}C. ^{1}H NMR ($CDCl_3$, 500 MHz), δ 3.29 (2H, d, *J* = 7.0 Hz), 3.95 (3H, s), 5.05-5.10 (2H, m), 5.80 (1H, s), 5.91 (1H, m), 6.97 (1H, s), 7.18 (1H, s), 10.76 (1H, s). ^{13}C NMR ($CDCl_3$, 125 MHz), δ 39.4, 52.3, 111.9, 116.0, 119.8, 120.4, 131.1, 137.0, 144.8, 147.2, 170.7. HRMS (DART): $[M+Na]^+$ *m/z* calcd., 231.0628; found, 231.0637.

5-Allyl-2,3-bis(benzyloxy)benzoic acid (21). Alkene **20** (2.18 g, 10.5 mmol), benzyl bromide (10.8 g, 60.3 mmol), and K_2CO_3 (24.5 g, 17.8 mmol) were combined in 200 mL of acetone at rt. The reaction was refluxed under N_2 for 16 h, which provided a yellow solution with

white solids, and the mixture was cooled to rt and filtered. The filtrate was concentrated under reduced pressure to afford a yellow oil. The oil was dissolved in a 375-mL mixture of 4:1 MeOH / 5 M NaOH (aq). The resulting solution was refluxed for 3.5 h and concentrated under reduced pressure to afford a white-yellow oil. Water (300 mL) was added to the oil, and the aqueous phase was washed with hexanes (4 x 100 mL). The pH of the aqueous phase was adjusted to ca. 1 by addition of 12 M HCl and the product precipitated as a white solid. A 100-mL portion of CH_2Cl_2 was added, and the resulting mixture was partitioned. The aqueous phase was extracted with additional CH_2Cl_2 (2 x 100 mL) and the combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to yield **21** as a white solid (3.91 g, 99%). TLC R_f = 0.55 (silica, 100% CH_2Cl_2); mp = 135-136 ^oC. ^1H NMR ($CDCl_3$, 300 MHz), δ 3.38 (2H, d, J = 6.6 Hz), 5.06-5.14 (2H, m), 5.17 (2H, s), 5.22 (2H, s), 5.92 (1H, m), 7.09 (1H, d, J = 2.1 Hz), 7.31-7.50 (10H, m), 7.58 (1H, m). ^{13}C NMR ($CDCl_3$, 125 MHz), δ 39.6, 71.4, 76.9, 116.7, 119.3, 122.6, 123.9, 127.8, 128.4, 128.7, 128.7, 129.1, 129.2, 134.8, 135.8, 136.2, 137.2, 145.5, 151.2, 165.6. HRMS (DART): $[M-H]^-$ *m/z* calcd., 373.1445; found, 373.1439.

(*E*)-2,3-Bis(benzyloxy)-5-(prop-1-en-1-yl)benzoic acid (22). A 30-mL portion of methanol (MeOH) was degassed with N_2 for 4 h at rt and **21** (750 mg, 2.00 mmol) was subsequently added. The mixture was stirred at rt until **21** dissolved and $PdCl_2$ (58 mg, 0.32 mmol) was added to give a cloudy brown solution. The reaction was stirred at rt for 24 h and filtered. The filtrate was concentrated and purified by column chromatography using silica gel (1:4:5 EtOAc/hexanes/CH_2Cl_2) to yield **22** as a light yellow solid (666 mg, 89%). TLC R_f = 0.4 (40% EtOAc/hexanes); mp = 140-142 ^oC. ^1H NMR ($CDCl_3$, 300 MHz), δ 1.88-1.90 (3H, m), 5.19 (2H, s), 5.23 (2H, s), 6.25 (1H, dq, J = 15.9, 6.0 Hz), 6.32-6.38 (1H, m), 7.22 (1H, d, J = 2.1 Hz), 7.32-7.51 (10H, m), 7.69 (1H, d, J = 2.1 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz), δ 18.3, 71.4, 77.0, 115.8, 121.6, 122.7, 127.4, 127.7, 128.4, 128.7, 129.1, 129.2, 129.3, 134.7, 135.0, 135.9, 145.7, 151.3, 165.5. HRMS (DART): $[M-H]^-$ *m/z* calcd., 373.1445; found, 373.1457.

***N,N'*-((3S,7S,11S)-11-(2,3-Bis(benzyloxy)-5-((E)-prop-1-en-1-yl)benzamido)-2,6,10-trioxo-1,5,9-trioxacyclododecane-3,7-diyl)bis(2,3-bis(benzyloxy)benzamide) (23).** Trilactone **4**

(740 mg, 2.00 mmol) and DIPEA (2.58 g, 20 mmol) were mixed in dry DMSO (8 mL) and stirred for 10 min at rt to give a clear solution. PyAOP (3.13 g, 6.07 mmol), **22** (748 mg, 2.00 mmol) and **6** (1.00 g, 2.99 mmol) were dissolved in dry DMSO (10 mL) and added to the solution containing **4**, and the reaction turned yellow and became orange after stirring for 2 h at rt. The orange solution was mixed with EtOAc (50 mL) and water (50 mL) and partitioned. The organic phase was washed with brine (3 x 50 mL), dried over Na_2SO_4, and concentrated to afford a yellow oil. Flash chromatography on silica gel with a solvent gradient (10% EtOAc/hexanes to 55% EtOAc/hexanes) yielded the product as a white foam (931 mg, 37%). TLC R_f = 0.3 (50% EtOAc/hexanes); mp = 100-102 ^oC (decomp). ^1H NMR ($CDCl_3$, 300 MHz), δ 1.88-1.91 (3H, m), 4.01-4.11 (3H, m), 4.16-4.22 (3H, m), 4.91-4.98 (3H, m), 5.03-5.19 (12H, m), 6.17-6.40 (2H, m), 7.10-7.47 (32H, m), 7.66-7.71 (3H, m), 8.51-8.53 (3H, m). ^{13}C NMR ($CDCl_3$, 125 MHz), δ 18.2, 40.6, 51.2, 63.9, 70.9, 76.0, 76.1, 114.2, 117.3, 120.4, 122.8, 124.1, 125.7, 126.1, 126.3, 127.4, 127.5, 127.9, 128.0, 128.2, 128.4, 128.4, 128.4, 128.7, 128.7, 129.6, 134.1, 135.8, 135.8, 136.0, 136.0, 145.5, 146.7, 151.4, 151.4, 164.7, 168.8, 168.8. HRMS (DART): $[M+H]^+$ *m/z* calcd., 1250.4645; found, 1250.4653.

***N,N'*-((3S,7S,11S)-11-(2,3-Bis(benzyloxy)-5-formylbenzamido)-2,6,10-trioxo-1,5,9-trioxacyclododecane-3,7-diyl)bis(2,3-bis(benzyloxy)benzamide) (24).** A portion of compound **23** (285 mg, 0.228 mmol) was dissolved in 1,4-dioxane (9 mL) at rt, and water (3 mL) was added to give a colorless solution. Osmium tetraoxide (OsO_4, 68 μL of 2.5% wt solution in 2-methyl-2 propanol, 6.7 μmol) was added and the reaction was stirred for 0.5 h at rt, which afforded a light brown solution. Sodium periodate ($NaIO_4$, 76.5 mg, 0.570 mmol) was then added and the reaction was stirred for another 2 h at rt. The suspension was partitioned in water (20 mL) and EtOAc (50 mL). The organic phase was washed with 0.1 M sodium thiosulfate ($Na_2S_2O_3$, 3 x 20 mL) and brine (2 x 20 mL), and dried over Na_2SO_4. Flash chromatography on silica gel with a solvent gradient (20% EtOAc/hexanes to 65% EtOAc/hexanes) yielded the product as white solid (165 mg, 58%). TLC R_f = 0.6 (70% EtOAc/hexanes); mp = 74 ^oC (decomp). ^1H NMR ($CDCl_3$, 300 MHz), δ 4.03-4.11 (3H, m), 4.18-4.26 (3H, m), 4.90-4.96 (3H,

m), 5.05-5.28 (12H, m), 7.09-7.44 (31H, m), 7.65-7.67 (2H, m), 8.14-8.15 (1H, m), 8.46-8.52 (3H, m), 9.86 (1H, s). ^{13}C NMR ($CDCl_3$, 125 MHz), δ 51.4, 51.4, 51.7, 64.1, 64.2, 71.0, 71.2, 76.2, 76.2, 76.5, 113.1, 117.3, 117.4, 122.9, 123.0, 124.2, 126.2, 126.3, 126.5, 127.5, 127.6, 127.8, 128.1, 128.3, 128.4, 128.4, 128.5,128.5, 128.5, 128.6, 128.8, 128.9, 132.1, 135.2, 135.3, 135.9, 135.9, 136.0, 146.7, 146.8, 151.5, 151.5, 151.7, 152.2, 163.7, 164.9, 164.9, 168.7, 168.9, 169.1, 190.6. HRMS (DART): $[M+H]^+$ *m/z* calcd., 1238.4287; found, 1238.4279.

3,4-Bis(benzyloxy)-5-(((3S,7S,11S)-7,11-bis(2,3-bis(benzyloxy)benzamido)-2,6,10-trioxo-1,5,9-trioxacyclododecan-3-yl)carbamoyl)benzoic acid (25). A portion of **24** (112 mg, 0.0903 mmol) was dissolved in 1,4-dioxane (3 mL) at rt. Sulfamic acid (NH_3SO_3, 15.8 mg, 0.162 mmol) was dissolved in water (0.75 mL) and added to the dioxane solution. Sodium chlorite ($NaClO_2$, 14.7 mg, 0.163 mmol) dissolved in 0.2 mL of water and the resulting solution was added to the reaction over the course of 10 min, and the reaction turned yellow. After stirring for 0.5 h at rt, the reaction was partitioned in water (10 mL) and EtOAc (20 mL), the aqueous phase was extracted with EtOAc (2 x 10 mL), and the combined organic phases were dried over Na_2SO_4. Flash chromatography on silica gel with a solvent gradient (CH_2Cl_2 to 10% MeOH/CH_2Cl_2) yielded the product as white solid (87 mg, 76%). TLC R_f = 0.5 (10% MeOH/ CH_2Cl_2); mp = 128-129 ^{o}C (decomp). ^{1}H NMR ($CDCl_3$, 500 MHz), δ 4.05-4.08 (3H, m), 4.22-4.25 (3H, m), 4.93-4.98 (3H, m), 5.06-5.25 (12H, m), 7.06-7.47 (31H, m), 7.67-7.69 (2H, m), 7.86 (1H, s), 8.44-8.47 (2H, m), 8.54-8.57 (2H, m). ^{13}C NMR ($CDCl_3$, 125 MHz), δ 51.4, 51.5, 51.6, 64.1, 71.1, 71.2, 76.2, 76.4, 117.5, 117.6, 123.0, 124.2, 125.4, 125.6, 126.2, 127.5, 127.6, 127.8, 128.1, 128.3, 128.4, 128.4, 128.5, 128.6, 128.7, 128.8, 128.9, 135.4, 135.6, 135.9, 136.1, 146.8, 150.7, 151.4, 151.5, 164.1, 165.0, 168.8, 168.9, 169.0, 169.3. HRMS (DART): $[M+H]^+$ *m/z* calcd., 1254.4230; found, 1254.4204.

Enantiomers 26-28. The D-isomers of the enterobactin alkene **23**, aldehyde **24**, and acid **25** were synthesized as described for the L-isomers except that triserine lactone **5** was employed instead of **4**. The synthetic procedures and characterization are provided as Supporting Information.

***Tert*-butyl(1-(3-(((3S,7S,11S)-7,11-bis(2,3-dihydroxybenzamido)-2,6,10-trioxo-1,5,9-trioxacyclododecan-3-yl)carbamoyl)-4,5-dihydroxyphenyl)-1-oxo-5,8,11-trioxa-2-azatridecan-13-yl)carbamate (29).** Compound **25** (50 mg, 40 ∫mol), PyAOP (34 mg, 60 ∫mol) and DIPEA (15.2 ∫L, 160 ∫mol) were mixed in 2 mL of dry CH_2Cl_2 at rt. A portion of **7** (15 mg, 48 ∫mol) was then added and the resulting yellow solution was stirred for 4 h at rt. The crude reaction was washed with 0.01N HCl (2 x 10 mL), dried over Na_2SO_4, and concentrated. The benzyl-protected product was purified by preparative TLC (10% MeOH/CH_2Cl_2) and obtained as a white viscous solid (46 mg, 75%). TLC R_f = 0.7 (10% MeOH/CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 500 MHz), δ 1.42 (9H, s), 3.27-3.28 (2H, m), 3.50-3.52 (2H, m), 3.59-3.66 (12H, m), 4.02-4.07 (3H, m), 4.15-4.18 (3H, m), 4.90-4.94 (3H, m), 5.03-5.20 (12H, m), 7.10-7.45 (36H, m), 7.65-7.67 (2H, m), 7.85-7.85 (1H, m), 7.99 (1H, bs), 8.49-8.54 (3H, m). ^{13}C NMR ($CDCl_3$, 125 MHz), δ 28.3, 39.9, 40.2, 51.3, 51.4, 63.9, 64.1, 69.7, 70.0, 70.2, 70.3, 70.4, 71.1, 71.2, 76.2, 76.3, 79.0, 116.7, 117.5, 120.3, 123.0, 124.2, 125.4, 126.1, 126.2, 127.6, 127.6, 127.8, 128.2, 128.3, 128.4, 128.4, 128.4, 128.5, 128.6, 128.6, 128.7, 128.8, 128.8, 129.0, 130.2, 135.4, 135.7, 135.9, 135.9, 136.1, 146.8, 146.9, 149.0, 151.5, 151.8, 155.9, 164.2, 164.8, 164.9, 165.8, 168.9, 169.0, 169.1. HRMS (ESI): $[M+Na]^+$ *m/z* calcd., 1550.5942; found, 1550.5977.

This benzyl-protected product was dissolved in 2 mL of 1:1 EtOAc/EtOH, the reaction flask was purged with N_2, and 45 mg Pd/C (10% wt) was added. The reaction was stirred under H_2 (1 atm) for 6 h at rt, and the Pd/C was removed by centrifugation (13,000 rpm, 10 min). The clear supernatant was decanted, concentrated, and re-dissolved in a 4:2:1 mixture of 1,4-dioxane/H_2O/MeOH, and purified by semi-preparative HPLC (20% B for 5 min followed by 20-70% B over 15 min, 4 mL/min). The product eluted at 15.8 min and was lyophilized to give **29** as white solid (15 mg, 50%). The analytical HPLC trace of the purified product is reported as Supporting Information. HRMS (ESI): $[M+Na]^+$ *m/z* calcd., 1010.3125; found, 1010.3173.

***N*^{3}-((3S,7S,11S)-7,11-Bis(2,3-dihydroxybenzamido)-2,6,10-trioxo-1,5,9-trioxacyclododecan-3-yl)-*N*^{1}-(1-cyclohexyl-1-oxo-5,8,11-trioxa-2-azatridecan-13-yl)-4,5-dihydroxyisophthalamide (30).** Compound **30** was synthesized as described for **29** except

that **14** (13.6 mg, 45.0 ⌠mol) was used instead of **7**. After purification by preparative TLC (10% MeOH/CH_2Cl_2), the benzyl-protected precursor of **30** was obtained as a white viscous solid (37 mg, 60%). TLC R_f = 0.6 (10% MeOH/CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 500 MHz), δ 1.17-1.21 (3H, m), 1.37-1.43 (2H, m), 1.62-1.63 (1H, m), 1.72-1.74 (2H, m), 1.78-1.81 (2H, m), 2.00-2.06 (1H, m), 3.39-3.42 (2H, m), 3.51-3.53 (2H, m), 3.59-3.61 (2H, m), 3.64-3.65 (10H, m), 4.01-4.06 (3H, m), 4.13-4.17 (3H, m), 4.88-4.93 (3H, m), 5.04-5.21 (12H, m), 6.23-6.25 (1H, m), 7.09-7.45 (35H, m), 7.64-7.66 (2H, m), 7.86 (1H, d, J = 2.0 Hz), 8.02 (1H, d, J = 2.0 Hz), 8.49-8.54 (3H, m). ^{13}C NMR ($CDCl_3$, 125 MHz), δ 25.6, 29.5, 38.8, 40.0, 45.3, 51.3, 51.4, 63.9, 64.1, 69.8, 69.8, 70.0, 70.3, 70.4, 70.4, 71.2, 71.2, 76.2, 76.3, 116.8, 117.5, 120.4, 123.0, 124.3, 125.4, 126.1, 126.2, 127.6, 127.6, 127.9, 128.2, 128.3, 128.4, 128.4, 128.5, 128.5, 128.6, 128.6, 128.8, 128.8, 128.9, 129.0, 130.1, 135.4, 135.7, 135.9, 136.0, 136.1, 146.8, 146.9, 149.1, 151.6, 151.8, 164.3, 164.9, 164.9, 165.8, 168.9, 169.0, 169.1, 176.2. HRMS (ESI): $[M+Na]^+$ *m/z* calcd., 1560.6150; found, 1560.6269. Compound **30** was purified by semi-preparative HPLC (20% B for 5 min followed by 20-70% B over 15 min, 4 mL/min). The product eluted at 15.1 min and was obtained as white solid (20 mg, 58%). The analytical HPLC trace of the purified product is reported as Supporting Information. HRMS (ESI): $[M+Na]^+$ *m/z* calcd., 1020.3333; found, 1020.3346.

***N*^{3}-((3R,7R,11R)-7,11-Bis(2,3-dihydroxybenzamido)-2,6,10-trioxo-1,5,9-trioxacyclododecan-3-yl)-*N*^{1}-(1-cyclohexyl-1-oxo-5,8,11-trioxa-2-azatridecan-13-yl)-4,5-dihydroxyisophthalamide (31).** Compound **31** was synthesized as described for **30** except that **28** (36 mg, 29 ⌠mol) was used instead of **25**. After purification by preparative TLC (10% MeOH/CH_2Cl_2), the benzyl-protected precursor of **31** was obtained as a white oily solid (29 mg, 65%). TLC R_f = 0.6 (10% MeOH/CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 500 MHz), δ 1.17-1.25 (3H, m), 1.38-1.44 (2H, m), 1.63 (1H, m), 1.72-1.81 (4H, m), 2.01-2.06 (1H, m), 3.40-3.41 (2H, m), 3.39-3.42 (2H, m), 3.51-3.53 (2H, m), 3.58-3.65 (12H, m), 4.01-4.06 (3H, m), 4.13-4.16 (3H, m), 4.87-4.95 (3H, m), 5.03-5.21 (12H, m), 6.22-6.23 (1H, m), 7.09-7.45 (35H, m), 7.65-7.66 (2H, m), 7.86 (1H, s), 8.02 (1H, s), 8.49-8.54 (3H, m). ^{13}C NMR ($CDCl_3$, 125 MHz), δ 25.6, 29.5, 38.8, 40.0, 45.3, 51.3, 51.4, 63.9, 64.1, 69.8, 69.8, 70.0, 70.3, 70.4, 70.4, 71.2, 71.2, 76.2, 76.3, 116.8, 117.5, 120.4, 123.0, 124.3,

125.4, 126.1, 126.2, 127.6, 127.6, 127.9, 128.2, 128.3, 128.4, 128.4, 128.5, 128.5, 128.6, 128.6, 128.8, 128.8, 128.9, 129.0, 130.1, 135.4, 135.7, 135.9, 136.0, 136.1, 146.8, 146.9, 149.1, 151.6, 151.8, 164.3, 164.9, 164.9, 165.8, 168.9, 169.0, 169.1, 176.2. HRMS (ESI): $[M+Na]^+$ *m/z* calcd., 1560.6150; found, 1560.6141. Compound **31** was purified by semi-preparative HPLC (20% B for 5 min followed by 20-70% B over 15 min, 4 mL/min). The product eluted at 14.8 min and was obtained as white solid (5.1 mg, 27% yield). The analytical HPLC trace of the purified product is reported as Supporting Information. HRMS (ESI): $[M+Na]^+$ *m/z* calcd., 1020.3333; found, 1020.3328.

***N*^{3}-((3S,7S,11S)-7,11-Bis(2,3-dihydroxybenzamido)-2,6,10-trioxo-1,5,9-trioxacyclododecan-3-yl)-4,5-dihydroxy-*N*^{1}-(1-(naphthalen-2-yl)-1-oxo-5,8,11-trioxa-2-azatridecan-13-yl)isophthalamide (32).** Compound **32** was synthesized as described for **29** except that **15** (20 mg, 44 ⌠mol) was used instead of **7**. After purification by preparative TLC (5% MeOH/CH_2Cl_2), the benzyl-protected precursor of **32** was obtained as a white-yellow oily solid (37 mg, 59%). TLC R_f = 0.6 (10% MeOH/CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 500 MHz), δ 3.44-3.74 (16H, m), 3.94-4.08 (4H, m), 4.12-4.16 (2H, m), 4.78-4.82 (1H, m), 4.87-4.92 (2H, m), 5.02-5.17 (12H, m), 7.01-7.52 (39H, m), 7.58-7.59 (1H, m), 7.64-7.66 (2H, m), 7.79-7.84 (3H, m), 7.94-7.94 (1H, m), 8.29-8.31 (1H, m), 8.47-8.50 (3H, m). ^{13}C NMR ($CDCl_3$, 125 MHz), δ 39.6, 39.9, 51.4, 51.4, 63.9, 64.1, 69.6, 69.7, 70.2, 70.4, 71.1, 71.2, 71.2, 76.2, 76.3, 76.3, 116.7, 117.5, 120.3, 123.1, 124.3, 124.6, 125.0, 125.2, 125.4, 126.1, 126.2, 126.2, 126.9, 127.6, 127.6, 127.9, 128.1, 128.2, 128.4, 128.4, 128.5, 128.6, 128.6, 128.8, 128.9, 128.9, 129.0, 130.0, 130.1, 130.3, 133.5, 134.5, 135.4, 135.7, 135.9, 136.0, 136.2, 146.9, 146.9, 149.0, 151.6, 151.7, 164.2, 164.9, 164.9, 165.7, 168.9, 169.0, 169.1, 169.6. HRMS (ESI): $[M+Na]^+$ *m/z* calcd., 1604.5837; found, 1604.5964. Compound **32** was purified by semi-preparative HPLC (20% B for 5 min followed by 30-55% B over 10 min, 4 mL/min) and eluted at 12.7 min. The isolated product was lyophilized and obtained as a white solid (4.4 mg, 18%). The analytical HPLC trace of the purified product is provided as Supporting Information. HRMS (ESI): $[M+Na]^+$ *m/z* calcd., 1064.3020; found, 1064.3084. Mass spectrometric analysis of the crude reaction indicated M+4 in addition to the

desired product **32** and suggested partial reduction of the naphthalene cargo under the deprotection conditions. From analysis of HPLC peak areas, the ratio between **32** and the partial reduction product is ca. 4:1.

***N*^{1}-(1-(3-Benzylphenyl)-1-oxo-5,8,11-trioxa-2-azatridecan-13-yl)-*N*^{3}-((3S,7S,11S)-7,11-bis(2,3-dihydroxybenzamido)-2,6,10-trioxo-1,5,9-trioxacyclododecan-3-yl)-4,5-dihydroxyisophthalamide (33).** Compound **33** was synthesized as described for **29** except that **16** (24 mg, 62 μmol) was used instead of **7**. Partial purification by preparative TLC (10% MeOH/CH_2Cl_2) afforded the benzyl-protected precursor of **33** as a white-yellow solid with a grease contamination (43 mg, 67%). TLC R_f = 0.6 (10% MeOH/CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 500 MHz), δ 3.57-3.61 (12H, m), 3.94-3.95 (2H, d, *J* = 6.0) 3.97-4.05 (3H, m), 4.07-4.15 (3H, m), 4.85-4.90 (3H, m), 5.01-5.17 (12H, m), 7.01-7.40 (30H, m) 7.62-7.70 (3H, m), 7.82 (1H, d, J=2.0), 7.99-8.00 (1H, d, *J* = 2.0), 8.47-8.51 (3H, m) HRMS (ESI): $[M+Na]^+$ *m/z* calcd., 1644.6150; found, 1644.6105. A portion (32.5 mg, 20.0 μmol) of this material was carried on without further purification or characterization. Compound **33** was purified by semi-preparative HPLC (20% B for 5 min followed by 20-70% B over 15 min, 4 mL/min). The product eluted at 15.8 min and was obtained as white solid (13.5 mg, 62%). The analytical HPLC trace of the purified product is provided as Supporting Information. HRMS (ESI): $[M+Na]^+$ *m/z* calcd., 1104.3333; found, 1104.3305.

***N*^{3}-((3S,7S,11S)-7,11-Bis(2,3-dihydroxybenzamido)-2,6,10-trioxo-1,5,9-trioxacyclododecan-3-yl)-4,5-dihydroxy-*N*^{1}-(1-oxo-1-(11-oxo-2,3,5,6,7,11-hexahydro-1*H*-pyrano[2,3-*f*]pyrido[3,2,1-*ij*]quinolin-10-yl)-5,8,11-trioxa-2-azatridecan-13-yl)isophthalamide (34).**Compound **34** was synthesized as described for **29** except that **17** (18 mg, 39 μmol) was used instead of **7**. After purification by preparative TLC (10% MeOH/CH_2Cl_2) the benzyl-protected precursor of **34** was obtained as an orange oily solid (18 mg, 26%). TLC R_f = 0.7 (10% MeOH/CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 500 MHz), δ 1.93-1.95 (4H, m), 2.71-2.83 (4H, m), 3.26-3.32 (4H, m), 3.56-3.69 (16H, m), 3.99-4.18 (6H, m), 4.88-4.94 (3H, m), 5.01-5.18 (12H, m), 6.94 (1H, s), 7.06-7.43 (35H, m), 7.62-7.66 (2H, m), 7.80-7.80 (1H, m), 7.97-7.97 (1H, m),

8.47-8.53 (4H, m), 9.02-9.03 (1H, m). ^{13}C NMR ($CDCl_3$, 125 MHz), δ 19.9, 20.0, 21.0, 27.3, 39.4, 40.1, 49.7, 50.2, 51.5, 64.1, 69.9, 71.1, 71.2, 76.3, 105.4, 108.1, 115.9, 117.5, 119.8, 123.0, 124.3, 125.7, 126.3, 127.2, 127.6, 127.6, 127.8, 128.1, 128.2, 128.5, 128.5, 128.6, 128.9, 128.9, 129.0, 130.0, 135.7, 136.0, 136.2, 146.9, 148.2, 148.3, 149.0, 151.6, 151.7, 152.6, 162.9, 164.4, 165.0, 165.0, 168.9, 169.1. HRMS (ESI): $[M+Na]^+$ *m/z* calcd.,1717.6313; found, 1717.6287. Compound **34** was purified by semi-preparative HPLC (20% B for 5 min followed by 20-70% B over 15 min, 4 mL/min). The product eluted at 17.1 min and was obtained as an orange solid (4.5 mg, 48%). The analytical HPLC trace of the purified product is provided as Supporting Information. HRMS (ESI): $[M+Na]^+$ *m/z* calcd., 1177.3496; found, 1177.3540.

7-(4-(1-(3-(((3S,7S,11S)-7,11-Bis(2,3-dihydroxybenzamido)-2,6,10-trioxo-1,5,9-trioxacyclododecan-3-yl)carbamoyl)-4,5-dihydroxyphenyl)-1-oxo-5,8,11-trioxa-2-azatetradecan-14-oyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (35). Compound **35** was synthesized as described for **29** except that **18** (26 mg, 48 ∫mol) was used instead of 7, and TMSCl (10 ∫L, 79 ∫mol) and DIPEA (15 µL, 160 µmol) was mixed with **18** before addition to the solution containing **25**. After purification by preparative TLC (10% MeOH/CH_2Cl_2), the benzyl-protected precursor of **35** was obtained as a yellow oily solid (46 mg, 65%). TLC R_f = 0.65 (10% MeOH/CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 500 MHz), δ 1.13 (2H, bs), 1.33 (2H, bs), 2.64 (2H, bs), 3.23-3.30 (4H, m), 3.51 (1H, bs), 3.63 (14H, bs), 3.79 (4H, bs), 3.99-4.04 (3H, m), 4.11-4.14 (3H, m), 4.86-4.91 (3H, m), 5.01-5.19 (12H, m), 7.06-7.43 (39H, m), 7.59-7.61 (2H, m), 7.83 (1H, s), 7.97-7.99 (2H, m), 8.45-8.49 (3H, m), 8.69 (1H, s). ^{13}C NMR ($CDCl_3$, 125 MHz), δ 8.2, 33.4, 35.4, 40.0, 41.1, 45.3, 49.3, 50.0, 51.3, 51.4, 51.4, 63.9, 64.1, 67.1, 69.7, 70.2, 70.3, 70.4, 70.5, 71.2, 71.3, 76.2, 76.3, 105.2, 108.0, 112.3, 112.4, 116.7, 117.5, 120.0, 120.0, 120.5, 123.0, 124.3, 125.6, 126.1, 126.1, 127.6, 127.6, 127.8, 128.2, 128.3, 128.4, 128.4, 128.5, 128.6, 128.6, 128.8, 128.8, 128.8, 129.0, 130.2, 135.5, 135.7, 135.9, 136.0, 136.1, 138.9, 145.2, 145.3, 146.8, 146.8, 147.4, 149.0, 151.6, 151.6, 151.8, 152.4, 154.4, 164.2, 164.9, 164.9, 165.8, 166.9, 168.9, 169.0, 169.1, 169.7, 176.9. ^{19}F NMR ($CDCl_3$, 282 MHz) *δ* -121.3. HRMS (ESI): $[M+Na]^+$ *m/z* calcd., 1792.6434; found, 1792.6337. Compound **35** was

purified by semi-preparative HPLC (20% B for 5 min followed by 20-70% B over 10 min, 4 mL/min) and eluted at 15.2 min. The isolated product was lyophilized and obtained as a white solid (2.5 mg, 9%). The HPLC trace of the purified product is provided as Supporting Information. HRMS (ESI): $[M+Na]^+$ *m/z* calcd., 1252.3617; found, 1252.3633.

7-(4-(6-Aminohexanoyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (36). Ciprofloxacin (**37**, 331 mg, 1.00 mmol) and DIPEA (1.0 mL, 5.7 mmol) were mixed in 6 mL of dry CH_2Cl_2, and TMSCl (370 μL , 2.91 mmol) was added to give a clear yellow solution. 6-((Tert-butoxycarbonyl)amino)hexanoic acid (**12**, 346 mg, 1.50 mmol), PyAOP (834 mg, 1.60 mmol), and DIPEA (700 μL, 4.02 mmol) were dissolved in 4 mL of dry CH_2Cl_2, and the two solutions were combined and stirred overnight at rt. The reaction was quenched with MeOH (10 mL), and the resulting solution was concentrated to dryness, and the crude product was redissolved in 40 mL of EtOAc. The organic phase was washed with 10 mM HCl (2 x 40 mL) and saturated aqueous $NaHCO_3$ (2 x 40 mL), dried over Na_2SO_4, and purified by flash chromatography on silica gel (3% MeOH/CH_2Cl_2) to give **38** as yellow solid (243 mg, 45%). TLC R_f = 0.7 (5% MeOH/CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 300 MHz), *δ* 1.14-1.20 (2H, m), 1.32-1.53 (13H, m), 1.59-1.69 (2H, m), 2.36 (2H, t, *J* = 6.0 Hz), 3.08 (2H, dt, *J* = 6.3, 6.3 Hz), 3.26-3.56 (4H, m), 3.51-3.59 (1H, m), 3.69-3.82 (4H, m), 4.68 (1H, bs), 7.32 (1H, d, *J* = 7.2 Hz), 7.82 (1H, d, *J* = 12.9 Hz), 8.60 (1H, s), 14.9 (1H, bs). ^{13}C NMR ($CDCl_3$, 125 MHz), δ 8.1, 24.7, 26.4, 28.3, 29.8, 32.9, 35.3, 40.2, 41.0, 45.1, 49.3, 49.9, 78.9, 105.0, 107.7, 111.9, 112.1, 119.6, 119.7, 138.8, 145.2, 145.3, 147.3, 152.4, 154.4, 155.9, 166.6, 171.4, 176.7. ^{19}F NMR ($CDCl_3$, 282 MHz), δ -121.1. HRMS (ESI): $[M+H]^+$ *m/z* calcd., 545.2775; found, 545.2768.

The TFA salt of **36** (202 mg, 98%) was obtained as a yellow solid from **38** (201 mg, 0.369 mmol) by stirring **38** in 40% TFA/CH_2Cl_2 at rt for 3 h and removing the solvent. TLC R_f = 0.1 (10% MeOH/CH_2Cl_2). ^{1}H NMR (CD_3OD, 300 MHz), δ 1.41-1.52 (4H, m), 1.65-1.77 (4H, m), 2.52 (2H, t, *J* = 7.2 Hz), 2.96 (2H, t, *J* = 7.2 Hz), 3.34-3.43 (4H, m), 3.82 (5H, m), 7.57 (1H, d, *J* = 7.5 Hz), 7.85 (1H, d, *J* = 13.2 Hz), 8.76 (1H, s). ^{13}C NMR ($CDCl_3$, 125 MHz), δ 7.8, 23.8, 25.4, 26.5, 26.6, 32.2, 35.4, 39.0, 39.1, 41.0, 45.0, 48.1, 48.3, 48.5, 48.6, 48.8, 49.0, 49.1, 49.5, 105.0,

107.0, 111.6, 111.8, 119.3, 119.4, 138.8, 145.1, 145.2, 147.4, 152.3, 154.3, 167.3, 171.8, 176.5. ^{19}F NMR ($CDCl_3$, 282 MHz), δ -76.0, -120.9. HRMS (ESI): $[M+H]^+$ *m/z* calcd., 445.2251; found, 445.2255.

7-(4-(6-(3-(((3S,7S,11S)-7,11-Bis(2,3-dihydroxybenzamido)-2,6,10-trioxo-1,5,9-trioxacyclododecan-3-yl)carbamoyl)-4,5-dihydroxybenzamido)hexanoyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (40). Compound **40** was synthesized as described for **35** except that DMSO (1.5 mL) was used as the solvent and compound **36** (19.4 mg, 34.8 ∫mol) was used instead of **18**. After preparative TLC purification (10% MeOH/CH_2Cl_2), **39** was obtained as white viscous solid (17 mg, 74%). TLC R_f = 0.6 (10% MeOH/CH_2Cl_2). ^1H NMR ($CDCl_3$, 300 MHz) δ 1.17-1.83 (10H, m), 2.40 (2H, bs), 3.29-3.44 (6H, m), 3.70-3.86 (5H, m), 4.02-4.17 (6H, m), 4.86-4.93 (3H, m), 5.04-5.21 (12H, m), 7.07-7.42 (33H, m), 7.60-7.64 (2H, m), 7.85-8.05 (3H, m), 8.47-8.50 (3H, m), 8.74 (1H, bs), 15.0 (1H, bs).^{13}C NMR ($CDCl_3$, 125 MHz), δ 8.1, 12.3, 17.2, 18.6, 24.4, 26.3, 26.4, 26.5, 29.0, 32.8, 34.7, 39.7, 41.2, 45.3, 46.2, 46.3, 51.4, 51.5, 51.5, 52.0, 54.8, 63.9, 64.1, 64.2, 71.1, 71.2, 71.2, 76.2, 76.3, 76.3, 105.2, 109.5, 113.0, 113.2, 116.6, 117.5, 120.1, 123.0, 124.3, 125.5, 126.1, 127.6, 127.8, 128.2, 128.3, 128.4, 128.4, 128.5, 128.6, 128.6, 128.6, 128.8, 128.9, 128.9, 129.0, 130.3, 135.5, 135.8, 135.9, 136.0, 136.1, 138.1, 145.4, 146.8, 148.4, 149.0, 151.6, 151.8, 152.3, 164.4, 164.9, 165.0, 165.8, 166.1, 168.8, 169.0, 169.1, 171.5. HRMS (ESI): $[M+H]^+$ *m/z* calcd., 1680.6303; found, 1680.6352. Compound **40** was purified by semi-preparative HPLC (20% B for 5 min followed by 20-70% B over 15 min, 4 mL/min) and eluted at 16.1 min. The isolated product was lyophilized and obtained as a white-yellow solid (6.7 mg, 59%). The HPLC trace of the purified product is provided as Supporting Information. HRMS (ESI): $[M+H]^+$ *m/z* calcd., 1140.3486; found, 1140.3482.

***N*$_1$-(2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethyl)-4,5-bis(benzyloxy)-*N*$_3$-((3S,7S,11S)-7,11-bis(2,3-bis(benzyloxy)benzamido)-2,6,10-trioxo-1,5,9-trioxacyclododecan-3-yl)isophthalamide (41).** 11-Azido-3,6,9-trioxaundecan-1-amine (**11**, 8.2 ∫L, 42 ∫mol) and **25** (40 mg, 32 μmol) were dissolved in 1 mL of dry CH_2Cl_2. PyAOP (33.2 mg, 63.8 ∫mol) and

DIPEA (22.2 ⌠L, 128 ⌠mol) were added to give a light yellow solution. The reaction was stirred for 4 h at rt and concentrated, and the crude product was purified by preparative TLC (50% EtOAc/CH_2Cl_2) to afford **41** as a light yellow oil (31 mg, 68%). TLC R_f = 0.3 (50% EtOAc/CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.34 (2H, t, *J* = 5.1 Hz), 3.61-3.69 (14H, m), 3.97-4.18 (6H, m), 4.88-4.94 (3H, m), 5.02-5.22 (12H, m), 7.08-7.46 (34H, m), 7.64-7.67 (2H, m), 7.85 (1H, d, *J* = 1.8 Hz), 7.99 (1H, d, *J* = 2.1 Hz), 8.48-8.52 (3H, m). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 40.0, 50.6, 51.4, 64.0, 64.1, 69.7, 69.9, 70.3, 70.6, 71.2, 71.2, 76.3, 116.7, 117.5, 120.4, 123.1, 124.3, 125.5, 126.2, 126.2, 127.6, 127.6, 127.9, 128.3, 128.4, 128.4, 128.5, 128.6, 128.7, 128.8, 128.9, 129.0, 130.2, 135.5, 135.8, 136.0, 136.0, 136.2, 146.9, 146.9, 149.1, 151.6, 151.8, 164.2, 164.9, 164.9, 165.9, 168.9, 169.1, 169.1. HRMS (ESI): $[M+Na]^+$ *m/z* calcd., 1476.5323; found, 1476.5345.

Vancomycin-PEG-Ent (42). A DMSO solution of **41** (19 mg/mL, 1.3 mM, 250 µL), an aqueous solution of **8** (20 mg/mL, 1.3 mM, 250 µL), a DMF solution of benzoic acid (49 mg/mL, 450 mM, 50 µL), and an aqueous solution of $CuSO_4$ (10 mg/mL, 45 mM, 50 µL) were mixed together, and an additional 400 µL of DMSO was added to yield a clear light blue solution. An aqueous solution of sodium ascorbate (NaAsc, 18 mg/mL, 90 mM, 50 µL) was subsequently added. The reaction become colorless to yellow and was stirred at rt for 15 min, at which time another 50 µL of aqueous NaAsc was added. After stirring for 15 min, the crude reaction was frozen and lyophilized to give a brown oil. The oil was dissolved in a 2:1:1 ratio of dioxane/MeOH/H_2O and purified by semi-preparative HPLC (50% B for 5 min followed by 50-100% B over 11 min, 4 mL/min). The benzyl-protected precursor of **42** eluted at 13 min and was obtained as white solid after lyophilization (3.5 mg, 36%). HRMS (ESI): $[M+2Na]^{2+}/2$ *m/z* calcd., 1520.5030; found, 1520.5171.

A portion of this precursor (14 mg, 4.7 ⌠mol; obtained from four 250-µL scale Click reactions) was dissolved in 30% H_2O/MeOH, the flask was purged with N_2, and 16 mg Pd/C (10% wt) was added. The reaction was stirred under H_2 (1 atm) for 24 h at rt, and the Pd/C was removed by centrifugation (13,000 rpm, 10 min). The supernatant was concentrated by

lyophilization and the resulting residue was dissolved in a 2:1:1 mixture of dioxane/MeOH/H_2O. HPLC purification (20% B for 5 min followed by 20-46% B over 8 min, 4 mL/min) gave **43** as white solid (6.3 mg, 55%). The HPLC trace of the purified product is reported in Supporting Information. HRMS (ESI): $[M+2H]^{2+}/2$ *m/z* calcd., 1228.37960; found, 1228.37961.

***tert*-Butyl(2-(((1-(1-(3-(((3S,7S,11S)-7,11-bis(2,3-dihydroxybenzamido)-2,6,10-trioxo-1,5,9-trioxacyclododecan-3-yl)carbamoyl)-4,5-dihydroxyphenyl)-1-oxo-5,8,11-trioxa-2-azatridecan-13-yl)-1H-1,2,3-triazol-4-yl)methyl)amino)-2-oxoethyl)carbamate (43).** Compound **43** was synthesized as described for **42** except that a DMSO solution of **7** (2.8 mg/mL, 13 mM, 25 μL) was used instead of **8**. HPLC purification gave 3.3 mg of the benzyl-protected precursor of **43** as a white solid (58%). HRMS (ESI): $[M+H]^+$ *m/z* calcd., 1688.6489; found, 1688.6421. Compound **43** (3.3 mg, 33%) was obtained from the precursor (13.3 mg, 7.88 μmol; obtained from four 25-μL scale Click reactions) following the same procedure as synthesizing **42**. HPLC purification (0% B for 5 min followed by 0-45% B over 8 min, 4 mL/min) afforded **43** as a white solid with a retention time of 12.8 min. The HPLC trace of the purified product is reported as Supporting Information. HRMS (ESI): $[M+H]^+$ *m/z* calcd., 1126.3853; found, 1126.3832.

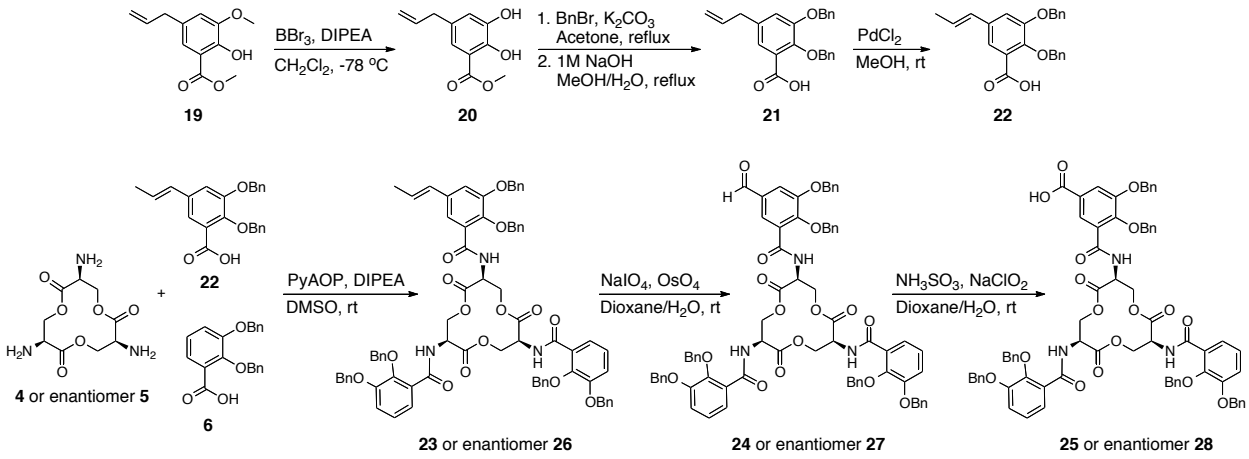
Growth Recovery Assays. General microbiology methods are included as Supporting Information. Overnight cultures were prepared by inoculating 5 mL of LB (*E. coli*) or LB base supplemented with 2.5 g/L NaCl (*P. aeruginosa*) with the appropriate freezer stocks and the cultures were incubated at 37 ^oC in a tabletop incubator shaker set at 150 rpm. The overnight culture was diluted 1:100 into 5 mL of fresh media with or without 200 μM 2,2'-dipyridyl (DP) and incubated at 37 ^oC with shaking at 150 rpm until the optical density at 600 nm (OD_{600}) reached 0.6. The cultures were diluted to an OD_{600} value of 0.001 in 50% reduced MHB medium (10.5 g/L) with or without 200 μM (*E. coli*) or 600 μM DP (*P. aeruginosa*). A 90-μL aliquot of the diluted culture was mixed with a 10-μL aliquot of a 10x solution of the siderophore or siderophore-cargo conjugate in a 96-well plate, which was wrapped in parafilm and incubated at 30 °C with shaking at 150 rpm for 19 h. Bacterial growth was assayed by measuring OD_{600}

using a BioTek Synergy HT plate reader. Each well condition was prepared in duplicate and three independent replicates of each assay were conducted on different days. The resulting mean OD_{600} are reported and the error bars are the standard deviation of the mean obtained from the three independent replicates.

Results and Discussion

Design and Synthesis of Monofunctionalized Enterobactin Platforms. We present a stepwise synthesis to monofunctionalized enterobactin scaffolds in Scheme 1. Guided by the structures of MGE and DGE (Figure 1A), we chose to install functional groups amenable to synthetic modification at the C5 position of one enterobactin catechol ring. This position is remote from the iron-binding hydroxyl groups in addition to the macrolactone (Figure 2). Prior studies of the salmochelins indicate that modification at this site compromises neither Fe(III) complexation nor the esterase-catalyzed hydrolysis of the macrolactone.[33,64] The structure of the antibiotic-siderophore conjugate MccE492m (Figure S1),[65] which exhibits a monoglucosylated enterobactin derivative attached to a ribosomal peptide, also influenced our decision to prepare monofunctionalized enterobactin platforms. We selected methyl-5-allyl-3-methoxysalicylate **19** as a starting material because of its commercial availability. This precursor was demethylated using BBr_3 in the presence of DIPEA to prevent HBr addition to the alkene moiety, and **20** was obtained in 53% yield as a white powder. Benzyl protection of **20** and subsequent hydrolysis of the methyl ester in refluxing sodium hydroxide was performed following a literature protocol[62] for catecholate protection of 2,3-dihydroxybenzoic acid and **21** was obtained in 99% yield as a white powder. Palladium-catalyzed isomerization of the alkene was achieved by using $PdCl_2$ in degassed methanol and **22** was obtained in 89% yield as a light yellow solid following workup. Next, a one-pot coupling reaction between the enterobactin trilactone **4**, **6** and **22** was performed with a 1:1.5:1 ratio and PyAOP as the coupling reagent. This reaction provided a mixture of **23**, its di- and tri-substituted analogs, and unmodified Ent. These products were separated by flash chromatography and afforded **23** in 37% yield as a

white foam. The 1:1.5:1 ratio of **4**/**6**/**22** was chosen based on several optimization trails and this ratio provided the highest yield of the desired monosubstituted product. Oxidation of alkene **23** by using OsO_4 and $NaIO_4$ in mixed 1,4-dioxane/water afforded **24** as a white foam in 58% yield. Further oxidation of **24** under mild conditions provided carboxylic acid **25** in 76% yield as a white powder. This step-wise synthesis provides gram quantities of **23-25** (L-isomers) in high purity, and these molecules are stable when stored as dry solids at 4 ^{o}C. The stepwise coupling and oxidations were also performed using triserine lactone **5** to afford the D-enantiomers alkene **26**, aldehyde **27**, and acid **28** (Supporting Information). The D-enantiomer of Ent is transported into *E. coli* by FepA, but it is not a substrate for the enterbactin esterase Fes.[66] We therefore reasoned that conjugates based on D-Ent would provide useful controls for conjugate uptake studies, and that this enantiomer may also be advantageous in antibacterial drug delivery applications because it provides an iron-starvation effect.

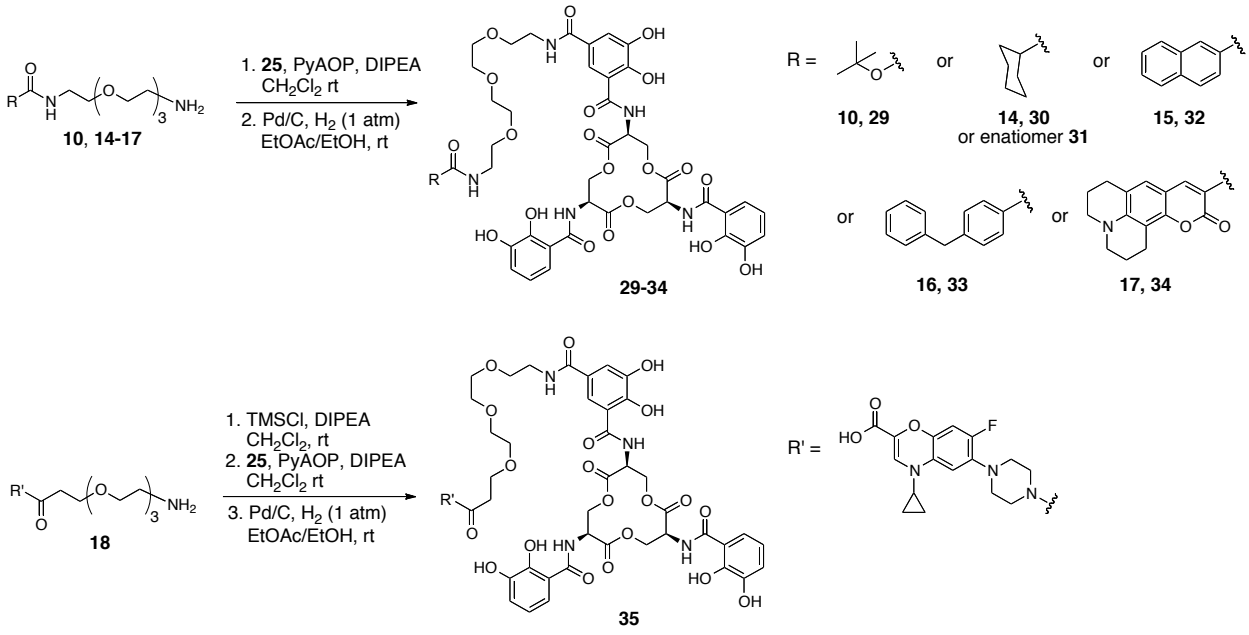


Scheme 1. Syntheses of **22** (top) and monofunctionalized enterobactin scaffolds (bottom).

This synthesis provides a family of enterobactin scaffolds amenable to functionalization. For instance, alkene **23** may be employed in olefin cross metathesis,[67] aldehyde **24** in reductive amination, and acid **25** in peptide coupling reactions. Moreover, other organic transformations of

22 or **23** may provide additional versatile functional groups (e.g. hydroxyl), affording more synthetic possibilities for enterobactin derivatives that can be utilized in various applications.

Design and Synthesis of Enterobactin-Cargo Conjugates. We selected carboxylic acid **25** as a key intermediate for the preparation of enterobactin-cargo conjugates, and evaluated two strategies for appending cargo to the enterobactin scaffold. In one thrust, standard peptide coupling chemistry was employed to link cargo to the enterobactin acid via an amide bond (Schemes 2 and 3). In the second approach, enterobactin-azide **41** was prepared and "Click" chemistry utilized for cargo attachment (Scheme 4). In both cases, benzyl deprotection unmasked the enterobactin catecholates in the final step.

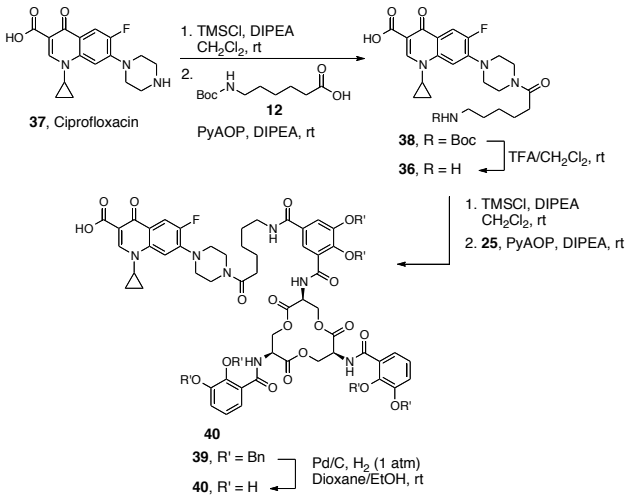


Scheme 2. Syntheses of enterobactin-cargo conjugates **29**-**35**. The syntheses of **14**-**18** are provided as Supporting Information.

We selected a variety of commercially available molecules housing carboxylic acids as cargos. The cargos presented in Scheme 2 vary in size and shape and include a simple Boc

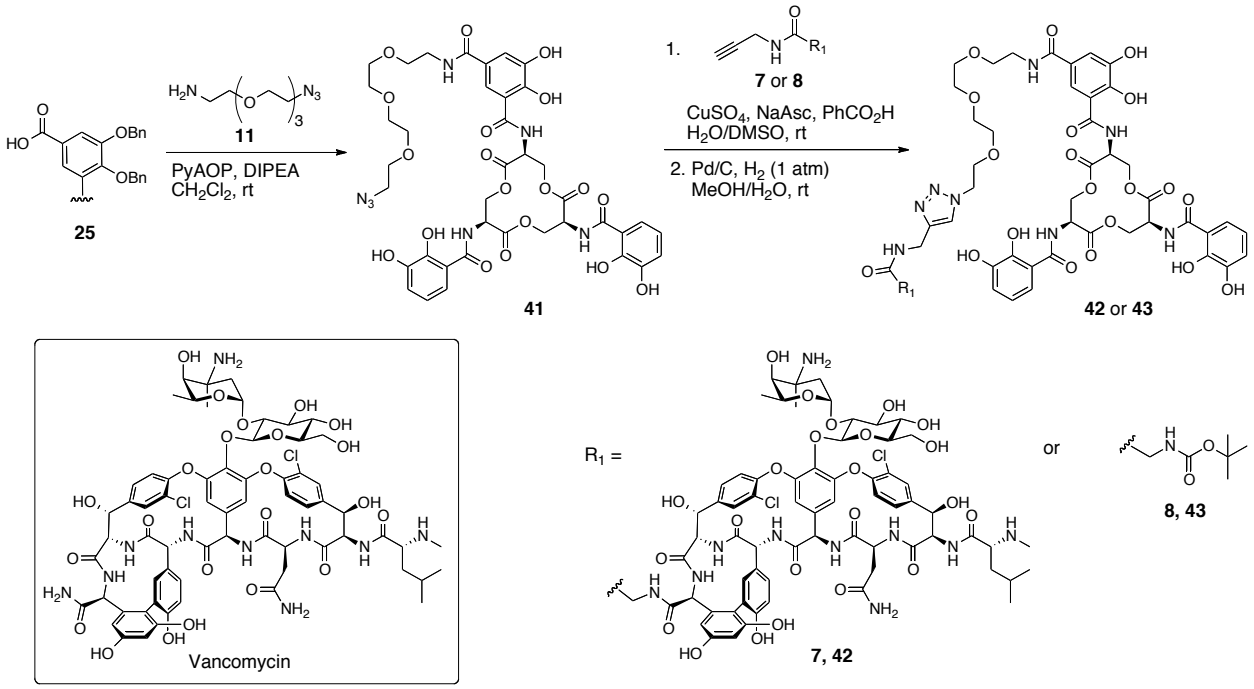
protecting group, cyclohexane, naphthalene, phenylmethylbenzene, ciprofloxacin, and coumarin 343. This selection includes cargo expected to be non-toxic (e.g. Boc, cyclohexane) in addition to an antibiotic (ciprofloxacin) and a fluorophore (coumarin 343). Next, we selected PEG_3 as a stable and water-compatible linker. It provides ca. 14-Å separation between enterobactin and the cargo. The conjugates depicted in Scheme 2 were prepared by coupling the PEG-derivatized cargo **10,14-18** to **25** using PyAOP as the coupling reagent. The resulting benzyl-protected conjugates were purified by preparative TLC and obtained in yields ranging from 26% (Bn-**34**) to 75% (Bn-**29**). Benzyl deprotection reactions were performed by hydrogenation over Pd/C and the resulting enterobactin-cargo conjugates were purified by reverse-phase semi-preparative HPLC. Conjugates **29-35** were obtained in milligram quantities and high purity judging by analytical HPLC (Figures S2-S11) and LC/MS analysis (Table S1). Conjugate **31** houses D-Ent and was prepared to probe the role of Fes-mediated hydrolysis in the bacterial growth recovery assays (*vide infra*).

Scheme 3 exhibits the synthesis of enterobactin-ciprofloxacin **40** where the PEG linker is substituted by a C_5 alkyl chain to probe the consequences of variable linker composition and hydrophilicity. The synthesis of **40** was carried out by reacting ciprofloxacin with 6-Boc-aminohexanoic acid **12** followed by Boc deprotection, coupling of the resulting free amine to **25**, and benzyl deprotection. The carboxylic acid of ciprofloxacin was protected *in situ* by using trimethylsilyl chloride (TMSCl) to prevent self-coupling in the syntheses of both **35** and **40**. In this general approach of attaching a carboxylic acid cargo, the linkers were first coupled to the cargo rather than to the Ent scaffold because the Ent macrolactone degrades in the presence of primary amines and under highly acidic conditions such as those required to remove Boc protecting groups.



Scheme 3. Synthesis of enterobactin-ciprofloxacin conjugate **40**.

In Scheme 4, we present the synthesis of **43**, an enterobactin-vancomycin conjugate assembled via Click chemistry. Vancomycin is a nonribosomal peptide antibiotic active against Gram-positive organisms that inhibits cell wall biosynthesis by binding to the D-Ala-D-Ala of lipid II and blocking peptidoglycan cross-linking.[68] It exhibits poor activity against Gram-negative bacteria because it is too large to cross the outer membrane. Because modification of the C-terminal carboxylic acid with a PEG chain did not perturb its antibacterial activity,[69] we selected this site as a point of attachment. Moreover, we employed Click chemistry for the conjugate assembly to avoid complications with the various functional groups exhibited by vancomycin. First, the azide-functionalized PEG linker **11** was coupled to **25** to afford the enterobactin-azide **41** in 68% yield. Copper(I)-catalyzed azide-alkyne cycloaddition of **41** with alkyne **8**[63] subsequently afforded enterobactin-vancomycin **42** in 55% yield following hydrogenation and purification. This synthetic approach was extended to **43**, a small analog of **42** that houses a *tert*-butyl cargo, and the strategy is also applicable to other alkyne-substituted cargos that are compatible with the benzyl deprotection conditions.



Scheme 4. Syntheses of enterobactin-cargo conjugates by Click chemistry.

Enterobactin-Cargo Conjugates Coordinate Fe(III). The optical absorption spectrum of each enterobactin-cargo conjugate exhibited catecholate absorption at ca. 316 nm (MeOH, rt). With the exception of **34**, which afforded a yellow solution because of the coumarin moiety, methanolic solutions of each conjugate turned from colorless to wine-colored following the addition of ca. one equiv of aqueous Fe(III), and the expected ligand-to-metal charge transfer (LMCT) bands were observed, indicating Fe(III) coordination to the enterobactin catecholates (Figure S12-S14).[70]

Enterobactin-Cargo Conjugate Delivery to the *E. coli* Cytoplasm. We employed three non-pathogenic *E. coli* strains that are defective in enterobactin synthesis, enterobactin transport, or ferric enterobactin utilization in growth recovery assays (Table S2). *E. coli* ATCC 33475 (*ent*-) cannot biosynthesize enterobactin, but retains the capacity to import and metabolize the siderophore.[71] *E. coli* H1187 (*fepA-)* lacks the outer membrane enterobactin

receptor. *E. coli* K-12 JW0576 (*fes*-) can accumulate ferric enterobactin, but cannot release the iron because it is deficient in the enterobactin esterase Fes. As a result of these defects in iron metabolism, all three strains grow poorly under conditions of iron limitation.[71] The iron chelator dipyridyl (DP) was used to generate iron-deficient conditions and promote expression of siderophore transport machinery in the growth recovery assays.

We first evaluated whether the enterobactin conjugates afforded growth recovery of *E. coli* (*ent*-) cultured under iron-deficient conditions (50% MHB, 200 μM DP). *E. coli* (*ent*-) grew to OD_{600} ~ 0.35 in 50% MHB medium (30 ^{o}C, t = 19 h), and this value decreased to <0.05 when 200 μM DP was added to the media. Low-micromolar concentrations of L-Ent restored growth, as expected,[70] and the *E. coli* cultures reached OD_{600} ~ 0.2 in the presence of 10 μM Ent (Figure 3). Likewise, low-micromolar concentrations of the enterobactin-cargo conjugates **29-33** and **43** exhibiting Boc (**29, 43**), cyclohexyl (**30**), napthyl (**32**), and phenylmethylbenzyl (**33**) cargos afforded growth recovery to similar levels (Figures 3 and S15). No growth restoration was observed when *E. coli* (*fepA*-) or *E. coli* (*fes*-) were cultured with **29** or **30** (Figures 5 and S16), which supports the notion that the growth recovery of *E. coli* (*ent*-) results from FepA-mediated cytoplasmic transport and Fes-catalyzed hydrolysis of the enterobactin moiety to release iron. Moreover, the D-enantiomer of enterobactin, D-Ent **9**, is not a substrate for Fes and does not provide growth recovery (Figures 4 and S17).[66] Indeed, no growth promotion occurred when *E. coli* (*ent*-) was treated with conjugate **31***,* the D-enantiomer of **30** (Figures 4 and S15). Taken together, these results demonstrate that the enterobactin transport machinery has the capacity to recognize and transport cargo-derivatized enterobactin scaffolds to the *E. coli* cytoplasm, and that these molecules are substrates for the cytoplasmic esterase Fes.

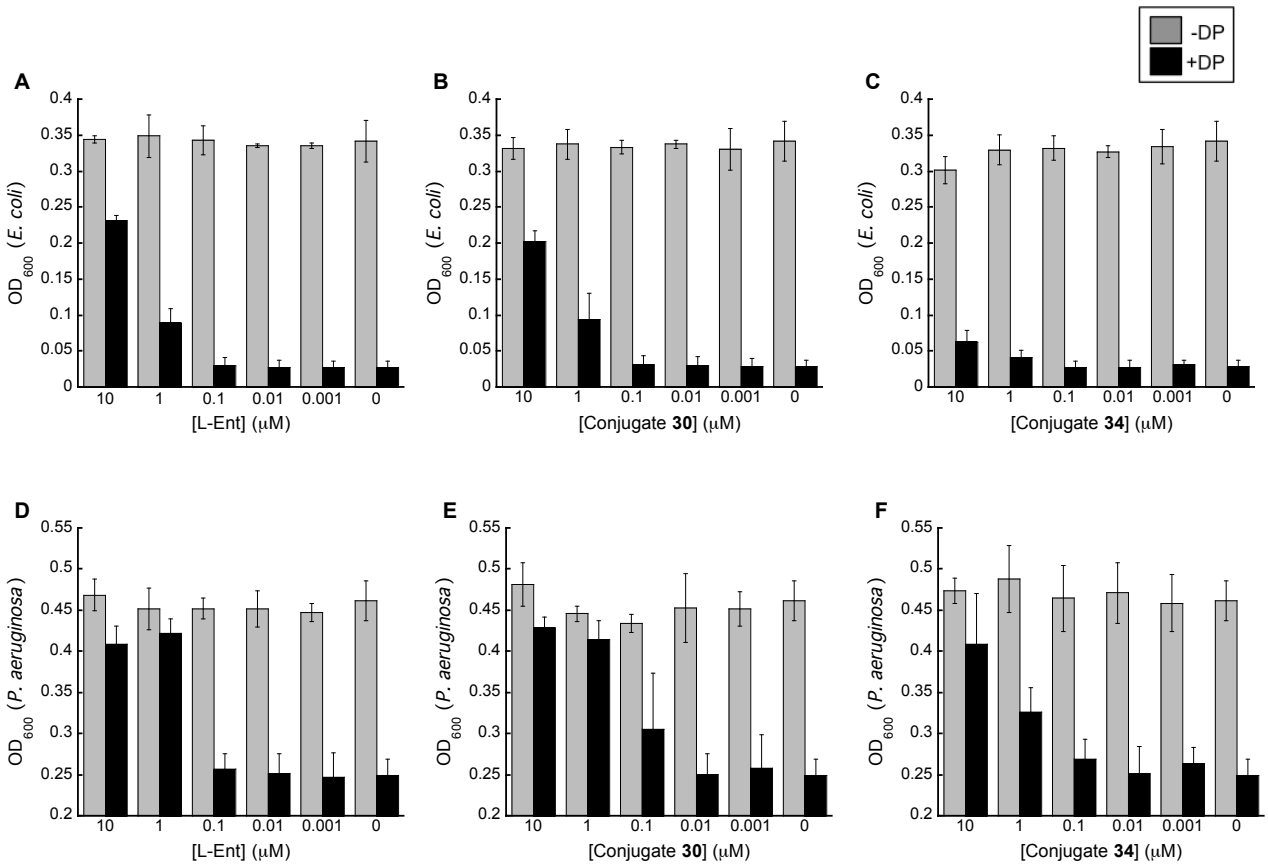


Figure 3. *E. coli* ATCC 33475 (*ent*-) and *P. aeruginosa* PAO1 (*pvd*-, *pch*-) growth recovery assays employing L-Ent and select enterobactin-cargo conjugates (50% MHB, ± 200 or 600 μM DP, t = 19 h, 30 °C). Grey bars: OD_{600} of bacteria cultured in the absence of DP. Black bars: OD_{600} of bacteria cultured in the presence of 200 (*E. coli*) or 600 (*P. aeruginosa*) μM DP. (A) L-Ent promotes growth recovery of *E. coli*. (B) Enterobactin conjugate **30** housing a cyclohexyl cargo affords growth recovery of *E. coli*. (C) Enterobactin conjugate **34** housing a coumarin moiety affords little-to-no growth recovery of *E. coli*. (D) L-Ent promotes growth recovery of *P. aeruginosa.* (E) Enterobactin conjugate **30** housing a cyclohexyl cargo affords growth recovery of *P. aeruginosa.* (F) Enterobactin conjugate **34** housing a coumarin moiety affords growth recovery of *P. aeruginosa*. Figures 4 and S15 contain the assay results for the other conjugates. Each bar indicates the average of three independent replicates (two wells per replicate) and the error bars are the standard deviation of the mean.

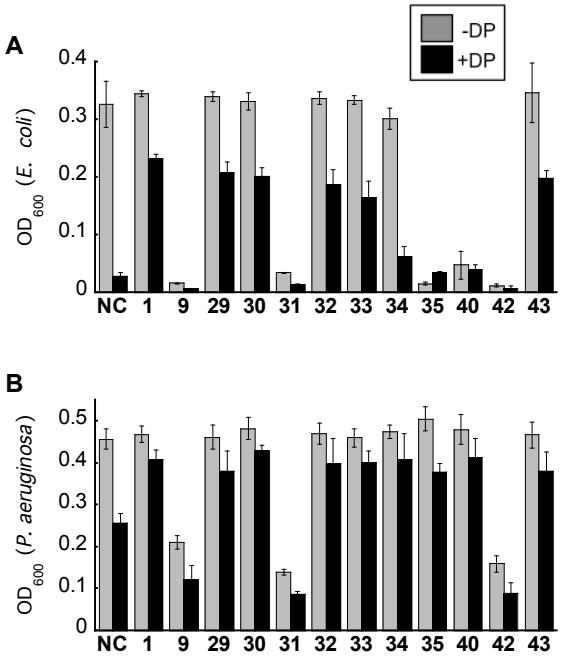


Figure 4. The comparative effects of enterobactin-cargo conjugates on bacterial cell growth. *E. coli* and *P. aeruginosa* were cultured in the presence of 10 μM of L-Ent **1**, D-Ent **9** and the enterobactin-cargo conjugates **29-35, 40, 42, 43** in the absence (grey bars) and presence (black bars) of DP (50% MHB, T = 30 °C, t = 19 h). (A) *E. coli* ATCC 33475 (*ent*-) and the DP concentration was 200 μM. (B) *P. aeruginosa* PAO1 (*pvd*-, *pch*-) and the DP concentration was 600 μM. NC refers to a no-conjugate control.

We observed no convincing evidence for marked uptake of larger cargos by *E. coli* ATCC 33475, which suggests that FepA of this *E. coli* strain has a cargo size limit. For instance, under iron limitation, negligible *E. coli* growth recovery and no toxicity was observed following treatment with the enterobactin-coumarin conjugate **34** (Figure 3), suggesting that *E. coli* (*ent*-) may not readily import **34**. Moreover, no growth recovery occurred following treatment of *E. coli* with either ciprofloxacin **35** or **40** (Figures 4 and S15). In the absence of DP, these conjugates afforded a concentration-dependent inhibition of *E. coli* growth. Likewise, 10 μM vancomycin **42**

inhibited the growth of *E. coli* (± DP, Figures 4 and S15). This behavior contrasts that of unmodified vancomycin, which is inactive against *E. coli* over the concentration range employed in this study. Two possible origins for inhibitory activity of the ciprofloxacin and vancomycin conjugates are (i) enterobactin-antibiotic uptake and resulting antibacterial action or (ii) a lack of active transport into *E. coli*, resulting in extracellular iron chelation and hence nutrient deprivation. Taking all observations into account, including those for *P. aeruginosa* described below, we contend that the latter option is the most probable explanation.

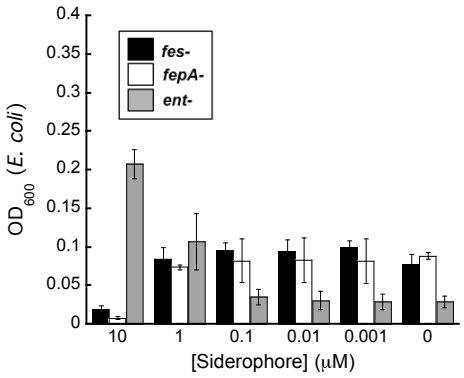


Figure 5. Comparison of growth recovery for *E. coli* (*ent-), E. coli* (*fepA*-), *and E. coli* (*fes*-) with conjugate **29** in the presence of 200 μM DP. Black bars: *E. coli* (*fes*-) cultured with conjugate **29**; white bars: *E. coli* (*fepA*-) cultured with conjugate **29** ; grey bars, *E. coli* (*ent*-) cultured with conjugate **29** in the presence of 200 μM DP. See Figures 4 and S16 for additional data.

Enterobactin-Cargo Conjugate Delivery to the *P. aeruginosa* Cytoplasm. We next sought to determine whether the enterobactin-cargo conjugates provided growth recovery for *Pseudomonas aeruginosa* PAO1. This Gram-negative opportunistic human pathogen synthesizes and exports two siderophores, pyoverdine (pvd) and pyochelin (pch), and employs

multiple additional mechanisms for iron acquisition.[72,73] *P. aeruginosa* utilizes enterobactin as a xenosiderophore, and the genes *pfeA*[74,75] and *pirA*[76] encode outer membrane enterobactin transporters. Similar to the *E. coli* experiments, we focused on using *P. aeruginosa* strains deficient in siderophore production or utilization in growth recovery assays. *P. aeruginosa* K648 (*pvd*-, *pch*-) is deficient in both pyoverdine and pyochelin biosynthesis, and shows attenuated growth in iron-deficient conditions, whereas *P. aeruginosa* K407 (*pvd*-, *pFr*-) is deficient in pyoverdine biosythesis and lacks the enterobactin transporter PfeA.[74]

In 50% MHB medium, *P. aeruginosa* (*pvd*-, *pch*-) grew to OD_{600} ~ 0.45 (30 ^{o}C, t = 19 h) and this value diminished to ca. 0.25 in the presence of 600 μM DP. Supplementation of the iron-limiting growth medium with low-micromolar concentrations of L-Ent resulted in the restoration of *P. aeruginosa* growth to OD_{600} ~ 0.40 (Figure 3). Comparable growth recovery was observed for cultures treated with eight of the nine conjugates based on L-Ent (Figures 3, 4 and S18). Vancomycin **42**, which exhibits the largest cargo, afforded a growth inhibitory effect (±DP) as observed for *E. coli* (*ent*-). In contrast to its L-Ent analog **30**, conjugate **31** based on D-Ent was growth inhibitory as was D-Ent (Figures 4 and S18). This result demonstrates that *P. aeruginosa* also requires the L-isomer for iron utilization. Lastly, no growth enhancement of *P. aeruginosa* (*pFr*-) was observed in the presence of L-Ent or conjugate **30** (600 μM DP); instead, these siderophores caused growth inhibition at micromolar concentrations (Figure S19). These results demonstrate that PfeA is necessary for conjugate-mediated growth recovery, supporting its role as a transporter for the enterobactin conjugates. In total, these assays demonstrate that the enterobactin transport machinery of *P. aeruginosa*, and PfeA in particular, recognizes and delivers various cargo-modified enterobactin scaffolds to the cytoplasm.

Ciprofloxacin is a fluoroquinolone antibiotic that acts in the cytoplasm and inhibits DNA gyrase.[77] The fact that ciprofloxacin conjugates **35** and **40** each restored *P. aeruginosa* growth demonstrated that the cargo was successfully delivered to the cytoplasm of this microbe with negligible impact of the variable linker composition, and that conjugation of ciprofloxacin to enterobactin attenuated its antibacterial activity. This observation is in general agreement with

reports of pyoverdine-fluoroquinoline[78] and pyochelin-fluoroquinoline[79,80] conjugates where the antibiotic was covalently attached to the siderophore and point to the need for appropriate linker design for fluoroquinolone delivery and release after cellular entry.[81] These pyoverdine/pyochelin-antibiotic conjugates afforded no antipseudomonal activity or diminished activity relative to the unmodified drug, and the pyoverdine-fluoroquinolone antibiotic exhibited decreased *E. coli* gyrase inhibitory activity *in vitro*.[78]

A comparison of the enterobactin-cargo growth recovery profiles for *E. coli* and *P. aeruginosa* (Figures 4, S15, S18) reveals that these particular microbes have different capacities for internalizing enterobactin-cargo conjugates, and that cargo size is an important factor. Vancomycin has a rigid dome-like structure and a molecular weight of ca. 1.4 KDa, and the assays presented in this work suggest that this molecule is too big for enterobactin-mediated transport into *E. coli* or *P. aeruginosa*. In contrast, small and malleable cargos such as a Boc protecting group and cyclohexane afforded growth recovery comparable to that of L-Ent for both strains. A comparison of OD_{600} values for bacterial cultures treated with such conjugates (e.g. **29**, **30**, **32**, **34**) shows that growth recovery to levels comparable to that of L-Ent occurs at a conjugate concentration of 1 μM for *P. aeruginosa* whereas 10 μM is required for *E. coli*. *P. aeruginosa* responds to lower Ent concentrations than *E. coli*, which indicates a higher uptake efficiency. Coumarin 343 is an example of a cargo that exhibits no signs of toxicity over the concentration range tested and affords markedly different results on microbial growth promotion for these two species. A comparison of the ciprofloxacin conjugate data for *E. coli* and *P. aeruginosa* also suggests differential uptake. For both the ciprofloxacin and coumarin cargo, the growth recovery assays indicate that the enterobactin transport machinery of *P. aeruginosa* imports these cargos whereas the *E. coli* system does not. These observations suggest that species-selective targeting may be possible with strategic cargo choice even when a siderophore is utilized by multiple microbial species.

Summary and Perspectives

We have designed and prepared a family of monofunctionalized enterobactin derivatives, and utilized these scaffolds for the preparation of enterobactin-cargo conjugates bearing cargos of varying size and complexity. Growth recovery assays employing *E. coli* and *P. aeruginosa* revealed that the enterobactin uptake machineries of these Gram-negative species recognize and transport enterobactin-cargo conjugates to the Gram-negative cytoplasm. These studies are significant in several respects. First, the notion of using siderophores for antibiotic delivery across the Gram-negative outer membrane, which serves as a permeability barrier, has achieved long-term interest.[6-8,13] Such "Trojan horse" antibiotics are largely inspired by the sideromycins,[11,12] a family of siderophore-antibiotic conjugates produced by the soil bacterium *Streptomyces*, and by early observations that catechol-modified β-lactams were recognized by the iron-uptake machinery of Gram-negative microbes.[43-46] Significant efforts have been made to prepare and characterize synthetic siderophore-antibiotic conjugates with the goal of targeting drug-resistant Gram-negative pathogens.[13,14] Timely examples of siderophore-antibiotic conjugates with antimicrobial activity include a mycobactin-artemisinin conjugate that kills *Mycobacterium tuberculosis* and *Plasmodium falciparum*,[82] and amoxicillin/ampicillin-appended tripodal triscatecholates that exhibit potent antipseudomonal activity relative to the parent β-lactam antibiotics.[49] One bottleneck with this general approach, and using siderophores in other applications, is that few synthetically tractable and modifiable native siderophores are available. DFO B and pyoverdine, which are readily obtained commercially (DFO B) or from bacterial cultures (pyoverdines), provide free amino groups useful for conjugation and are most commonly derivatized for application-based work.[18] Syntheses of modified pyochelin,[15] petrobactin,[19] and mycobactin[82,83] platforms that house functional groups amenable to site-specific elaboration have been reported, and these scaffolds are important contributions to the toolkit of siderophores that can be modified without compromising Fe(III) coordination in addition to recognition by siderophore-binding proteins. The syntheses described in this work provide enterobactin with a functional handle for versatile chemical modifications,

and will allow strategic use of this canonical siderophore in a multitude of chemical biology and biotechnology initiatives.

Unanswered questions regarding the antibacterial activity and fate of reported synthetic siderophore-antibiotic conjugates exist. Whether a given conjugate is actively transported into the bacterial cell is oftentimes unclear. Because FepA recognizes relatively large biomolecules including MccE492m (84-aa) and colicin B (324-aa), it is tempting to predict that FepA may accommodate almost any cargo appended to an enterobactin or catecholate platform. The results presented in this work challenge this notion and indicate that cargo size is an important and species-specific parameter. Our assays indicate that *P. aeruginosa* PAO1 has a greater capacity to import enterobactin-cargo conjugates than *E. coli* ATCC 33475. It will be interesting to determine the cargo scope of other *E. coli* strains and bacterial species that utilize enterobactin for iron acquisition, and understand the molecular and physiological basis for such variations. Colicins are largely α-helical[40] and MccE492m shares some sequence homology with colicins.[84] It is likely that some enterobactin receptors have decreased propensity to transport synthetic small molecules or natural products with less structural malleability (i.e. vancomycin) than an α-helical peptide.

The mechanisms of iron release from siderophores, which vary tremendously for the myriad of siderophores produced by different bacterial species, are another important consideration in siderophore-cargo conjugate design. Guided by studies of chiral recognition in enterobactin transport, which demonstrated that D-Ent is transported into *E. coli* but cannot be hydrolyzed by Fes,[71] we designed the monofunctionalized D-Ent scaffolds to probe cytosolic delivery. This design feature prevents esterase-catalyzed iron release from enterobactin-based conjugates in the cytoplasm and may have practical utility. From the standpoint of drug delivery, a tug-of-war may result from utilizing an iron-supplying siderophore that confers a growth advantage for delivering a toxic payload to a bacterial cell, and preventing iron release may be beneficial. In other applications, siderophore-fluorophore conjugates are of interest for bacterial

detection and diagnostics, and Fe(III) binding to and release from the siderophore will likely influence the photophysical properties of such molecules.

In summary, these investigations reveal that the enterobactin transport machineries of *E. coli* (e.g. FepABCDG and TonB-ExbB-ExbD) and *P. aeruginosa* will deliver enterobactin-modified cargo to the Gram-negative cytoplasm. Moreover, the preparative work affords a new siderophore platform amenable to synthetic elaboration and an entry route for employing the native enterobactin scaffold in a multitude of application-based initiatives that include intracellular cargo delivery, iron sensing, siderophore labeling, protein and pathogen detection, and therapeutic development.

Acknowledgements. The Searle Scholars Program (Kinship Foundation), the Department of Chemistry and the Undergraduate Research Opportunities Program (UROP) at MIT, and the Amgen Scholars Program (J.L.B) are gratefully acknowledged for financial support. We thank Professor Keith Poole for providing the *Pseudomonas aeruginosa* strains employed in this work, Professor Klaus Hantke for providing the *E. coli* H1178 (*fepA*-) strain, Professor Stephen J. Lippard for use an IR spectrophotometer and a melting point apparatus, and Dr. Andrew Wommack for carefully proof-reading the manuscript. *E. coli* K-12 JW0576 was obtained from the Keio Collection.[85] NMR instrumentation maintained by the MIT DCIF is supported by NSF grants CHE-9808061 and DBI-9729592.

Supporting Information. Syntheses and characterization of **14-18** and **26-28**, general liquid chromatography, mass spectrometry and microbiology methods, summary of enterobactin-cargo conjugate characterization (Table S1), summary of bacterial strains and sources (Table S2), structure of MccE492m (Figure S1), HPLC traces for the purified conjugates (Figures S2-S11), optical absorption spectra (Figures S12-S14), growth recovery assays (Figures S15-19), ^{1}H and ^{13}C NMR spectra, and IR spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

1. Hider, R. C.; Kong, X. *Nat. Prod. Rep.* **2010,** *27*, 637-657.
2. Miethke, M.; Marahiel, M. A. *Microbiol. Mol. Biol. Rev.* **2007,** *71*, 413-451.
3. Rajkumar, M.; Ae, N.; Prasad, M. N. V.; Freitas, H. *TRENDS Biotechnol.* **2010,** *28*, 142-149.
4. Bernhardt, P. V. *Dalton Trans.* **2007,** 3214-3220.
5. Manning, T.; Kean, G.; Thomas, J.; Thomas, K.; Corbitt, M.; Gosnell, D.; Ware, R.; Fulp, S.; Jarrard, J.; Phillips, D. *Curr. Med. Chem.* **2009,** *16*, 2416-2429.
6. Miller, M. J., *Chem. Rev.* **1989,** *89*, 1563-1579.
7. Roosenberg, J. M., II; Lin, Y.-M.; Lu, Y.; Miller, M. J. *Curr. Med. Chem.* **2000,** *7*, 159-197.
8. Budzikiewicz, H. *Curr. Top. Med. Chem.* **2001,** *1*, 73-82.
9. Ballouche, M.; Cornelis, P.; Baysse, C. *Recent Pat. Anti-infect. Drug Discovery* **2009,** *4*, 190-205.
10. Möllmann, U.; Heinisch, L.; Bauernfeind, A.; Köhler, T.; Ankel-Fuchs, D. *Biometals* **2009,** *22*, 615-624.
11. Braun, V.; Pramanik, A.; Gwinner, T.; Köberle, M.; Bohn, E. *Biometals* **2009,** *22*, 3-13.
12. Braun, V., *Drug Resist. Update* **1999,** *2*, 363-369.
13. Ji, C.; Juárez-Hernández, R. E.; Miller, M. J. *Future Med. Chem.* **2012,** *4*, 297-313.
14. Miller, M. J.; Zhu, H.; Xu, Y.; Wu, C.; Walz, A. J.; Vergne, A.; Roosenberg, J. M.; Moraski, G.; Minnick, A. A.; McKee-Dolence, J.; Hu, J.; Fennell, K.; Kurt Dolence, E.; Dong, L.; Franzblau, S.; Malouin, F.; Möllmann, U. *Biometals* **2009,** *22*, 61-75.
15. Nöel, S.; Guillon, L.; Schalk, I. J.; Mislin, G. L. A. *Org. Lett.* **2011,** *13*, 844-847.
16. Espósito, B. P.; Epsztejn, S.; Breuer, W.; Cabantchik, Z. I. *Anal. Biochem.* **2002,** *304*, 1-18.
17. Lam, C. K. S. C. C.; Jickells, T. D.; Richardson, D. J.; Russell, D. A. *Anal. Chem.* **2006,** *78*, 5040-5045.

18. Zheng, T.; Nolan, E. M. *Metallomics* **2012,** *4*, 866-880.

19. Bugdahn, N.; Peuckert, F.; Albrecht, A. G.; Miethke, M.; Marahiel, M. A.; Oberthür, M., *Angew. Chem. Int. Ed.* **2010,** *49*, 10210-10213.

20. Doorneweerd, D. D.; Henne, W. A.; Reifenberger, R. G.; Low, P. S. *Langmuir* **2010,** *26*, 15424-15429.

21. Kim, Y.; Lyvers, D. P.; Wei, A.; Reifenberger, R. G.; Low, P. S. *Lab Chip* **2012,** *12*, 971-976.

22. Raymond, K. N.; Dertz, E. A.; Kim, S. S. *Proc. Natl. Acad. Sci. U. S. A.* **2003,** *100*, 3584-3588.

23. Crosa, J. H.; Walsh, C. T. *Microbiol. Mol. Biol. Rev.* **2002,** *66*, 223-249.

24. Loomis, L. D.; Raymond, K. N. *Inorg. Chem.* **1991,** *30*, 906-911.

25. Buchanan, S. K.; Smith, B. S.; Venkatramani, L.; Xia, D.; Esser, L.; Palnitkar, M.; Chakraborty, R.; van der Helm, D.; Deisenhofer, J. *Nat. Struct. Biol.* **1999,** *6*, 56-63.

26. Newton, S. M. C.; Igo, J. D.; Scott, D. C.; Klebba, P. E. *Mol. Microbiol.* **1999,** *32*, 1153-1165.

27. Stephens, D. L.; Choe, M. D.; Earhart, C. F. *Microbiology-UK* **1995,** *141*, 1647-1654.

28. Chenault, S. S.; Earhart, C. F. *Mol. Microbiol.* **1991,** *5*, 1405-1413.

29. Shea, C. M.; Mcintosh, M. A. *Mol. Microbiol.* **1991,** *5*, 1415-1428.

30. Chakraborty, R.; Storey, E.; van der Helm, D. *Biometals* **2007,** *20*, 263-274.

31. Krewulak, K. D.; Vogel, H. J. *Biochim. Biophys. Acta.* **2008,** *1778*, 1781-1804.

32. Chu, B. C.; Garcia-Herrero, A.; Johanson, T. H.; Krewulak, K. D.; Lau, C. K.; Peacock, R. S.; Slavinskaya, Z.; Vogel, H. J. *Biometals* **2010,** *23*, 601-611.

33. Lin, H.; Fischbach, M. A.; Liu, D. R.; Walsh, C. T. *J. Am. Chem. Soc.* **2005,** *127*, 11075-11084.

34. Miethke, M.; Hou, J.; Marahiel, M. A. *Biochemistry* **2011,** *50*, 10951-10964.

35. Bäumler, A. J.; Norris, T. L.; Lasco, T.; Voigt, W.; Reissbrodt, R.; Rabsch, W.; Heffron, F., *J. Bacteriol.* **1998,** *180*, 1446-1453.

36. Lagos, R.; Baeza, M.; Corsini, G.; Hetz, C.; Strahsburger, E.; Castillo, J. A.; Vergara, C.; Monasterio, O. *Mol. Microbiol.* **2001,** *42*, 229-243.

37. Nolan, E. M.; Fischbach, M. A.; Koglin, A.; Walsh, C. T. *J. Am. Chem. Soc.* **2007,** *129*, 14336-14347.

38. Fischbach, M. A.; Lin, H. N.; Liu, D. R.; Walsh, C. T., *Proc. Natl. Acad. Sci. U. S. A.* **2005,** *102*, 571-576.

39. Müller, S.; Valdebenito, M.; Hantke, K. *Biometals* **2009,** *22*, 691-695.

40. Cao, Z.; Klebba, P. E. *Biochimie* **2002,** *84*, 399-412.

41. Lagos, R.; Tello, M.; Mercado, G.; Garciá, V.; Monasterio, O. *Curr. Pharm. Biotechnol.* **2009,** *10*, 74-85.

42. Rabsch, W.; Ma, L.; Wiley, G.; Najar, F. Z.; Kaserer, W.; Schuerch, D. W.; Klebba, J. E.; Roe, B. A.; Gomez, J. A. L.; Schallmey, M.; Newton, S. M. C.; Klebba, P. E. *J. Bacteriol.* **2007,** *189*, 5658-5674.

43. Katsu, K.; Kitoh, K.; Inoue, M.; Mitsuhashi, S. *Antimicrob. Agents Chemother.* **1982,** *22*, 181-185.

44. Watanabe, N.-A.; Nagasu, T.; Katsu, K.; Kitoh, K. *Antimicrob. Agents Chemother.* **1987,** *31*, 497-504.

45. Nakagawa, S.; Sanada, M.; Matsuda, K.; Hazumi, N.; Tanaka, N. *Antimicrob. Agents Chemother.* **1987,** *31*, 1100-1105.

46. Hashizume, T.; Sanada, M.; Nakagawa, S.; Tanaka, N. *J. Antibiot.* **1990,** *43*, 1617-1620.

47. Möllmann, U.; Ghosh, A.; Dolence, E. K.; Dolence, J. A.; Ghosh, M.; Miller, M. J.; Reissbrodt, R. *Biometals* **1998,** *11*, 1-12.

48. Mckee, J. A.; Sharma, S. K.; Miller, M. J. *Bioconjugate Chem.* **1991,** *2*, 281-291.

49. Ji, C.; Miller, P. A.; Miller, M. J. *J. Am. Chem. Soc.* **2012,** *134*, 9898-9901.

50. Diarra, M. S.; Lavoie, M. C.; Jacques, M.; Darwish, I.; Dolence, E. K.; Dolence, J. A.; Ghosh, A.; Ghosh, M.; Miller, M. J.; Malouin, F. *Antimicrob. Agents Chemother.* **1996,** *40*, 2610-2617.

51. Ghosh, A.; Ghosh, M.; Niu, C.; Malouin, F.; Möllmann, U.; Miller, M. J. *Chem. Biol.* **1996,** *3*, 1011-9.

52. Corey, E. J.; Bhattacharyya, S. *Tetrahedron Lett.* **1977,** *45*, 3919-3922.

53. Rastetter, W. H.; Erickson, T. J.; Venuti, M. C. *J. Org. Chem.* **1981,** *46*, 3579-3590.

54. Shanzer, A.; Libman, J. *J. Chem. Soc., Chem. Commun.* **1983,** *15*, 846-847.

55. Ramirez, R. J. A.; Karamanukyan, L.; Ortiz, S.; Gutierrez, C. G. *Tetrahedron Lett.* **1997,** *38*, 749-752.

56. Marinez, E. R.; Salmassian, E. K.; Lau, T. T.; Gutierrez, C. G. *J. Org. Chem.* **1996,** *61*, 3548-3550.

57. Rodgers, S. J.; Lee, C.-W.; Ng, C. Y.; Raymond, K. N. *Inorg. Chem.* **1987,** *26*, 1622-1625.

58. Tor, Y.; Libman, J.; Shanzer, A.; Felder, C. E.; Lifson, S. *J. Am. Chem. Soc.* **1992,** *114*, 6661-6671.

59. Ecker, D. J.; Loomis, L. D.; Cass, M. E.; Raymond, K. N. *J. Am. Chem. Soc.* **1988,** *110*, 2457-2464.

60. Stack, T. D. P.; Hou, Z.; Raymond, K. N. *J. Am. Chem. Soc.* **1993,** *115*, 6466-6467.

61. Yu, X.; Dai, Y.; Yang, T.; Gagné, M. R.; Gong, H. *Tetrahedron* **2011,** *67*, 144-151.

62. Gardner, R. A.; Kinkade, R.; Wang, C.; Phanstiel IV, O. *J. Org. Chem.* **2004,** *69*, 3530-3537.

63. Arnusch, C. J.; Bonvin, A. M. J. J.; Verel, A. M.; Jansen, W. T. M.; Liskamp, R. M. J.; de Kruijff, B.; Pieters, R. J.; Breukink, E. *Biochemistry* **2008,** *47*, 12661-12663.

64. Luo, M.; Lin, H.; Fischbach, M. A.; Liu, D. R.; Walsh, C. T.; Groves, J. T. *ACS Chem. Biol.* **2006,** *1*, 29-32.

65. Thomas, X.; Destoumieux-Garzón, D.; Peduzzi, J.; Afonso, C.; Blond, A.; Birlirakis, N.; Goulard, C.; Dubost, L.; Thai, R.; Tabet, J.-C.; Rebuffat, S., *J. Biol. Chem.* **2004,** *279*, 28233-28242.

66. Abergel, R. J.; Zawadzka, A. M.; Hoette, T. M.; Raymond, K. N. *J. Am. Chem. Soc.* **2009,** *131*, 12682-12692.

67. Zheng, T.; Nolan, E. *Unpublished results*.

68. Hubbard, B. K.; Walsh, C. T. *Angew. Chem. Int. Ed.* **2003,** *42*, 730-765.

69. Lawson, M. C.; Shoemaker, R.; Hoth, K. B.; Bowman, C. N.; Anseth, K. S. *Biomacromolecules* **2009,** *10*, 2221-2234.

70. Scarrow, R. C.; Ecker, D. J.; Ng, C.; Liu, S.; Raymond, K. N. *Inorg. Chem.* **1991,** *30*, 900-906.

71. Wayne, R.; Frick, K.; Neilands, J. B. *J. Bacteriol.* **1976,** *126*, 7-12.

72. Mossialos, D.; Amoutzias, G. D. *Future Microbiol.* **2007,** *2*, 387-395.

73. Cornelis, P. *App. Microbiol. Biotechnol.* **2010,** *86*, 1637-1645.

74. Poole, K.; Young, L.; Neshat, S. *J. Bacteriol.* **1990,** *172*, 6991-6996.

75. Dean, C. R.; Neshat, S.; Poole, K. *J. Bacteriol.* **1996,** *178*, 5361-5369.

76. Ghysels, B.; Ochsner, U.; Möllman, U.; Heinisch, L.; Vasil, M.; Cornelis, P.; Matthijs, S. *FEMS Microbiol. Lett.* **2005,** *246* (2), 167-174.

77. Collin, F.; Karkare, S.; Maxwell, A. *App. Microbiol. Biotechnol.* **2011,** *92*, 479-497.

78. Hennard, C.; Truong, Q. C.; Desnottes, J.-F.; Paris, J. M.; Moreau, N. J.; Abdallah, M. A. *J. Med. Chem.* **2001,** *44*, 2139-2151.

79. Rivault, F.; Liébert, C.; Burger, A.; Hoegy, F.; Abdallah, M. A.; Schalk, I. J.; Mislin, G. L. A. *Bioorg. Med. Chem. Lett.* **2007,** *17*, 640-644.

80. Nöel, S.; Gasser, V.; Pesset, B.; Hoegy, F.; Rognan, D.; Schalk, I. J.; Mislin, G. L. A. *Org. Biomol. Chem.* **2011,** *9* (24), 8288-8300.

81. Ji, C.; Miller, M. J. *Bioorg. Med. Chem.* **2012,** *20*, 3828-3836.

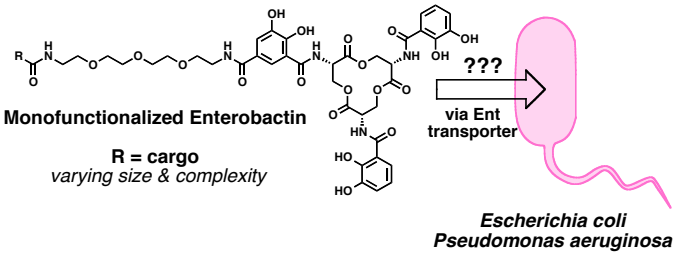
82. Miller, M. J.; Walz, A. J.; Zhu, H.; Wu, C.; Moraski, G.; Möllmann, U.; Tristani, E. M.; Crumbliss, A. L.; Ferdig, M. T.; Checkley, L.; Edwards, R. L.; Boshoff, H. I. *J. Am. Chem. Soc.* **2011,** *133*, 2076-2079.

83. Xu, Y.; Miller, M. J. *J. Org. Chem.* **1998,** *63*, 4314-4322.

84. Pons, A.-M.; Zorn, N.; Vignon, D.; Delalande, F.; Dorsselaer, A. V.; Cottenceau, G. *Antimicrob. Agents Chemother.* **2002**, *46*, 229-230.

85. Baba, T.; Ara, T.; Hasegawa, M.; Takai, Y.; Okumura, Y.; Baba, M.; Datsenko, K. A.; Tomita, M.; Wanner, B. L.; Mori, H., *Mol. Sys. Biol.* **2006**, 2006.0008.

TOC Graphic


???
via Ent
transporter
Monofunctionalized Enterobactin
R = cargo
varying size & complexity
Escherichia coli
Pseudomonas aeruginosa

Supporting Information for

Siderophore-Mediated Cargo Delivery to the Cytoplasm of *Escherichia coli* and *Pseudomonas aeruginosa*: Syntheses of Monofunctionalized Enterobactin Scaffolds and Evaluation of Enterobactin-Cargo Conjugate Uptake

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This Supporting Information includes:

Supplementary Experimental Section

***N*-(2-(2-(2-(2-Aminoethoxy)ethoxy)ethoxy)ethyl)cyclohexanecarboxamide (14).** Cyclohexanecarboxylic acid (64 mg, 0.50 mmol) and **7** (192 mg, 0.599 mmol) were combined in 5 mL of dry CH_2Cl_2, and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC,143 mg, 0.751 mmol), 4-dimethylaminopyridine (DMAP, 30 mg, 0.25 mmol), and DIPEA (435 μL, 2.52 mmol) were added. The reaction was stirred for 4 h at rt, and the organic phase was washed with 50 mM HCl (3 x 20 mL) and brine (1 x 20 mL). The organic phase was dried over Na_2SO_4 and concentrated. Flash chromatography on silica gel with a solvent gradient (CH_2Cl_2 to 10% MeOH/CH_2Cl_2) afforded the Boc-protected product as a colorless oil (190 mg, 94%). TLC R_f = 0.65 (10% MeOH/CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 300 MHz), δ 1.13-1.37 (14H, m), 1.58-1.60 (1H, m), 1.68-1.78 (4H, m), 1.97-2.05 (1H, m), 3.23-3.24 (2H, m), 3.34-3.40 (2H, m), 3.45-3.50 (4H, m), 3.53-3.59 (8H, m), 5.09 (1H, bs), 6.11 (1H, bs). ^{13}C NMR ($CDCl_3$, 125 MHz), δ 25.5, 28.2, 29.4, 38.7, 40.1, 45.2, 69.7, 69.9, 70.0, 70.2, 78.9, 155.8, 176.0. HRMS (ESI): $[M+Na]^+$ *m/z* calcd., 425.2622; found, 425.2654.

A portion of this Boc-protected product (118 mg, 0.278 mmol) was dissolved in 2.5 mL of 40% TFA/CH_2Cl_2 and the light red solution was stirred at rt for 2.5 h. The reaction was concentrated to give **14** as a light-yellow oil in quantitative yield. TLC R_f = 0.2 (10% MeOH/CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 500 MHz), δ 1.13-1.37 (5H, m), 1.62-1.64 (1H, m), 1.71-1.77 (4H, m), 2.10-2.15 (1H, m), 3.16 (2H, bs), 3.36 (2H, bs), 3.48-3.65 (10H, m), 3.76 (2H, bs), 6.93 (1H, bs), 7.88 (2H, 3s). ^{13}C NMR ($CDCl_3$, 125 MHz), δ 25.4, 25.5, 29.4, 39.1, 39.7, 45.1, 66.6, 69.6, 69.8, 69.9, 145.8. HRMS (ESI): $[M+Na]^+$ *m/z* calcd., 325.2098; found, 325.2119.

***N*-(2-(2-(2-(2-Aminoethoxy)ethoxy)ethoxy)ethyl)-2-naphthamide (15).** Compound **15** was synthesized as described for **14** except that 2-naphthoic acid (86 mg, 0.50 mmol) was used instead of cyclohexanecarboxylic acid. The Boc-protected product was obtained as light yellow oil (178 mg, 80%). TLC R_f = 0.7 (10% MeOH/CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 500 MHz), δ 1.36 (9H, s), 3.11-3.12 (2H, m), 3.28 (2H, t, *J* = 5.2 Hz), 3.34-3.35 (2H, m), 3.45 (2H, t, *J* = 4.5 Hz), 3.54-3.60 (4H, m), 3.65 (4H, bs), 4.99 (1H, bs), 6.82 (1H, bs), 7.37 (1H, dd, *J* = 7.8, 7.8 Hz), 7.43-

7.49 (2H, m), 7.55 (1H, d, *J* = 7.0 Hz), 7.79-7.83 (2H, m), 8.26 (1H, d, *J* = 8.0 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz), δ 28.2, 39.5, 39.9, 69.5, 69.7, 70.0, 70.1, 70.2, 78.9, 124.5, 124.9, 125.2, 126.1, 126.7, 128.0, 129.9, 130.2, 133.4, 134.3, 155.7, 169.4. HRMS (ESI): $[M+Na]^+$ *m/z* calcd., 469.2309; found, 469.2335.

Compound **15** was obtained as light orange oil (quantitative yield from 91.6 mg, 0.205 mmol of the Boc-protected precursor). TLC R_f = 0.15 (10% MeOH/CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 300 MHz), δ 3.06 (2H, bs), 3.56-3.66 (14H, m), 7.15 (1H, bs), 7.40-7.56 (6H, m), 7.85-7.94 (2H, m), 8.08-8.11 (1H, m). ^{13}C NMR ($CDCl_3$, 125 MHz), δ 39.3, 39.6, 66.4, 69.6, 69.6, 69.7, 69.9, 124.7, 125.0, 125.1, 126.3, 127.0, 128.3, 129.8, 130.6, 133.5, 133.7, 170.7. *δ* HRMS (ESI): $[M+Na]^+$ *m/z* calcd., 369.1785; found, 369.1806.

***N*-(2-(2-(2-(2-Aminoethoxy)ethoxy)ethoxy)ethyl)-4-benzylbenzamide (16).** Compound **16** was synthesized as described for **14** except that 4-benzylbenzoic acid (106 mg, 0.50 mmol) was used instead of cyclohexanecarboxylic acid. The Boc-protected product was obtained as light yellow oil (220 mg, 90%). TLC R_f = 0.7 (10% MeOH/CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 500 MHz), δ 1.48 (9H, s), 3.32 (2H, s), 3.51-3.54 (1H, m), 3.61-3.71(11H, m), 4.07 (2H, s), 5.10 (1H, s), 6.82 (1H, s), 7.21-7.34 (6H, m), 7.78-7.81 (2H, m). ^{13}C NMR ($CDCl_3$, 125 MHz), δ 28.3, 39.6, 40.2, 41.7, 69.8, 70.1, 70.1, 70.2, 70.4, 70.4, 79.1, 94.0, 126.2, 127.2, 128.5, 128.8, 128.9, 132.4, 140.3, 144.7, 145.8, 145.8, 155.9, 167.3. HRMS (ESI): $[M+Na]^+$ *m/z* calcd., 509.2622; found, 509.2628.

Compound **16** was obtained as brown oil (quantitative yield from 220 mg of the Boc-protected precursor). TLC R_f = 0.4 (10% MeOH/CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 500 MHz), δ 3.12 (2H, s), 3.55-3.62 (12H, m), 3.63-3.71 (2H, m), 4.00 (2H, s), 7.15-7.30 (8H, m), 7.68-7.73 (5H, m), 8.49 (3H, m). ^{13}C ($CDCl_3$, 125 MHz), δ 39.2, 39.5, 41.6, 67.1, 66.8, 69.6, 70.09, 126.2, 127.4, 128.5, 128.7, 128.9, 131.5, 140.3, 145.1. $[M+Na]^+$ *m/z* calcd., 409.2098; found, 409.2093.

***N*-(2-(2-(2-(2-Aminoethoxy)ethoxy)ethoxy)ethyl)-11-oxo-2,3,5,6,7,11-hexahydro-1H-pyrano[2,3-f]pyrido[3,2,1-ij]quinoline-10-carboxamide (17).** Coumarin 343 (142 mg, 0.50

mmol), EDC (143 mg, 0.751 mmol), DMAP (30 mg, 0.25 mmol), and DIPEA (435 μL, 2.52 mmol) were mixed in 15 mL of CH_2Cl_2. A portion (280 μL, 1.50 mmol) of 2,2'-((oxybis(ethane-2,1-diyl))bis(oxy))diethanamine was added and the reaction mixture was stirred overnight at rt. The solvent was removed under reduced pressure and **17** was purified by preparative TLC (15% MeOH/CH_2Cl_2 with 1% TEA) and obtained as an orange oil (86 mg, 38%). TLC R_f = 0.8 (10% MeOH/CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 300 MHz), δ 1.95 (4H, bs), 2.75 (2H, t, *J* = 6.0 Hz), 2.85 (2H, t, *J* = 6.3 Hz), 3.22 (2H, bs), 3.29-3.34 (4H, m), 3.56-3.70 (14H, m), 3.89 (2H, bs), 7.03 (1H, s), 7.65 (2H, bs), 8.55 (1H, s), 9.10 (1H, s). ^{13}C NMR ($CDCl_3$, 125 MHz), δ 19.8, 19.9, 20.8, 27.2, 39.3, 39.9, 49.6, 50.0, 66.5, 69.8, 69.9, 70.0, 105.3, 107.8, 108.0, 119.6, 127.0, 148.1, 148.2, 152.4, 162.8, 164.1. HRMS (ESI): $[M+H]^+$ *m/z* calcd., 460.2442; found, 460.2435.

7-(4-(3-(2-(2-(2-Aminoethoxy)ethoxy)ethoxy)propanoyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (18). Ciprofloxacin (115 mg, 0.50 mmol) and DIPEA (0.5 mL, 2.8 mmol) were mixed in 5 mL of CH_2Cl_2, and TMSCl (135 μL, 1.45 mmol) was added to give a clear yellow solution. Fmoc-PEG-CO_2H **13** (333 mg, 1.50 mmol), EDC (144 mg, 1.50 mmol), DMAP (30 mg, 0.050 mmol), and DIPEA (0.35 mL, 2 mmol) were dissolved in 2 mL of dry CH_2Cl_2, and the two solutions were combined and stirred at rt overnight. The resulting solution was washed with water (1x10 mL), 0.1M HCl (2x20 mL), and brine (1x20 mL), dried over Na_2SO_4, and purified by flash chromatography on silica gel (3% isopropanol/CH_2Cl_2) to give the product as yellow solid (206 mg, 54%). TLC R_f = 0.6 (10% MeOH/CH_2Cl_2); mp = 83 ^{o}C (decomp). ^{1}H NMR ($CDCl_3$, 300 MHz), δ 1.11-1.16 (2H, m), 1.30-1.34 (2H, m), 2.64 (2H, t, *J* = 6.6 Hz), 3.23-3.69 (19H, m), 3.78-3.83 (4H, m), 4.17 (1H, t, *J* = 6.9 Hz), 4.35 (2H, d, *J* = 6.9 Hz), 5.64-5.66 (1H, m), 7.26-7.31 (3H, m), 7.34-7.39 (2H, m), 7.57-7.59 (2H, m), 7.70-7.73 (2H, m), 7.90-7.94 (1H, m), 8.66 (1H, s), 14.9 (1H, s). ^{13}C NMR ($CDCl_3$, 125 MHz), δ 7.7, 33.0, 35.0, 40.5, 40.7, 44.9, 46.8, 49.5, 66.1, 66.8, 69.7, 69.8, 70.0, 70.0, 104.7, 170.2, 111.3, 111.6, 119.0, 119.1, 119.5, 124.7, 126.7, 127.3, 138.5, 140.7, 143.5, 144.8, 145.0, 146.9, 151.3, 154.6, 156.2, 166.4, 169.3, 176.2, 176.3. ^{19}F NMR ($CDCl_3$, 282 MHz) *δ* -121.2. HRMS (ESI): $[M+Na]^+$ *m/z* calcd., 779.3063; found, 779.3052.

A portion of this product (182 mg, 0.240 mmol) was dissolved in CH_2Cl_2 (2 mL) and diethylamine (2 mL, 19.3 mmol) was added. The solution was stirred for 2 h and concentrated under reduced pressure, and this procedure was repeated. A portion of the crude yellow product was dissolved in 3:7 H_2O/DMSO and purified by semi-preparative HPLC (20% B for 5 min followed by 20%-70% B over 20 min, 4 mL/min). Compound **18** eluted at 11 min and a yellow powder was obtained after lyophilization (38 mg). TLC R_f = 0.15 (10% MeOH/CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 500 MHz), δ 1.15 (2H, bs), 1.41 (2H, bs), 2.70 (2H, bs), 3.19 (2H, bs), 3.30-3.37 (4H, m), 3.62-3.82 (17 H, m), 7.37 (1H, bs), 7.83-7.85 (1H, m), 8.16 (3H, bs), 8.65 (1H, s). ^{13}C NMR ($CDCl_3$, 125 MHz), δ 8.1, 33.1, 35.5, 40.0, 41.3, 45.2, 49.0, 49.6, 66.4, 67.1, 69.9, 70.1, 70.2, 105.4, 107.7, 111.9, 112.1, 119.8, 119.8, 139.0, 145.2, 145.3, 147.4, 152.4, 152.4, 167.0, 170.4, 176.8. ^{19}F NMR ($CDCl_3$, 282 MHz) *δ* -121.4, -75.6. HRMS (ESI): $[M+H]^+$ *m/z* calcd., 535.2563; found, 535.2578.

***N*,*N*'-((3*S*,7*S*,11*S*)-11-(2,3-bis(benzyloxy)-5-((*E*)-prop-1-en-1-yl)benzamido)-2,6,10-trioxo-1,5,9-trioxacyclododecane-3,7-diyl)bis(2,3-bis(benzyloxy)benzamide) (26).** Compound **5** (0.441 g, 1.19 mmol) was dissolved in 8 mL of DMSO and DIPEA (2.28 mL, 13.1 mmol) was added. In a separate flask, compounds **6 (**0.591 g, 1.78 mmol), **22** (0.669 g, 1.78 mmol), and PyAOP(2.48 g, 4.76 mmol) were combined in 15 mL of DMSO. This mixture was added drop wise to the solution of **5**, and the resulting solution was stirred for 2 h at rt during which time it turned dark red-brown. The reaction was diluted with 50 mL of EtOAc and 25 mL of water. The layers were partitioned, and the organic phase was washed with saturated brine (2 x 25 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure, which yielded a red-orange oil. Flash chromatography on silica gel with a solvent gradient (10% EtOAc/hexanes to 50% EtOAc/hexanes) afforded **26** as a white-yellow solid (207 mg, 15%). TLC R_f = 0.4 (50% EtOAc/Hexanes). ^{1}H NMR ($CDCl_3$, 500 MHz), δ 1.88-1.89 (3H, m) 4.01-4.04 (3H, m) 4.14-4.16 (3H, m) 4.91-4.92 (3H, m) 5.02-5.27 (12H, m) 6.18-6.22 (1H, m) 6.35-6.40 (1H, m) 7.12-7.68 (36H, m) 8.48-8.49 (3H, d, J=7.5) ^{13}C NMR ($CDCl_3$, 500 MHz) δ 18.4, 51.4, 51.4, 64.1, 71.2, 76.3, 76.4, 76.7, 117.6, 120.7, 123.2, 124.3, 126.0, 126.4, 126.5, 127.6, 127.7,

127.8, 128.2, 128.4, 128.6, 128.6, 128.8, 128.8, 128.9, 128.9, 129.3, 129.8, 134.3, 136.0, 136.3, 147.0, 151.6, 164.9, 169.1. HRMS (ESI): $[M+Na]^+$ *m/z* calcd., 1272.4464; found, 1272.4434.

***N*,*N*'-((3*S*,7*S*,11*S*)-11-(2,3-bis(benzyloxy)-5-formylbenzamido)-2,6,10-trioxo-1,5,9-trioxacyclododecane-3,7-diyl)bis(2,3-bis(benzyloxy)benzamide) (27)**: Compound **26** (159 mg, 0.127 mmol) was dissolved in 6 mL of 1,4-dioxane. Water (2 mL) was slowly added drop wise to the solution. With each drop of water, a white cloudy precipitate appeared and then disappeared. OsO_4 (39 μL of a 2.5 wt % solution in 2-methyl-2-propanol, 3.8 μmol) was then added to the solution, and the reaction was stirred for 0.5 h, which yielded a brown solution. $NaIO_4$ (67 mg, 0.42 mmol) was added to the reaction, which was stirred for 2 h and a white precipitate formed. The reaction was diluted with EtOAc (25 mL) and water (10 mL). The organic phase was washed with 0.1 M NaS_2O_3 (3 x 20 mL) and brine (1 x 20 mL). The organic phase gained a yellow tint with the addition of NaS_2O_3. The organic phase was dried over Na_2SO_4 and concentrated in vacuo. Flash chromatography on silica gel using a solvent gradient (20% EtOAc/hexanes to 70% EtOAc/hexanes) yielded the product as a white foam (101 mg, 64%). TLC R_f = 0.7 (10% MeOH/CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 500 MHz), δ 4.00-4.08 (3H, m), 4.15-4.22 (3H, m), 4.88-4.93 (3H, m), 5.03-5.26 (12H, m), 7.08-7.43 (31H, m) 7.65-7.65 (2H, m), 8.14 (1H, s), 8.41-8.47 (3H, m), 9.88 (1H, s). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 51.4, 51.5, 51.5, 51.7, 64.1, 64.3, 71.2, 71.3, 76.3, 76.6, 113.2, 117.5, 117.5, 123.1, 123.2, 124.3, 126.3, 126.3, 126.6, 127.6, 127.6, 127.8, 128.2, 128.2, 128.4, 128.4, 128.5, 128.6, 128.6, 128.7, 128.9, 128.9, 129.0, 132.2, 135.3, 135.4, 136.0, 136.0, 136.2, 146.8, 146.9, 151.6, 151.8, 152.3, 163.8, 165.0, 165.0, 168.8, 169.0, 169.2, 190.7. HRMS (ESI): $[M+Na]^+$ *m/z* calcd., 1260.4101; found, 1260.4094.

3,4-Bis(benzyloxy)-5-(((3*S*,7*S*,11*S*)-7,11-bis(2,3-bis(benzyloxy)benzamido)-2,6,10-trioxo-1,5,9-trioxacyclododecan-3-yl)carbamoyl)benzoic acid (28): Aldehyde **27** (0.092 g, 0.074 mmol) was dissolved in 2 mL of 1,4-dioxane. In a separate flask, NH_3SO_3 (12.8 mg, 0.132 mmol) was dissolved in 0.5 mL of H_2O. The sulfamic acid solution was added to the 1,4-dioxane solution. The reaction turned cloudy shortly after the addition of NH_3SO_3. $NaClO_2$ (12

mg, 0.13 mmol) was dissolved in 0.4 mL of H_2O and added drop wise over ten minutes to the dioxane solution. The reaction was stirred for 30 min and diluted with 10mL of H_2O and 10 mL of EtOAc. The aqueous phase was back extracted with EtOAc (2 x 10 mL), and the combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. Flash chromatography on silica gel with an solvent gradient (CH_2Cl_2 to 10% MeOH/CH_2Cl_2) yielded a white solid (83 mg, 89%). TLC R_f = 0.6 (10% MeOH/CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 500 MHz), δ 4.08-4.11 (3H, m), 4.25-4.28 (3H, m), 4.96-5.00 (3H, m), 5.08-5.29 (12H, m), 7.14-7.51 (30H, m), 7.70-7.72 (2H, m), 7.89 (1H, s), 8.45-8.48 (2H, m), 8.53-8.59 (2H, m). ^{13}C NMR ($CDCl_3$, 125 MHz), δ 51.5, 51.5, 51.6, 53.6, 64.2, 71.2, 71.3, 76.3, 76.5, 117.5, 117.7, 123.1, 124.3, 125.7, 126.3, 127.6, 127.6, 127.9, 128.2, 128.4, 128.5, 128.6, 128.7, 128.8, 128.9, 129.0, 135.5, 135.6, 136.0, 136.2, 145.9, 146.9, 150.8, 151.4, 151.6, 164.1, 165.1, 168.9, 169.0, 169.1, 169.6. HRMS (DART): $[M+H]^+$ *m/z* calc., 1254.4230; found, 1254.4225.

General Liquid Chromatography and Mass Spectrometry Methods. HPLC-grade acetonitrile (MeCN) and trifluoroacetic acid (TFA) were purchased from EMD. LC-MS grade MeCN containing 0.1% formic acid and water containing 0.1% formic acid were obtained from J. T. Baker. Semi-preparative and analytical high-performance liquid chromatography (HPLC) were performed by using an Agilent 1200 series HPLC system outfitted with an Agilent Zorbax reverse-phase C18 column (5 μm pore size, 9.4 x 250 mm) at a flow rate of 4 mL/min and a Clipeus reverse-phase C18 column (5 μm pore size, 4.6 x 250 mm; Higgins Analytical, Inc.) at a flow rate of 1 mL/min, respectively. The multi-wavelength detector was set to read the absorption at 220, 280, and 316 (catecholate absorption) nm using a reference wavelength of 500 nm. For all HPLC runs, solvent A was 0.1% TFA/H_2O and solvent B was 0.1% TFA/MeCN. Each semi-preparative or analytical run began with a five-minute equilibration at the %B used at the start of the gradient followed by a gradient of increasing %B. The HPLC solvents were prepared with HPLC-grade MeCN and TFA, and Milli-Q water (18.2 mΩcm^{-1}), and filtered through a 0.2-μm filter before use. For analytical HPLC to evaluate conjugate purity, the entire portion of each HPLC-purified compound was dissolved in a mixture of 1:1:1 1,4-

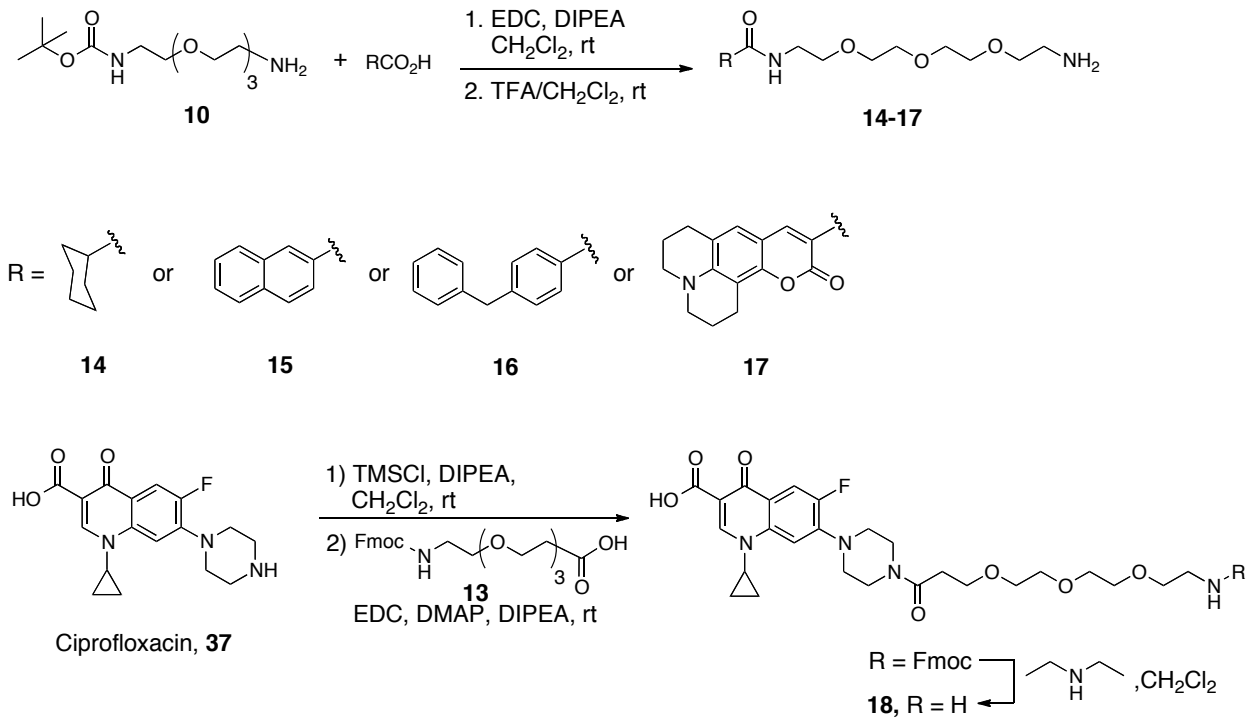
dioxane/methanol/water and an aliquot was taken for HPLC analysis, and the solution was subsequently lyophilized. Conjugate **40** was an exception, and this molecule was dissolved in DMSO prior to analytical HPLC. Most high-resolution mass spectrometry was performed by using an Agilent LC-MS system comprised of an Agilent 1260 series LC system outfitted with an Agilent Poroshell 120 EC-C18 column (2.7 μm pore size) and an Agilent 6230 TOF system housing an Agilent Jetstream ESI source. For all LC-MS analyses, solvent A was 0.1% formic acid / H_2O and solvent B was 0.1% formic acid / MeCN. The samples were run using a gradient of 5-95% B over five min with a flow rate of 0.4 mL/min. In some instances, high-resolution mass spectrometry was performed by staff at the MIT Department of Chemistry Instrumentation Facility, which houses a Bruker Daltonics APEXIV 4.7 Tesla Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FT-ICR-MS) with a direct analysis in real time (DART) ionization source.

General Microbiology Materials and Methods. *E. coli* 33475 (*ent*-) was purchased from American Type Culture Collection (ATCC). *E. coli* K-12 JW0576 (*fes*-) was obtained from the Keio Collection (Japan). *Pseudomonas aeruginosa* K648 (pvd$^-$/pch$^-$) and K407 (p*vd*-/*pFr*-) were gifts from Professor Keith Poole (Department of Biomedical and Molecular Sciences, Queen's University, Canada). Freezer stocks of all *E. coli* strains were prepared in 25% glycerol/Luria Broth (LB) medium. Freezer stocks of all *Pseudomonas aeruginosa* strains were prepared in 7.5% DMSO/LB base medium supplemented with 2.5 g/L NaCl. Luria Broth (tryptone 10 g/L, yeast extract 5 g/L, NaCl 10 g/L), Luria Broth base (pancreatic digest of casein 10 g/L, yeast extract 5 g/L, NaCl 0.5 g/L), Mueller Hinton Broth (MHB, beef extract powder 2.0 g/L, acid digest of casein 17.5 g/L, soluble starch 1.5 g/L), and agar were purchased from BD. All growth media and Milli-Q water used for bacterial cultures or for preparing solutions of the enterobactin-cargo conjugates were sterilized by using an autoclave. The iron chelator 2,2'-dipyridyl (DP) was purchased from Sigma-Aldrich. A 200 mM DP stock solution was prepared in DMSO and used in the bacteria growth assays. All enterobactin-cargo conjugates, L-Ent and D-Ent were stored as DMSO stock solutions at -20 °C. With the exception of the coumarin 343 conjugate, the stock

solution concentrations were determined by using the reported extinction coefficient for enterobactin (316 nm, 9,500 $M^{-1}cm^{-1}$)[S1] with the assumption that the cargo had no effect on catecholate absorption. Working dilutions of the Ent conjugates, L-Ent, and D-Ent were prepared in 20% DMSO/H_2O, and the growth recovery assay cultures all contained 2% v/v DMSO. Sterile polypropylene culture tubes and sterile polystyrene 96-well plates used for culturing were manufactured by VWR and Corning Incorporated, respectively. OD_{600} values were recorded on an Agilent 8453 diode array spectrophotometer or by using a BioTek Synergy HT plate reader.

References

(S1) Scarrow, R. C.; Ecker, D. J.; Ng, C.; Liu, S.; Raymond, K. N. *Inorg. Chem.* **1991**, *30*, 900-906.



Scheme S1. Syntheses of PEG-derivatized cargos **14**-**18**.

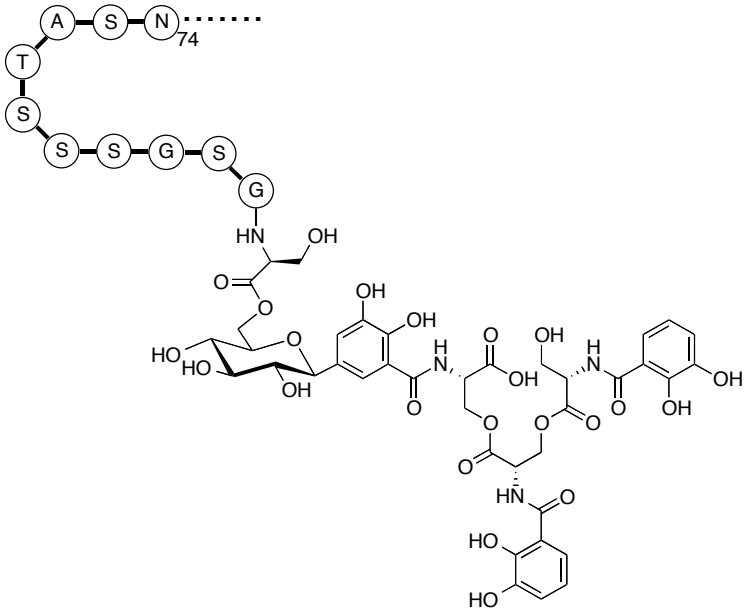
Table S1. Characterization of enterobactin-cargo conjugates.

No.	Cargo	HPLC retention time (min) [a]	*m/z* obs	*m/z* cald.[b]
29	Boc	25.3	1010.3173	1010.3125
30	Cyclohexyl	24.6	1020.3346	1020.3333
31	Cyclohexyl (D-Ent)	24.6	1020.3328	1020.3333
32	Napthyl	25.3	1064.3086	1064.3020
33	Phenylmethylbenzyl	25.2	1104.3305	1104.3333
34	Coumarin 343	27.2	1177.3570	1177.3496
35	Ciprofloxacin (PEG)	24.9	1252.3633	1252.3617
40	Ciprofloxacin (alkyl)	26.7	1140.3482	1140.3486 [d]
42	Vancomycin (triazole)	20.4	1228.3796	1228.3796 [c]
43	Boc-glycine (triazole)	23.8	1126.3775	1126.3832 [d]

[a] HPLC gradient used for all compounds is 0% B for 5 min followed by 0-100% B over 30 min, 1 mL/min. [b] All *m/z* values correspond to $[M+Na]^+$ unless specified otherwise. [c] The *m/z* value corresponds to $[M+2Na]^{2+}/2$. [d] The *m/z* value corresponds to $[M+H]^+$.

Table S2. Bacterial strains used in this study.

Bacterial strain	Description	Source
E. coli ATCC 33475	*ent-*	ATCC
E. coli JW0576	*fes-*	Kieo Collection
E. coli H1187	*fepA-*	Professor Klaus Hantke Universtät Tübingen
P. aeruginosa K648	*pvd-, pch-*	Professor Keith Poole Queen's University, Canada
P. aeruginosa K407	*pvd-, pFr-*	Professor Keith Poole Queen's University Canada



Microcin E492m (MccE492m)

Figure S1. Structure of MccE492m. The C-terminal amino acids are depicted by the cartoon.

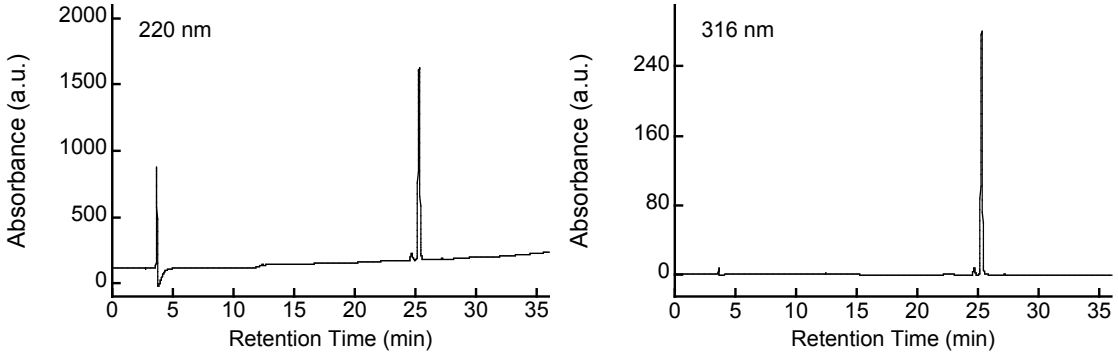


Figure S2. Analytical HPLC traces of purified **29** (0% B for 5 min following by 0-100% B over 30 min, 1 mL/min). The minor peak at ca. 24.6 min is attributed to loss of the Boc group resulting from the acidic HPLC conditions.

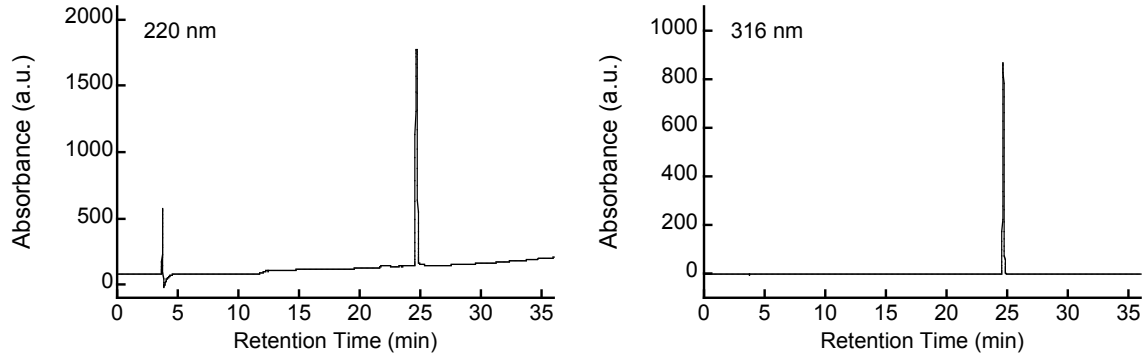


Figure S3. Analytical HPLC traces of purified **30** (0% B for 5 min followed by 0-100% B over 30 min, 1 mL/min).

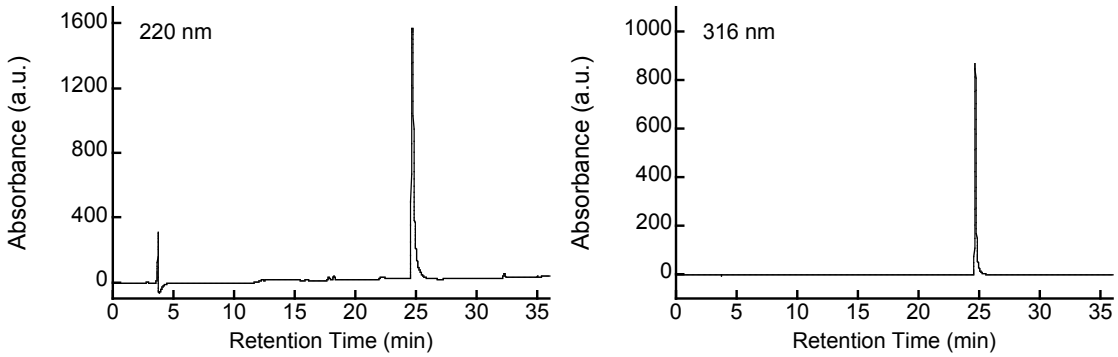


Figure S4. Analytical HPLC traces of purified **31** (0% B for 5 min followed by 0-100% B over 30 min, 1 mL/min).

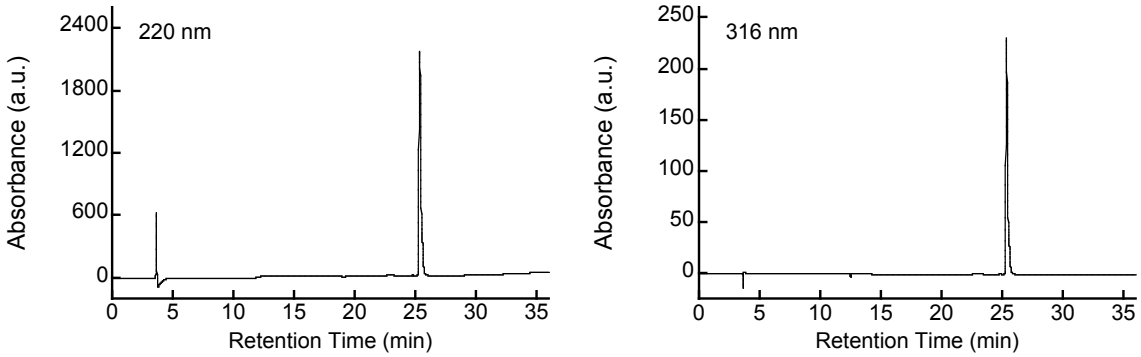


Figure S5. Analytical HPLC traces of purified **32** (0% B for 5 min followed by 0-100% B over 30 min, 1 mL/min).

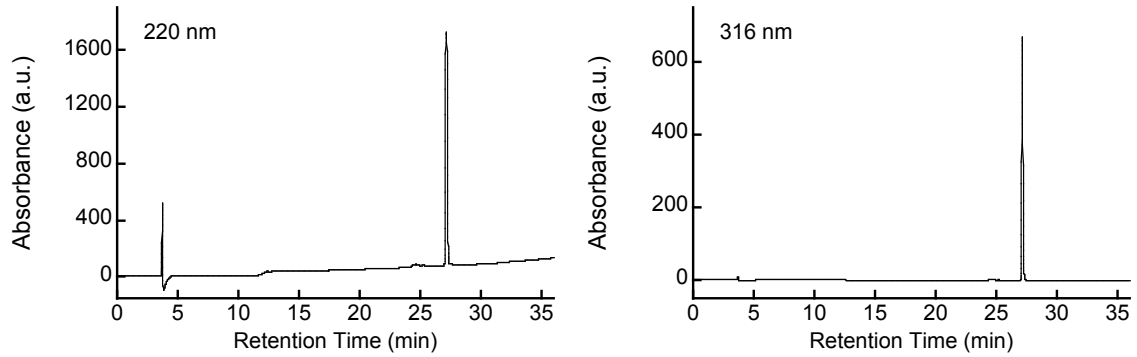


Figure S6. Analytical HPLC traces of purified **33** (0% B for 5 min followed by 0-100% B over 30 min, 1 mL/min).

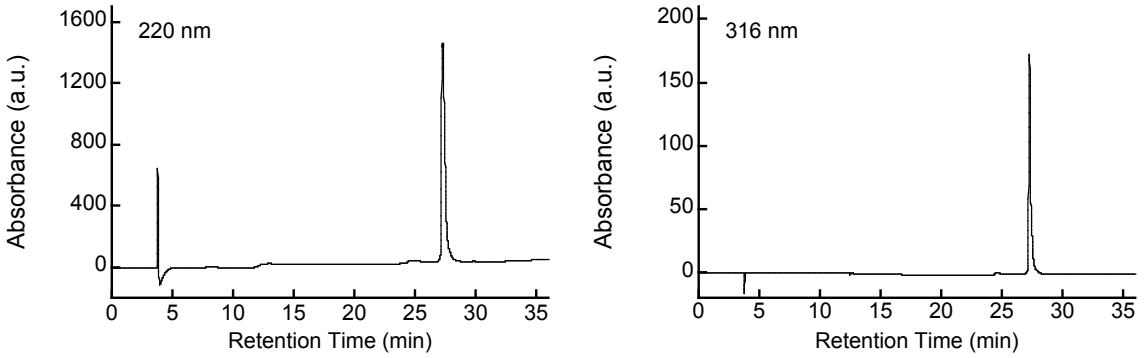


Figure S7. Analytical HPLC traces of purified **34** (0% B for 5 min followed by 0-100% B over 30 min, 1 mL/min).

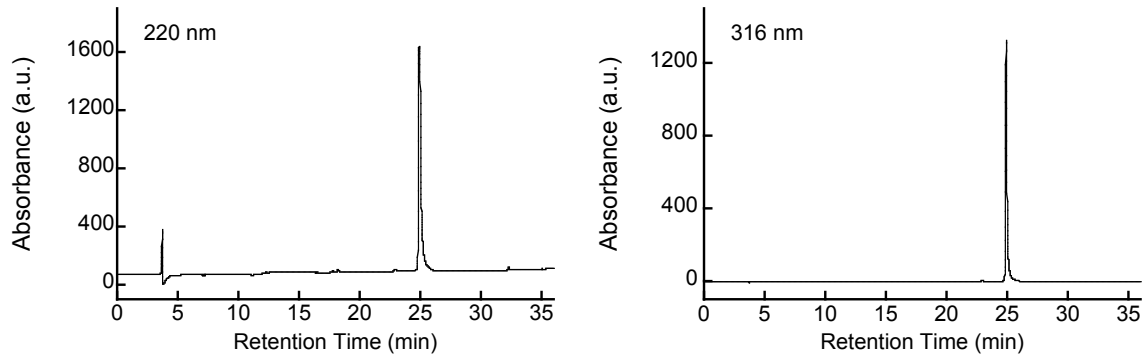


Figure S8. Analytical HPLC traces of purified **35** (0% B for 5 min followed by 0-100% B over 30 min, 1 mL/min).

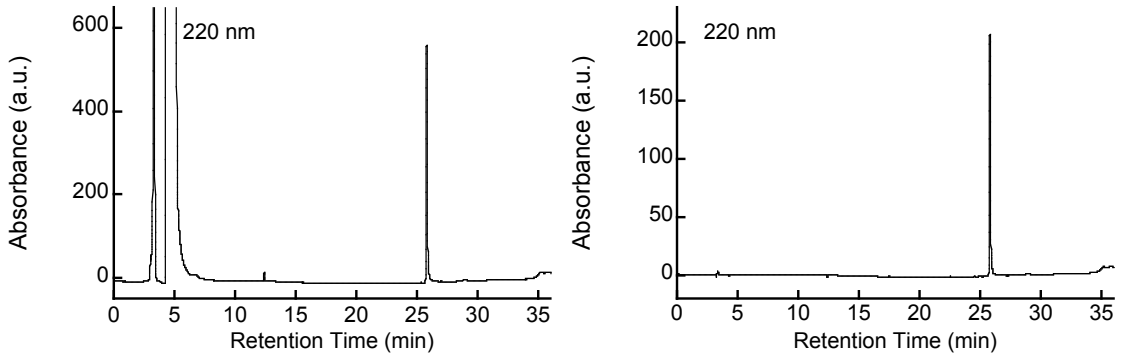


Figure S9. Analytical HPLC traces of purified **40** (0% B for 5 min followed by 0-100% B over 30 min, 1 mL/min). The peak at ca. 5 min in the 220 nm trace is because of the DMSO solvent.

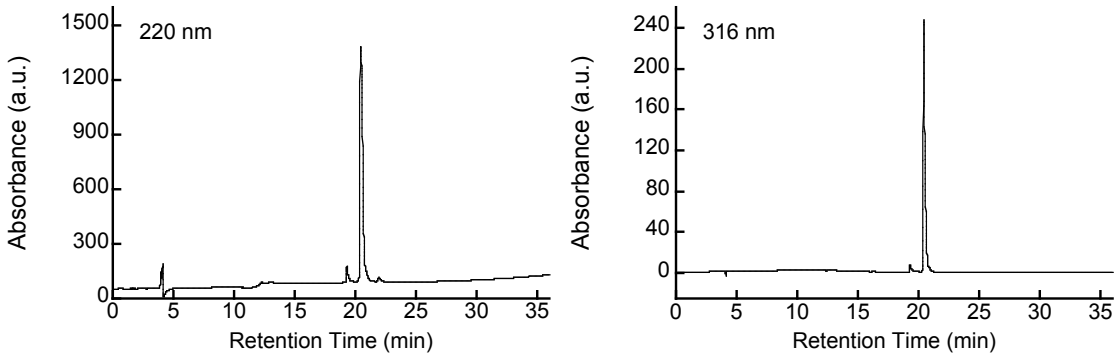


Figure S10. Analytical HPLC traces of purified **42** (0% B for 5 min followed by 0-100% B over 30 min, 1 mL/min).

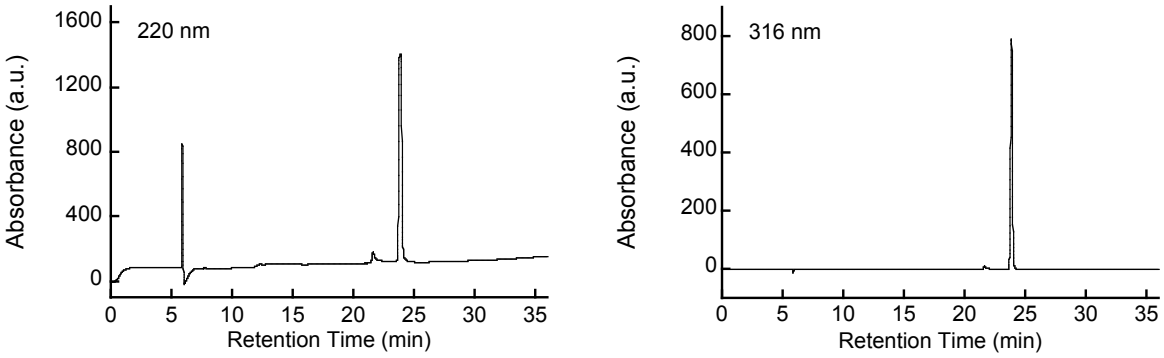


Figure S11. Analytical HPLC traces of purified **43** (0% B for 5 min followed by 0-100% B over 30 min, 1 mL/min). The minor peak at ca. 22 min is attributed to loss of the Boc group resulting from the acidic HPLC conditions.

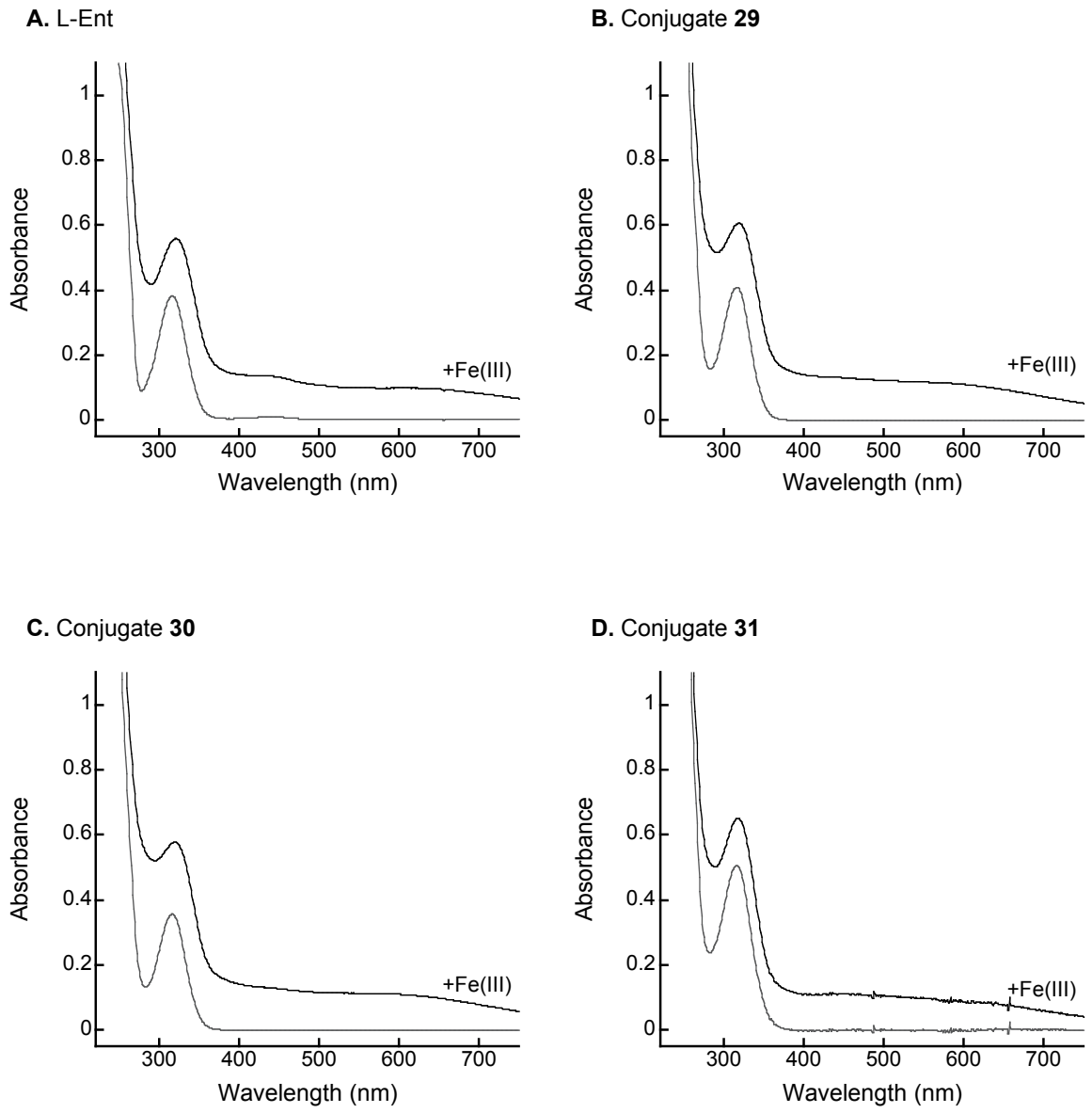


Figure S12. Optical absorption spectra of L-Ent and enterobactin-cargo conjugates **29**-**31** in the absence (grey) and presence (black) of Fe(III). (MeOH, rt). The concentration of ligand was ca. 40 μM and ca. one equiv of Fe(III) was added. (A) L-Ent; (B) conjugate **29**, Boc cargo; (C) conjugate **30**, cyclohexyl cargo; (D) conjugate **31**, cyclohexyl cargo and D-Ent.

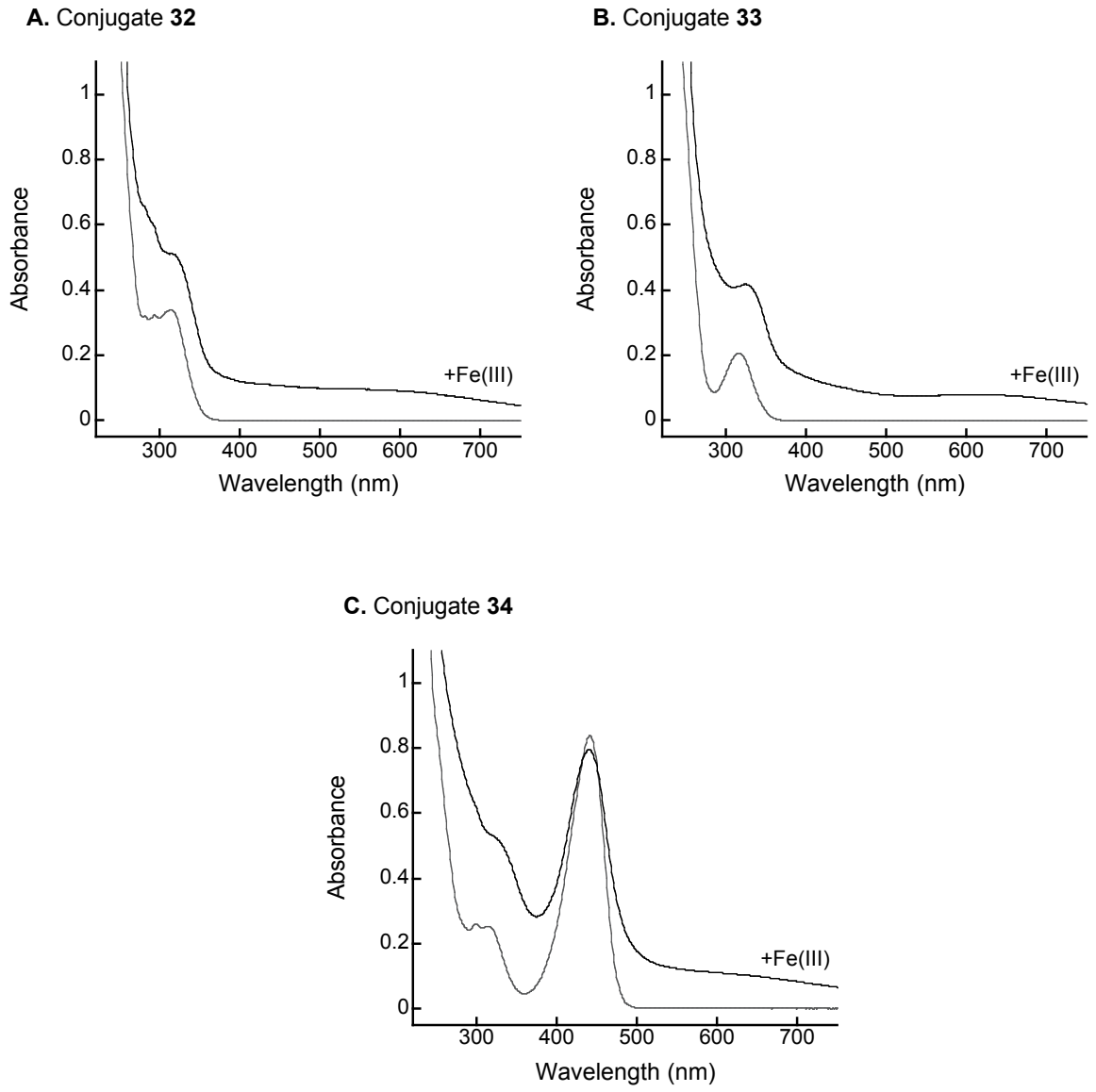


Figure S13. Optical absorption spectra of enterobactin-cargo conjugates **32-34** in the absence (grey) and presence (black) of Fe(III). (MeOH, rt). The concentration of ligand was ca. 40 μM and ca. one equiv of Fe(III) was added. (A) Conjugate **32**, napthyl cargo; (B) conjugate **33**, phenylmethylbenzyl cargo; (C) conjugate **34**, coumarin 343 cargo. The absorption feature at ca. 440 nm is from the coumarin moiety.

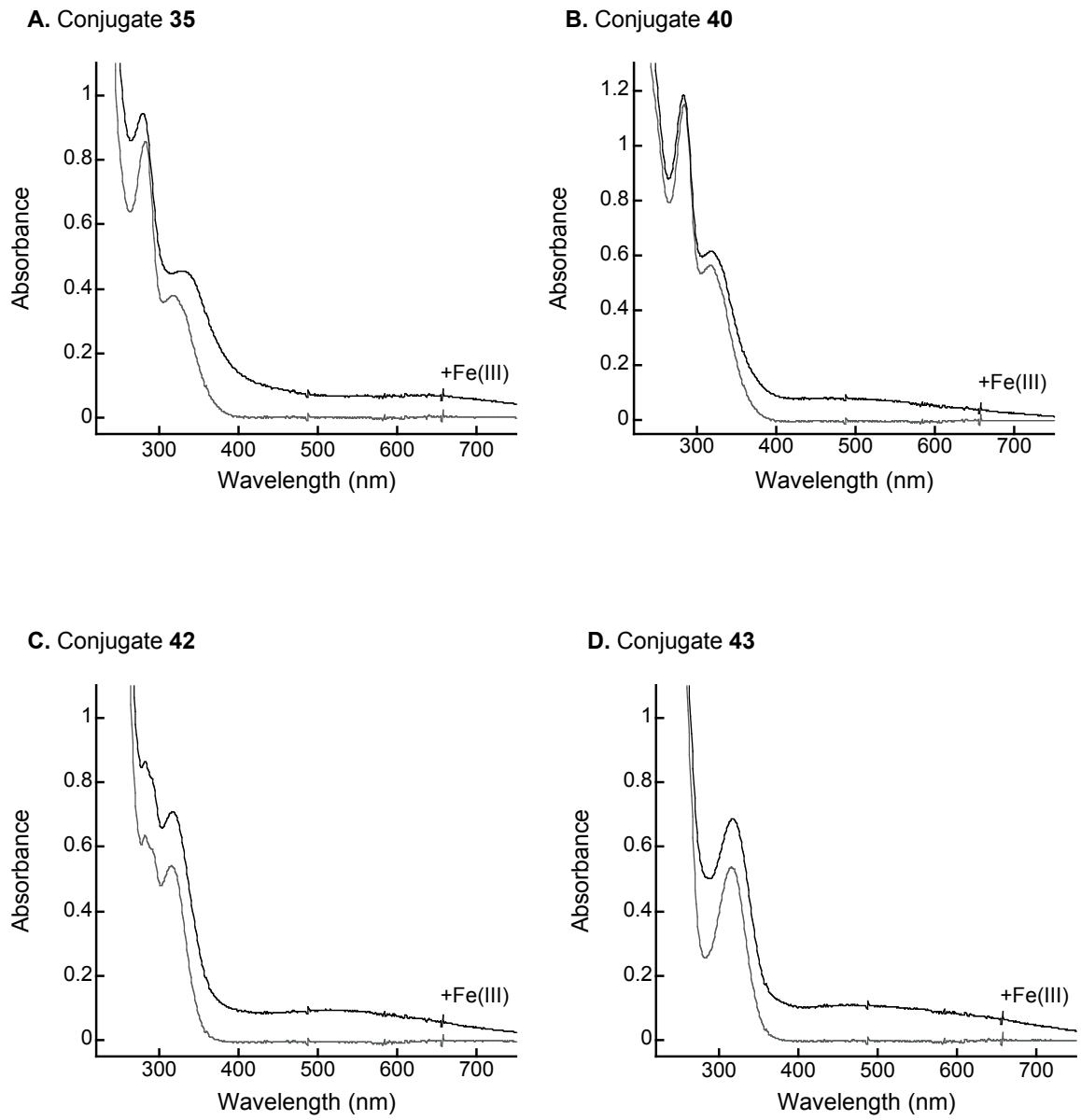


Figure S14. Optical absorption spectra of enterobactin-cargo conjugates **35, 40, 42** and **43** in the absence (grey) and presence (black) of Fe(III). (MeOH, rt). The concentration of ligand was ca. 40 μM and ca. one equiv of Fe(III) was added. (A) Conjugate **35**, ciprofloxacin cargo; (B) conjugate **40**, ciprofloxacin cargo; (C) conjugate **42**, vancomycin cargo. (D) conjugate **43**, Boc-glycine-triazole cargo.

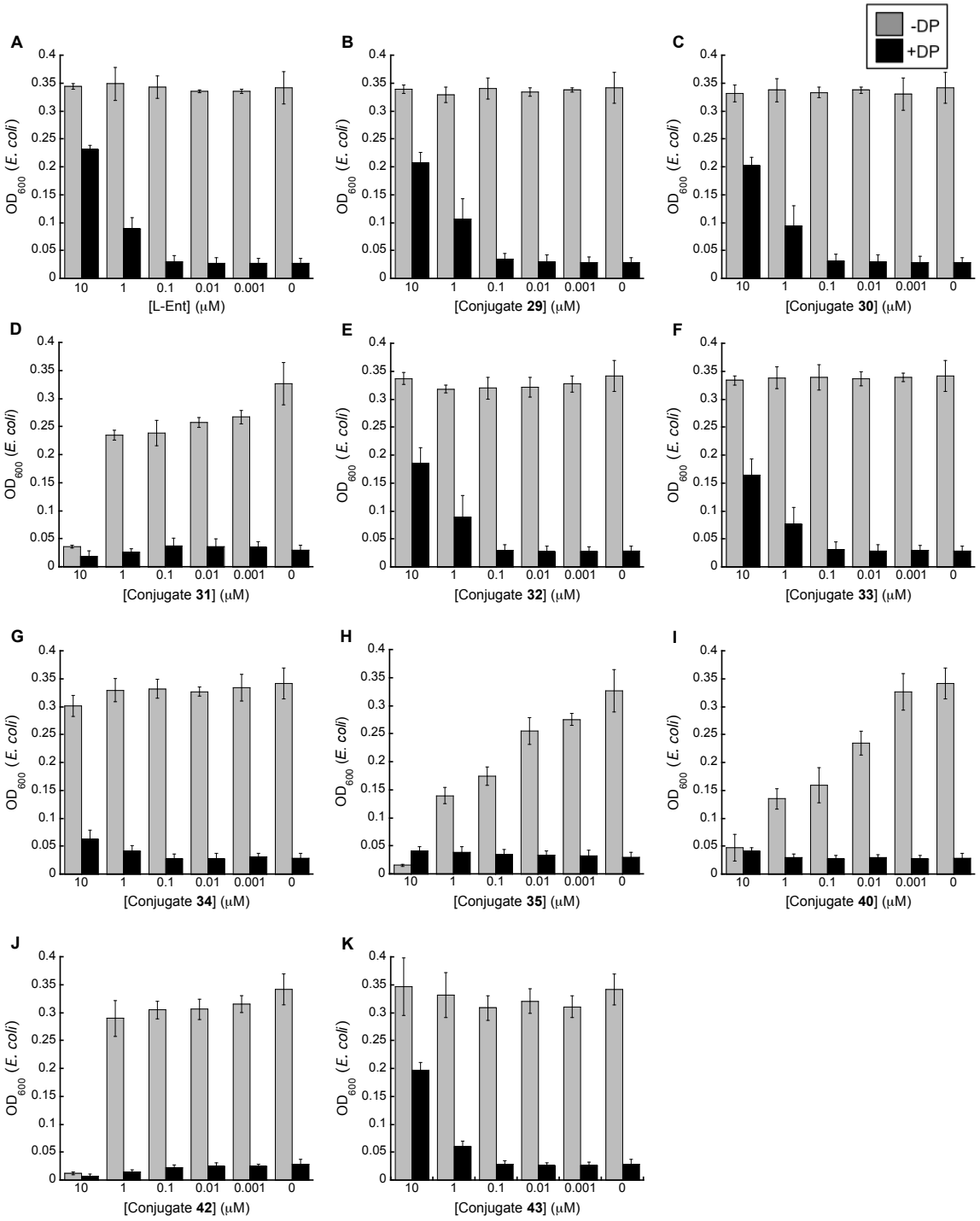


Figure S15. *E. coli* ATCC 33475 (*ent*-) growth recovery assays employing enterobactin-cargo conjugates (50% MHB, ± 200 μM DP, t = 19 h, T = 30 °C). Grey bars: *E. coli* cultured in the absence of DP. Black bars: *E. coli* cultured in the presence of 200 μM DP. (A) L-Ent; (B) conjugate **29**; (C) conjugate **30**; (D) conjugate **31**; (E) conjugate **32**; (F) conjugate **33**; (G) conjugate **34**; (H) conjugate **35**; (I) conjugate **40**; (J) conjugate **42**; (K) conjugate **43**.

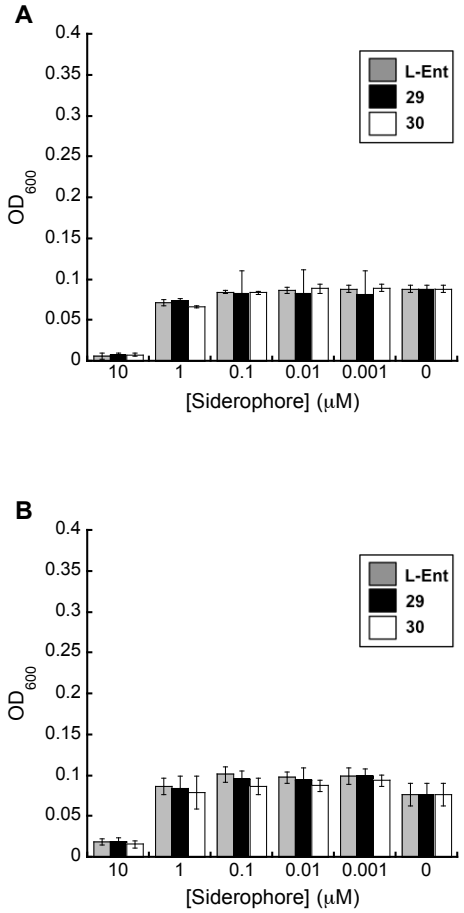


Figure S16. *E. coli* H1187 (*fepA*-) and *E. coli* K-12 JW0576 (*fes*-) growth recovery assays employing L-Ent and enterobactin-cargo conjugates **29** and **30** (50% MHB, 200 μM DP, t = 19 h, T = 30 ^{o}C). (A) *E. coli* H1187 (*fepA-)*. This strain grows to an OD_{600} of ~ 0.2 in the absence of DP (data not shown). (B) *E. coli* JW0576 (*fes*-). This strain grows to an OD_{600} of ~0.2 in the absence of DP (data not shown). Grey bars: L-Ent; black bars, conjugate **29**; white bars, conjugate **30**.

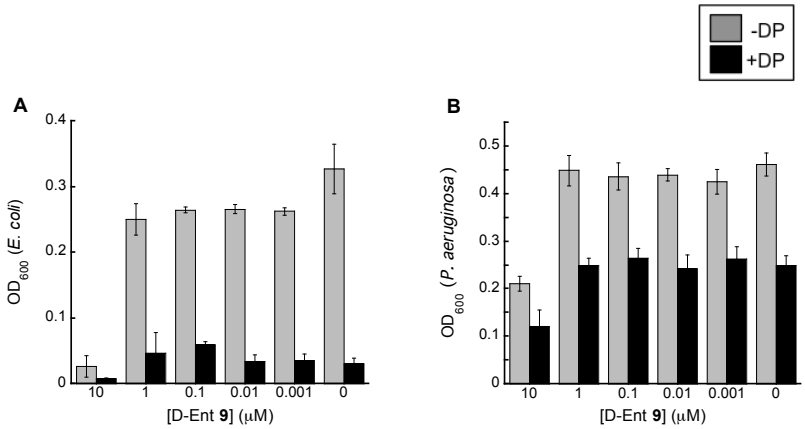


Figure S17. *E. coli* ATCC 33475 (ent-) and *P. aeruginosa* PAO1 K648 (*pvd*-, *pch*-) growth assays with the D-isomer of enterobactin (50% MHB, ± 200 or 600 μM DP, t = 19 h, T = 30 °C). Grey bars: bacteria cultured in the absence of DP. Black bars: bacteria cultured in the presence of 200 (*E. coli*) or 600 (*P. aeruginosa*) μM DP. (A) *E. coli*. (B) *P. aeruginosa.*

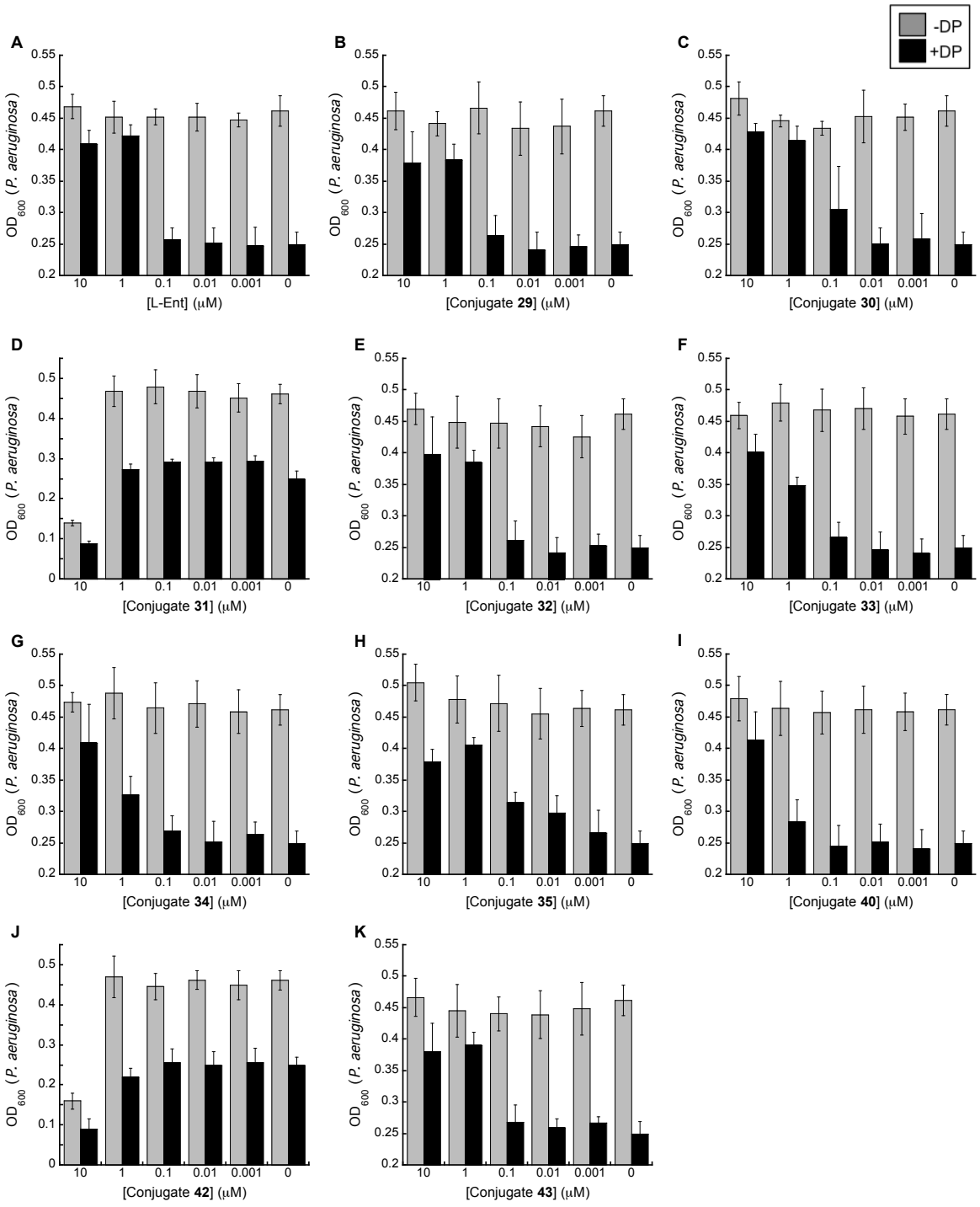


Figure S18. *P. aeruginosa* PAO1 K648 (*pvd*-, *pch*-) growth recovery assays employing enterobactin-cargo conjugates (50% MHB, ± 600 μM DP, t = 19 h, T = 30 °C). Grey bars: In the absence of DP. Black bars: In the presence of 600 μM DP. (A) L-Ent; (B) conjugate **29**; (C) conjugate **30**; (D) conjugate **31**; (E) conjugate **32**; (F) conjugate **33**; (G) conjugate **34**; (H) conjugate **35**; (I) conjugate **40**; (J) conjugate **42**;(K) conjugate **43**.

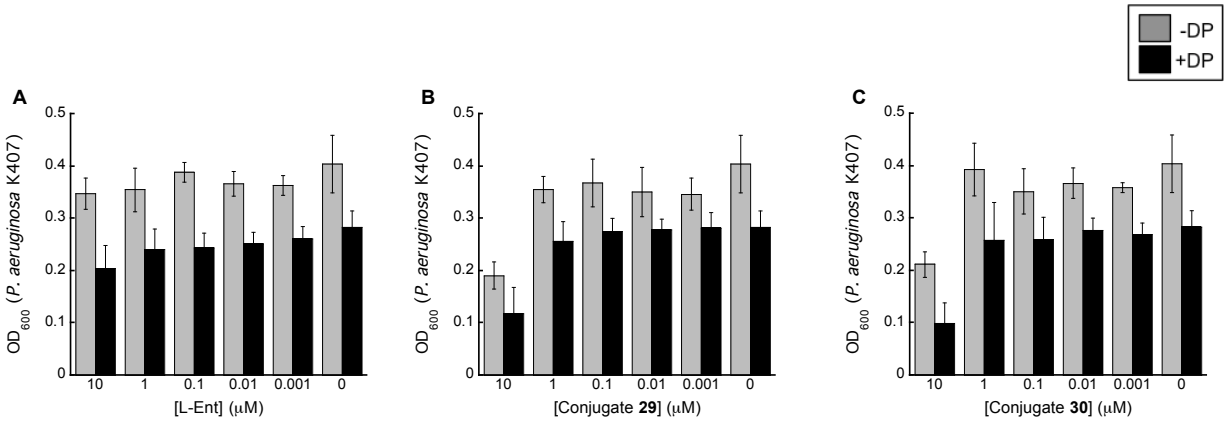
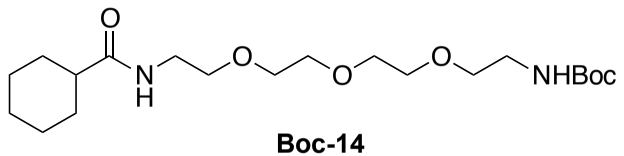


Figure S19. *P. aeruginosa* PAO1 K407 (*pvd*-, *pFr*-) growth recovery assays employing L-Ent and enterobactin-cargo conjugates **29** and **30** (50% MHB, ± 600 μM DP, t = 19 h, T = 30 ^{o}C). Grey bars: In the absence of DP. Black bars: In the presence of 600 μM DP. (A) L-Ent; (B) conjugate **29**; (C) conjugate **30**.



Boc-14

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    ex-PEG-Boc.fid   dmm              c
   ACQUISITION       dmf            200
sfrq        300.108       PROCESSING
tn               H1  wtfile
at            4.003  proc           ft
np            48052  fn         131072
sw           6002.4
fb        not used  werr
bs               4  wexp
tpwr            54  wbs
pw             8.0  wnt
d1           0.050
tof          867.7
nt               8
ct               8
alock            n
gain      not used
      FLAGS
il               n
in               n
dp               y
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sp          -294.9
wp          4014.6
vs             151
sc               0
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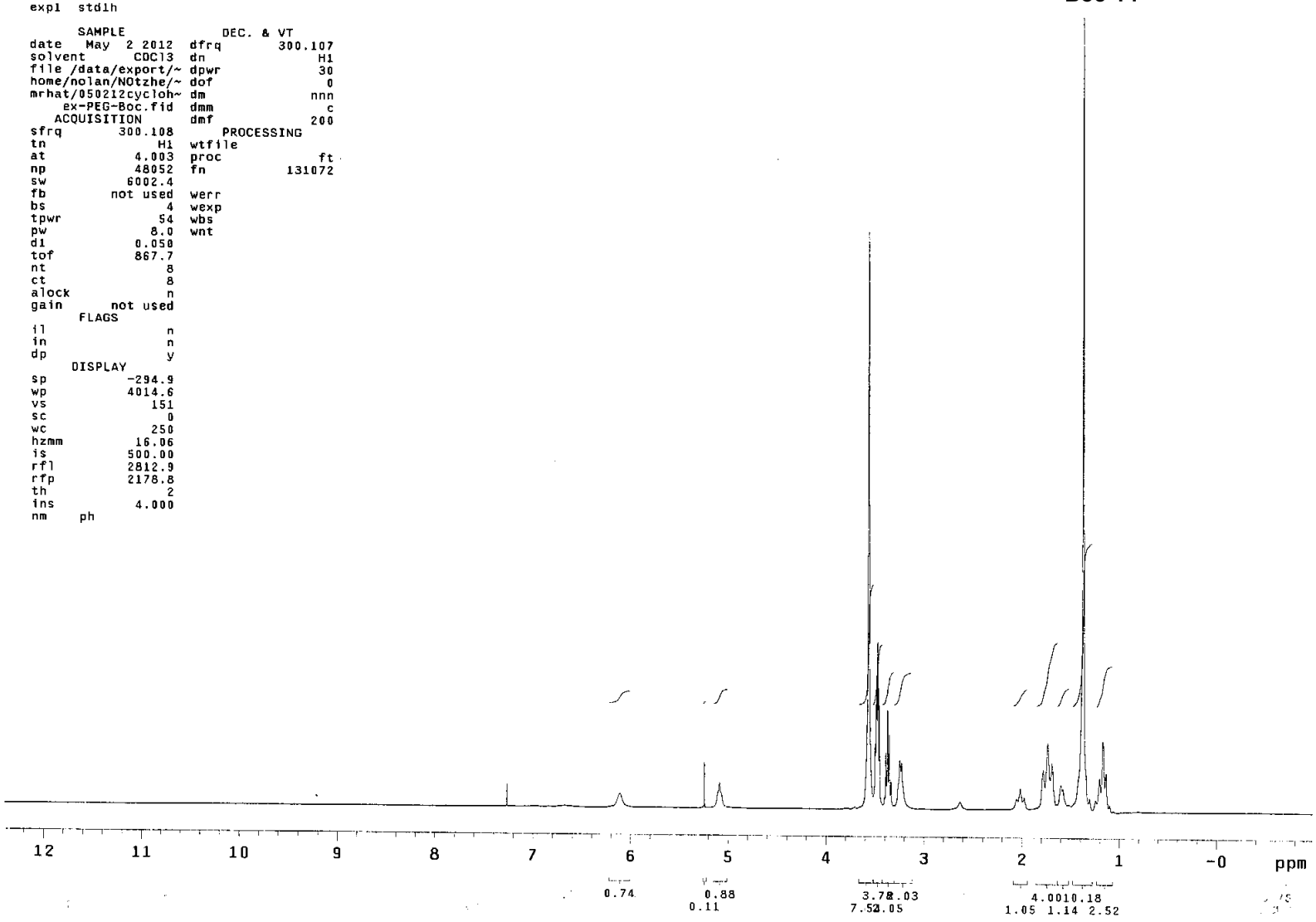

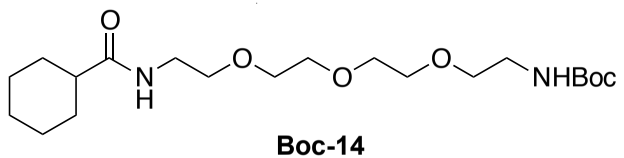

Boc-14

050512Cyclohex-PEG-Boc-C13

expl s2pul

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np	125588	dseq	
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fb	not used	homo	n
bs	16	DEC2	
tpwr	58	dfrq2	0
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tof	0	dof2	0
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ct	2416	dmm2	c
alock	n	dmf2	10000
gain	not used	dseq2	
FLAGS		dres2	1.0
il	n	homo2	n
in	n	DEC3	
dp	y	dfrq3	0
hs	nn	dn3	
DISPLAY		dpwr3	1
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wp	31396.7	dm3	n
vs	357	dmm3	c
sc	0	dmf3	10000
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is	500.00	homo3	n
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rfp	9675.8	lb	1.00
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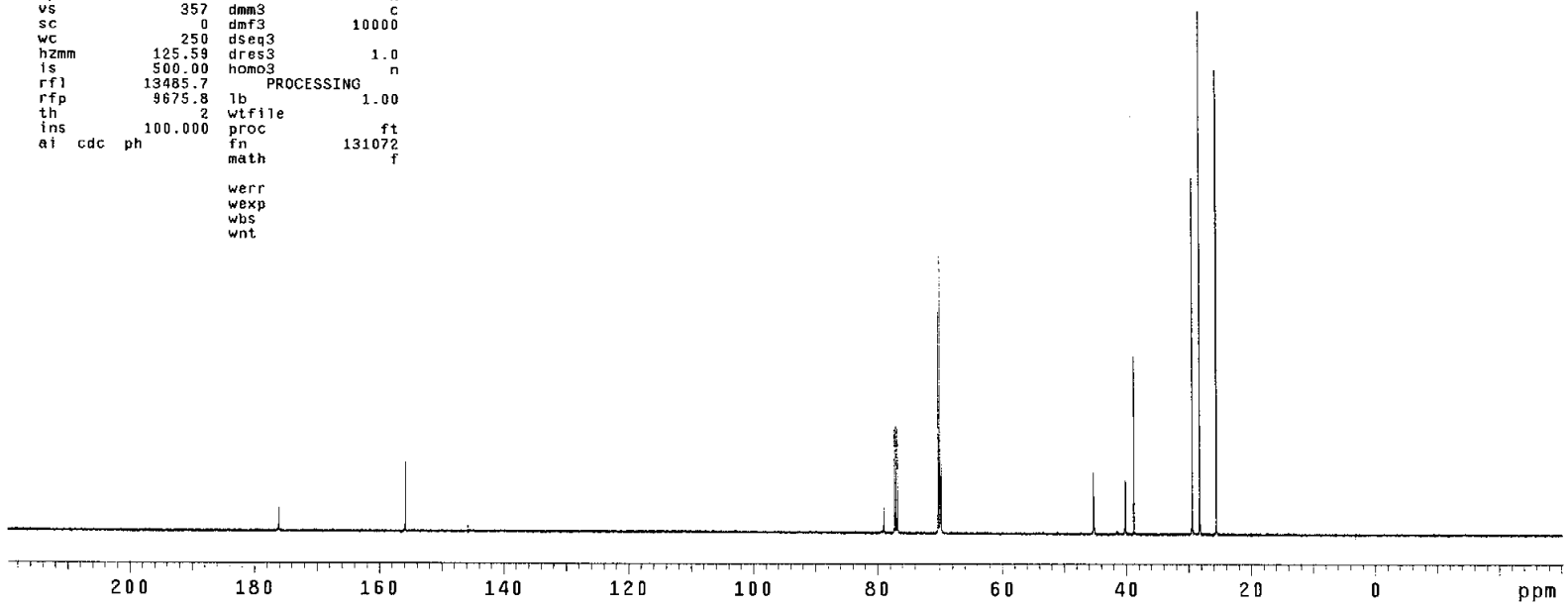

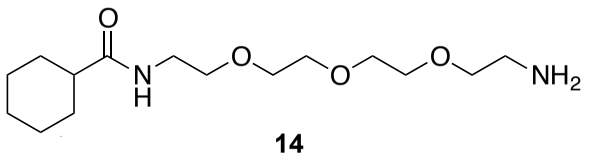

14

050512Cyclohex-PEG-NH2-H1

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FLAGS		dres2	1.0
il	n	homo2	n
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dp	y	dfrq3	0
hs	nn	dn3	
DISPLAY		dpwr3	1
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hzmm	19.99	dres3	1.0
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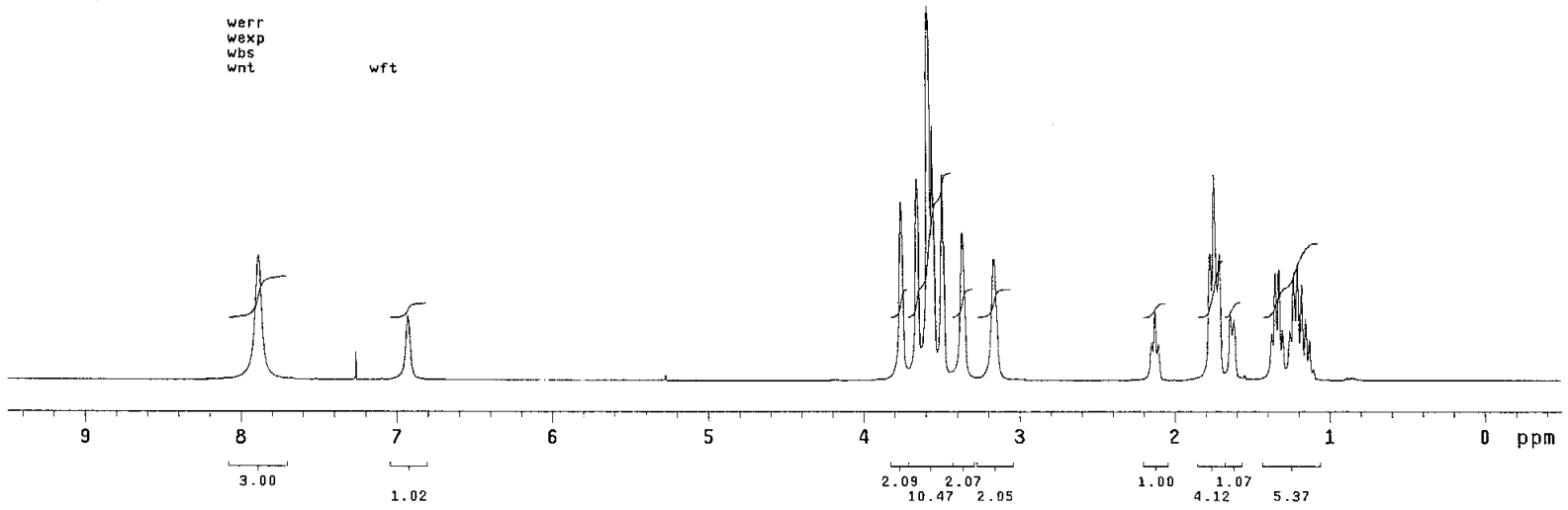

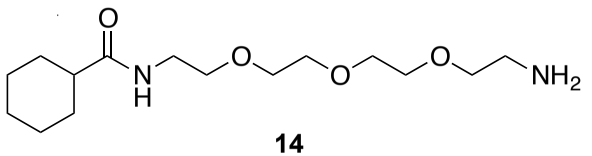

14

050512Cyclohex-PEG-NH2-C13

expl s2pul

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at	2.000	dmf	10400
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sw	31397.2	dres	1.0
fb	not used	homo	n
bs	16	DEC2	
tpwr	59	dfrq2	0
pw	6.7	dn2	
d1	1.000	dpwr2	1
tof	0	dof2	0
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ct	128	dmm2	c
alock	n	dmf2	10000
gain	not used	dseq2	
FLAGS		dres2	1.0
il	n	homo2	n
in	n	DEC3	
dp	y	dfrq3	0
hs	nn	dn3	
DISPLAY		dpwr3	1
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wp	31396.7	dm3	n
vs	413	dmm3	c
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th	4	wtfile	
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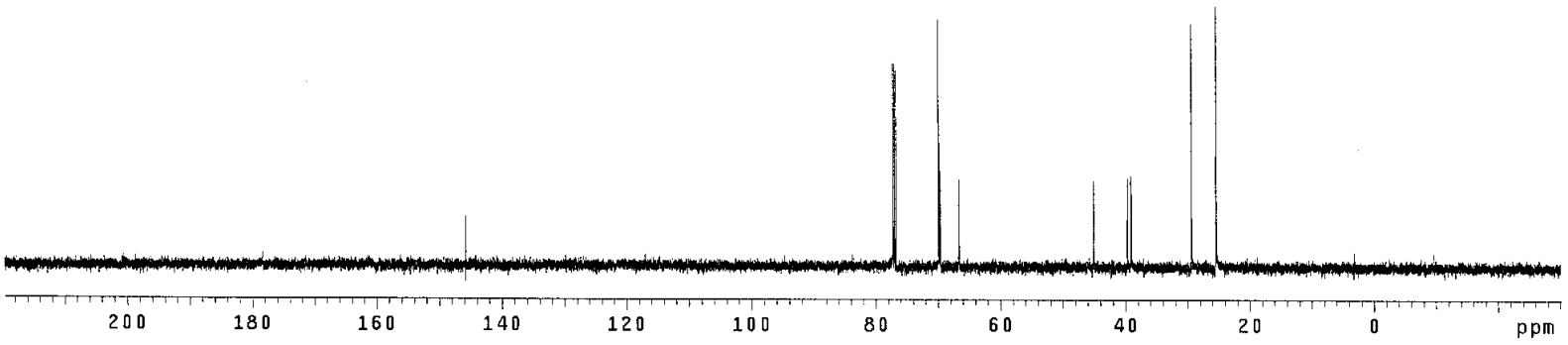

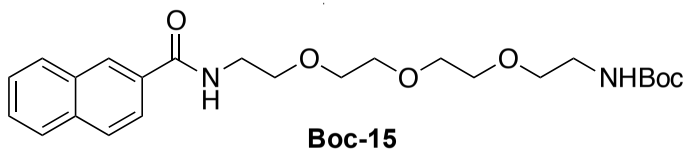

Boc-15

STANDARD PROTON PARAMETERS

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alock	n	dmf2	200
gain	not used	dseq2	
FLAGS		dres2	1.0
il	n	homo2	n
in	n	DEC3	
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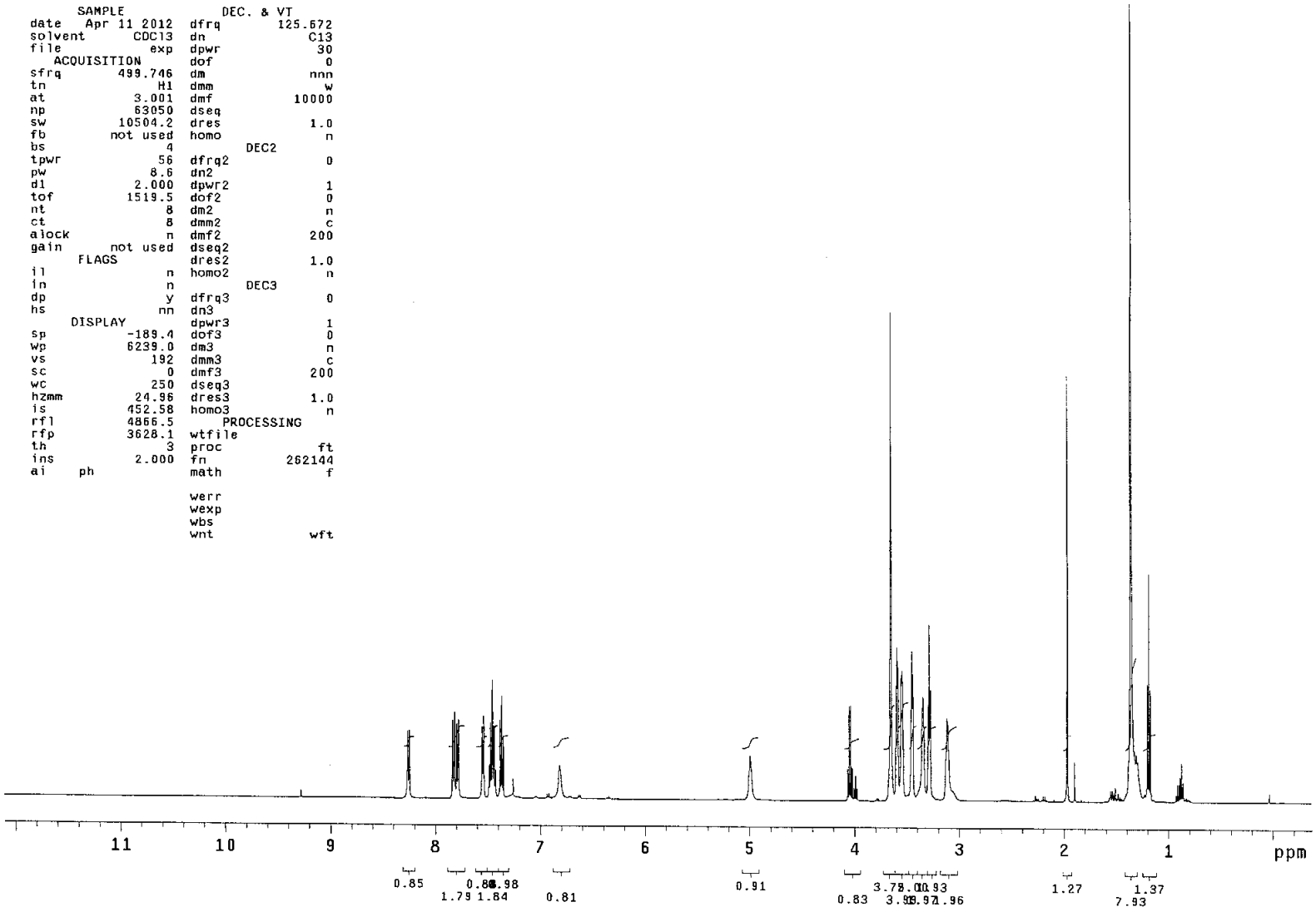

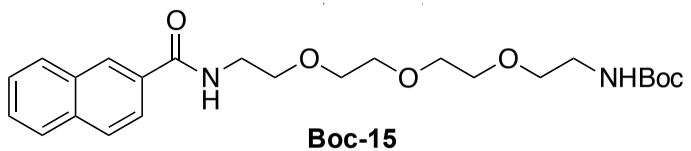

Boc-15

STANDARD CARBON PARAMETERS

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at	2.000	dres	1.0
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sw	31397.2	DEC2	
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bs	16	dn2	
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nt	99999	dmf2	10000
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il	n	dfrq3	0
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dp	y	dpwr3	1
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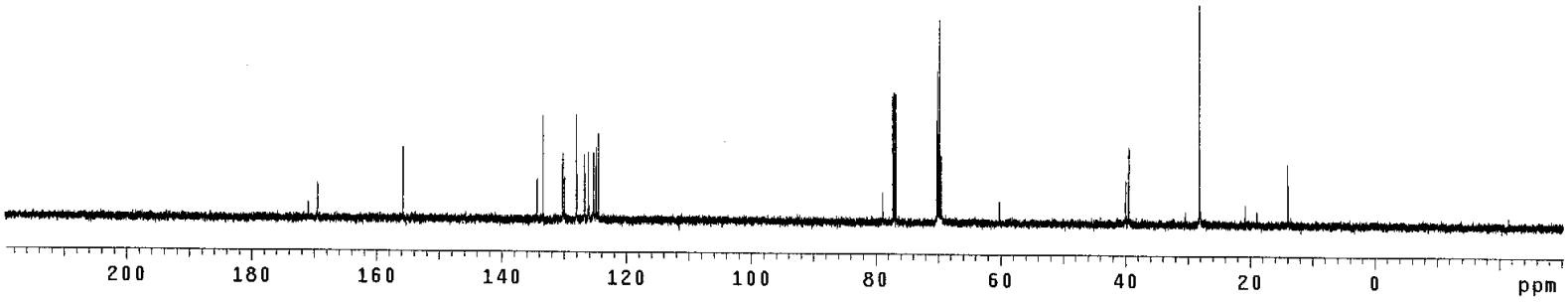

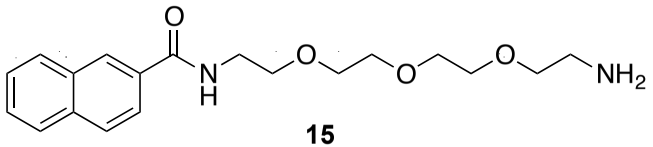

15

STANDARD 1H OBSERVE

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sfrq	300.108	PROCESSING	
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at	4.003	proc	ft
np	48052	fn	131072
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fb	not used	werr	
bs	4	wexp	
tpwr	54	wbs	
pw	8.0	wnt	
d1	0.050		
tof	867.7		
nt	32		
ct	16		
alock	n		
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
DISPLAY			
sp	-319.0		
wp	4566.7		
vs	151		
sc	0		
wc	250		
hzmm	18.27		
is	154.25		
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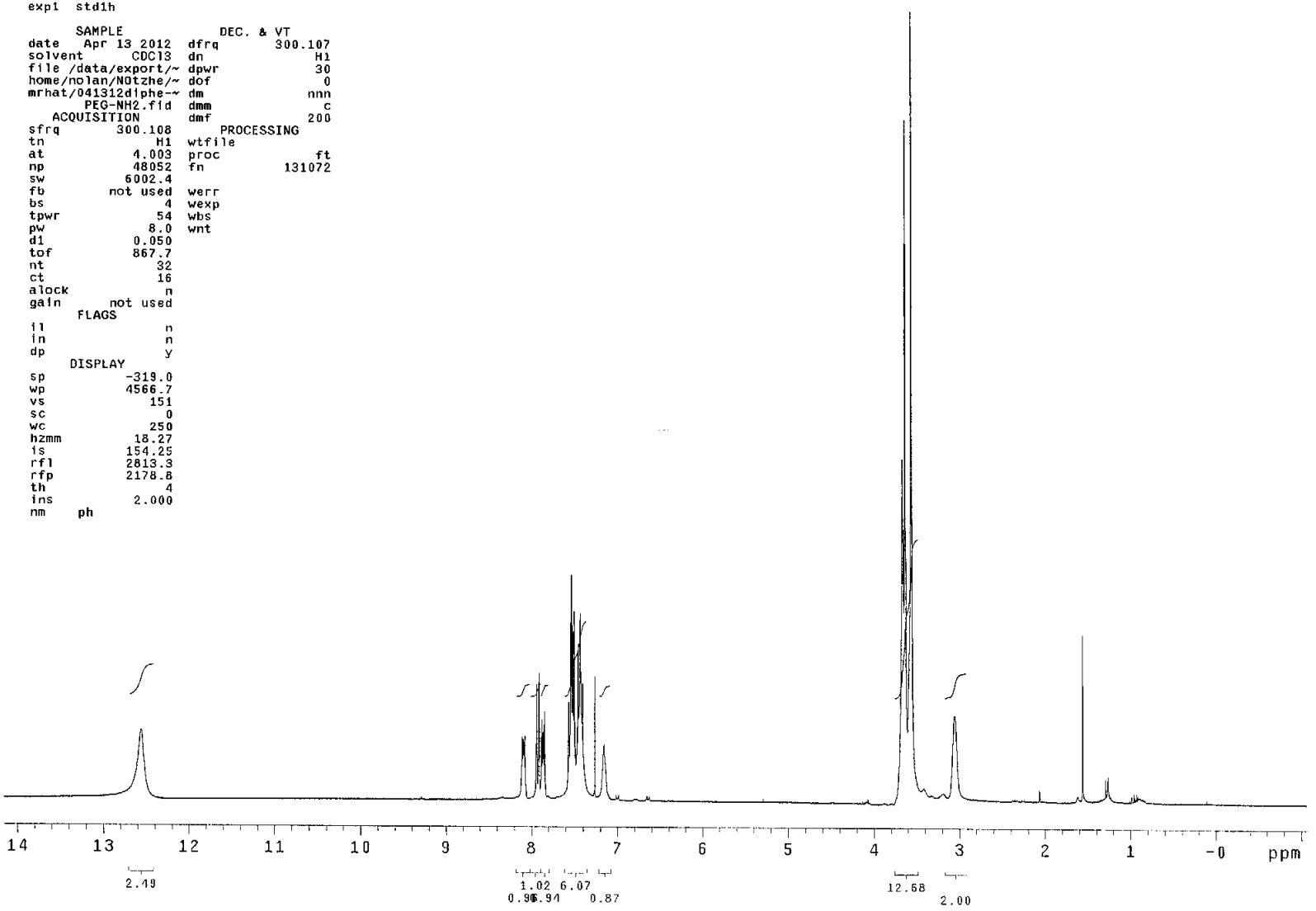
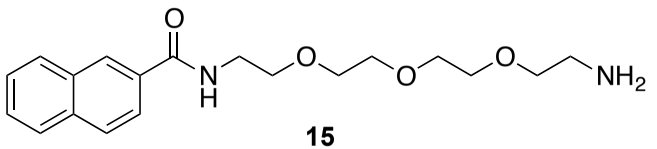
rfp	2178.8		
th	4		
ins	2.000		
nm	ph		



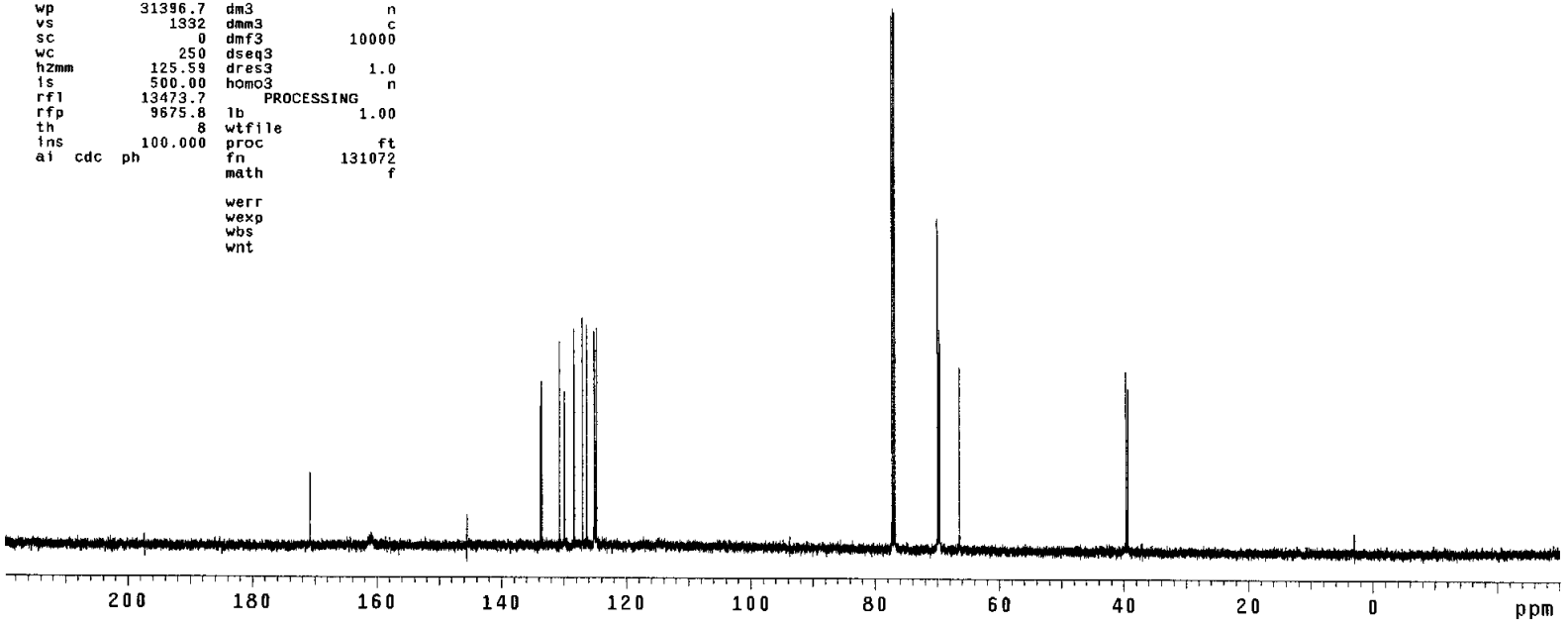


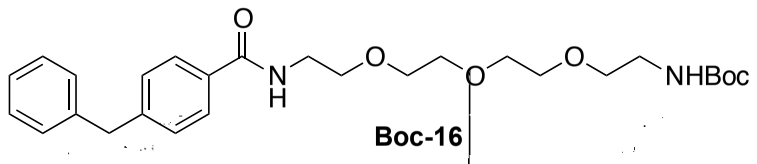
15

080112_Napha_PEG_amine_13C

exp3 s2pul

SAMPLE		DEC. & VT	
date	Aug 1 2012	dfrq	499.744
solvent	CDCl3	dn	H1
file	exp	dpwr	34
ACQUISITION		dof	0
sfrq	125.672	dm	yyy
tn	C13	dmm	w
at	2.000	dmf	10400
np	125588	dseq	
sw	31397.2	dres	1.0
fb	not used	homo	n
bs	16	DEC2	
tpwr	59	dfrq2	0
pw	6.7	dn2	
d1	0	dpwr2	1
tof	0	dof2	0
nt	99999	dm2	n
ct	2080	dmm2	c
alock	n	dmf2	10000
gain	not used	dseq2	
FLAGS		dres2	1.0
il	n	homo2	n
in	n	DEC3	
dp	y	dfrq3	0
hs	nn	dn3	
DISPLAY		dpwr3	1
sp	-3797.4	dof3	0
wp	31396.7	dm3	n
vs	1332	dmm3	c
sc	0	dmf3	10000
wc	250	dseq3	
hzmm	125.59	dres3	1.0
is	500.00	homo3	n
rfl	13473.7	PROCESSING	
rfp	9675.8	lb	1.00
th	8	wtfile	
ins	100.000	proc	ft
ai cdc ph		fn	131072
		math	f
		werr	
		wexp	
		wbs	
		wnt	



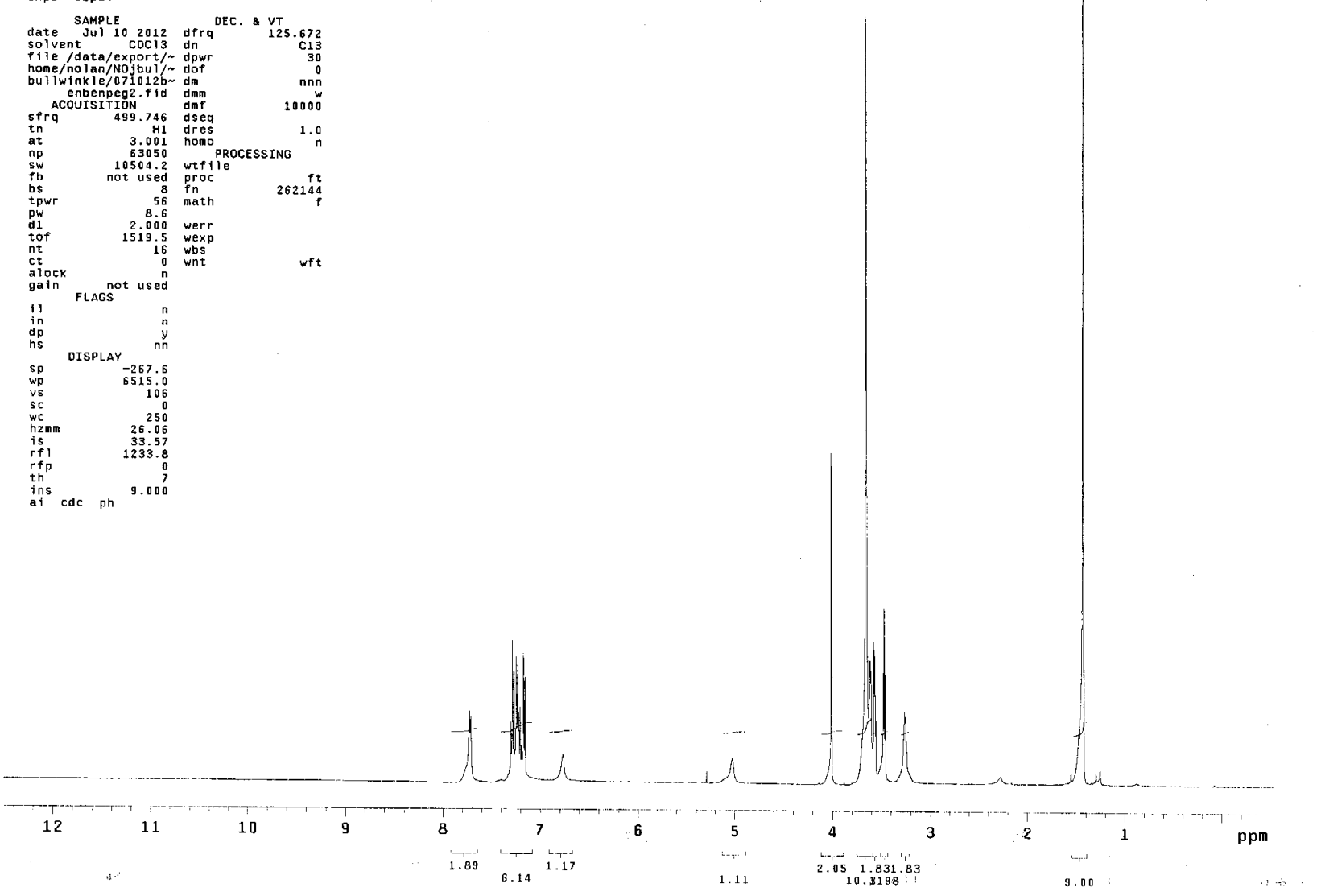


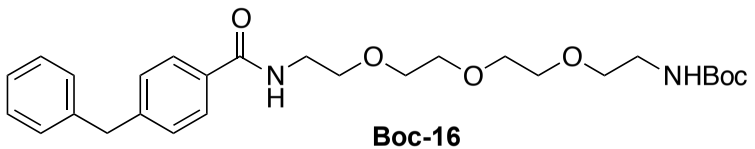
Boc-16

STANDARD PROTON PARAMETERS

exp1 s2pul

SAMPLE		DEC. & VT	
date	Jul 10 2012	dfrq	125.672
solvent	CDCl3	dn	C13
file	/data/export/~	dpwr	30
home/nolan/NOjbul/~		dof	0
bullwinkle/071012b~		dm	nnn
enbenpeg2.fid		dmm	w
ACQUISITION		dmf	10000
sfrq	499.746	dseq	
tn	H1	dres	1.0
at	3.001	homo	n
np	63050	PROCESSING	
sw	10504.2	wtfile	
fb	not used	proc	ft
bs	8	fn	262144
tpwr	56	math	f
pw	8.6		
d1	2.000	werr	
tof	1519.5	wexp	
nt	16	wbs	
ct	0	wnt	wft
alock	n		
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
hs	nn		
DISPLAY			
sp	-267.6		
wp	6515.0		
vs	106		
sc	0		
wc	250		
hzmm	26.06		
is	33.57		
rfl	1233.8		
rfp	0		
th	7		
ins	9.000		
ai cdc ph			





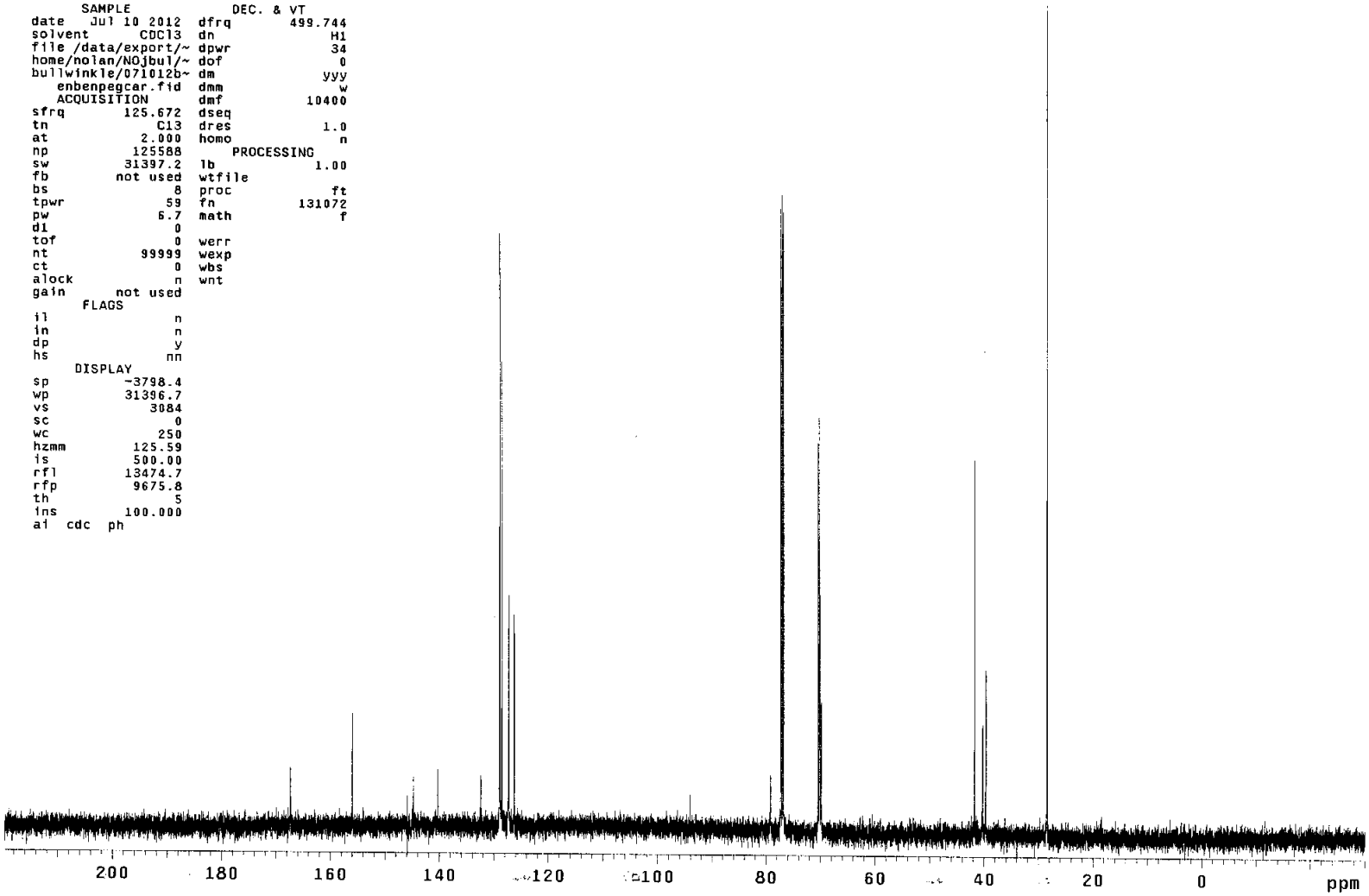
Boc-16

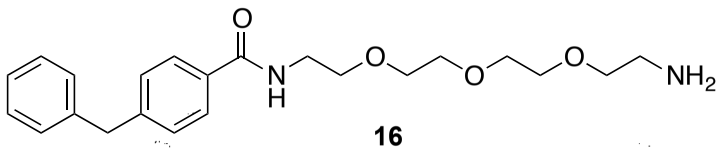
STANDARD CARBON PARAMETERS

expl s2pul

SAMPLE		DEC. & VT	
date	Jul 10 2012	dfrq	499.744
solvent	CDCl3	dn	H1
file	/data/export/~	dpwr	34
home/nolan/NOjbul/~		dof	0
bullwinkle/071012b~		dm	yyy
enbenpegcar.fid		dmm	w
ACQUISITION		dmf	10400
sfrq	125.672	dseq	
tn	C13	dres	1.0
at	2.000	homo	n
np	125588	PROCESSING	
sw	31397.2	lb	1.00
fb	not used	wtfile	
bs	8	proc	ft
tpwr	59	fn	131072
pw	6.7	math	f
dl	0		
tof	0	werr	
nt	99999	wexp	
ct	0	wbs	
alock	n	wnt	
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
hs	nn		
DISPLAY			
sp	-3798.4		
wp	31396.7		
vs	3084		
sc	0		
wc	250		
hzmm	125.59		
is	500.00		
rfl	13474.7		
rfp	9675.8		
th	5		
ins	100.000		

ai cdc ph





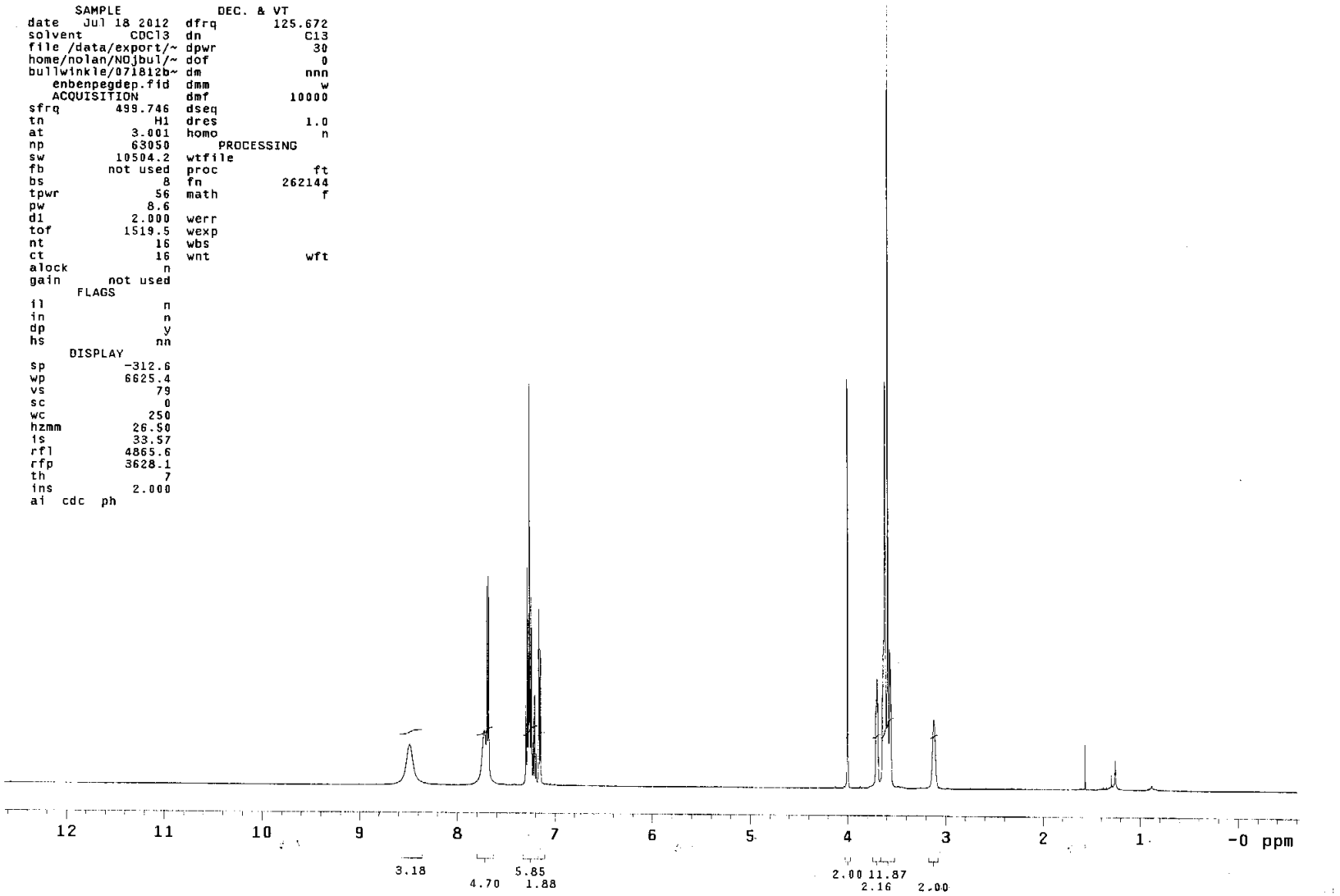
16

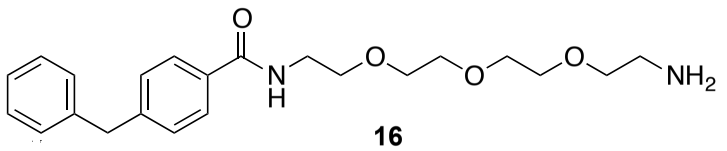
STANDARD PROTON PARAMETERS

expl s2pul

SAMPLE		DEC. & VT	
date	Jul 18 2012	dfrq	125.672
solvent	CDCl3	dn	C13
file	/data/export/~	dpwr	30
home/nolan/NOjbul/~		dof	0
bullwinkle/071812b~		dm	nnn
enbenpegdep.fid		dmm	w
ACQUISITION		dmf	10000
sfrq	499.746	dseq	
tn	H1	dres	1.0
at	3.001	homo	n
np	63050	PROCESSING	
sw	10504.2	wtfile	
fb	not used	proc	ft
bs	8	fn	262144
tpwr	56	math	f
pw	8.6		
d1	2.000	werr	
tof	1519.5	wexp	
nt	16	wbs	
ct	16	wnt	wft
alock	n		
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
hs	nn		
DISPLAY			
sp	-312.6		
wp	6625.4		
vs	79		
sc	0		
wc	250		
hzmm	26.50		
is	33.57		
rfl	4865.6		
rfp	3628.1		
th	7		
ins	2.000		

ai cdc ph



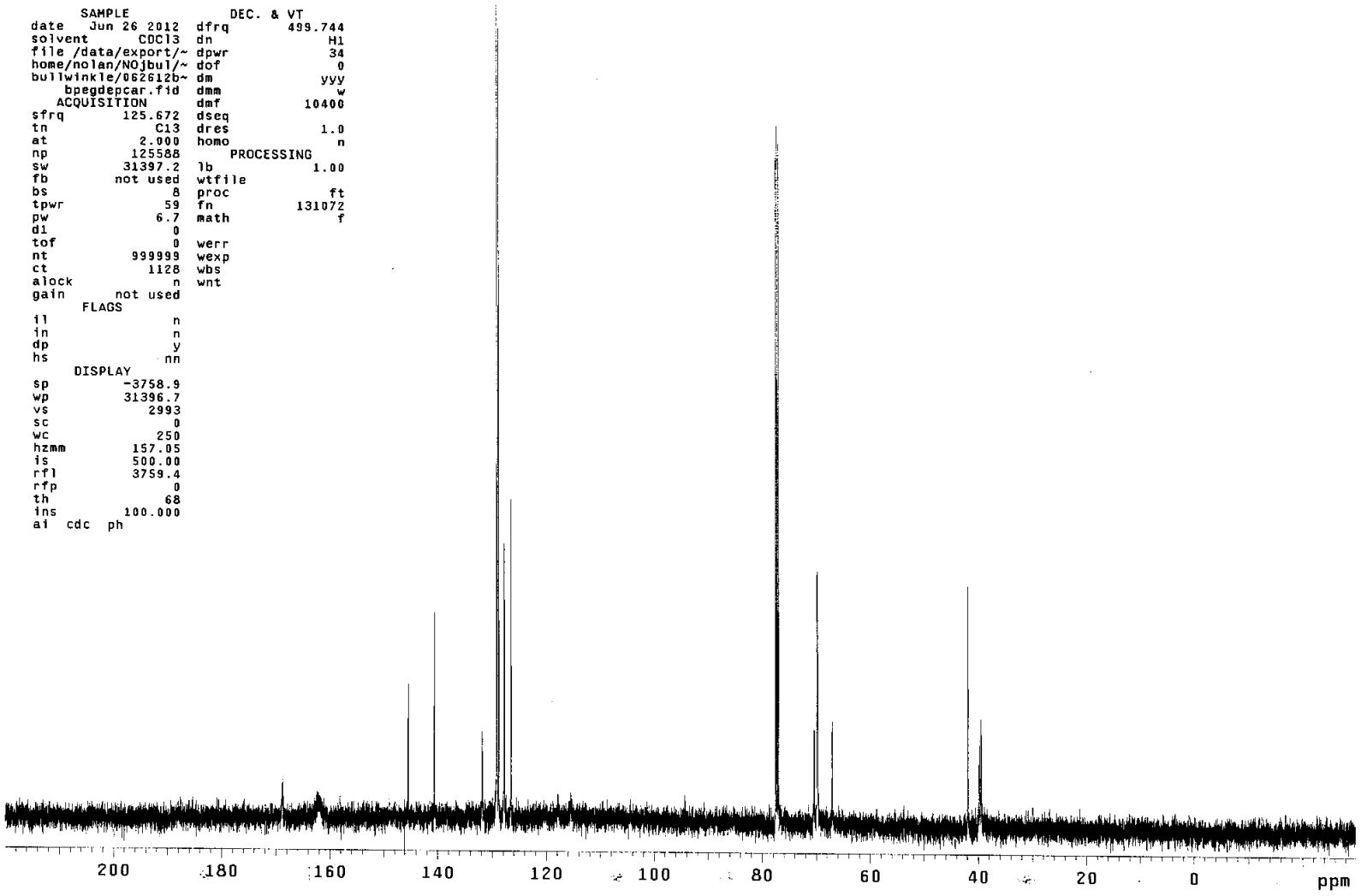


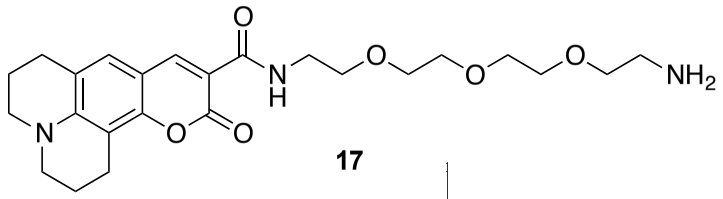
16

STANDARD CARBON PARAMETERS

exp1 s2pul

SAMPLE		DEC. & VT	
date	Jun 26 2012	dfrq	499.744
solvent	CDCl3	dn	H1
file	/data/export/~	dpwr	34
	home/nolan/NOjbul/~	dof	0
	bullwinkle/062612b~	dm	yyy
	bpegdepcar.fid	dmm	w
ACQUISITION		dmf	10400
sfrq	125.672	dseq	
tn	C13	dres	1.0
at	2.000	homo	n
np	125588	PROCESSING	
sw	31397.2	lb	1.00
fb	not used	wtfile	
bs	8	proc	ft
tpwr	59	fn	131072
pw	6.7	math	f
d1	0		
tof	0	werr	
nt	999999	wexp	
ct	1128	wbs	
alock	n	wnt	
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
hs	nn		
DISPLAY			
sp	-3758.9		
wp	31396.7		
vs	2993		
sc	0		
wc	250		
hzmm	157.05		
is	500.00		
rfl	3759.4		
rfp	0		
th	68		
ins	100.000		
ai cdc ph			





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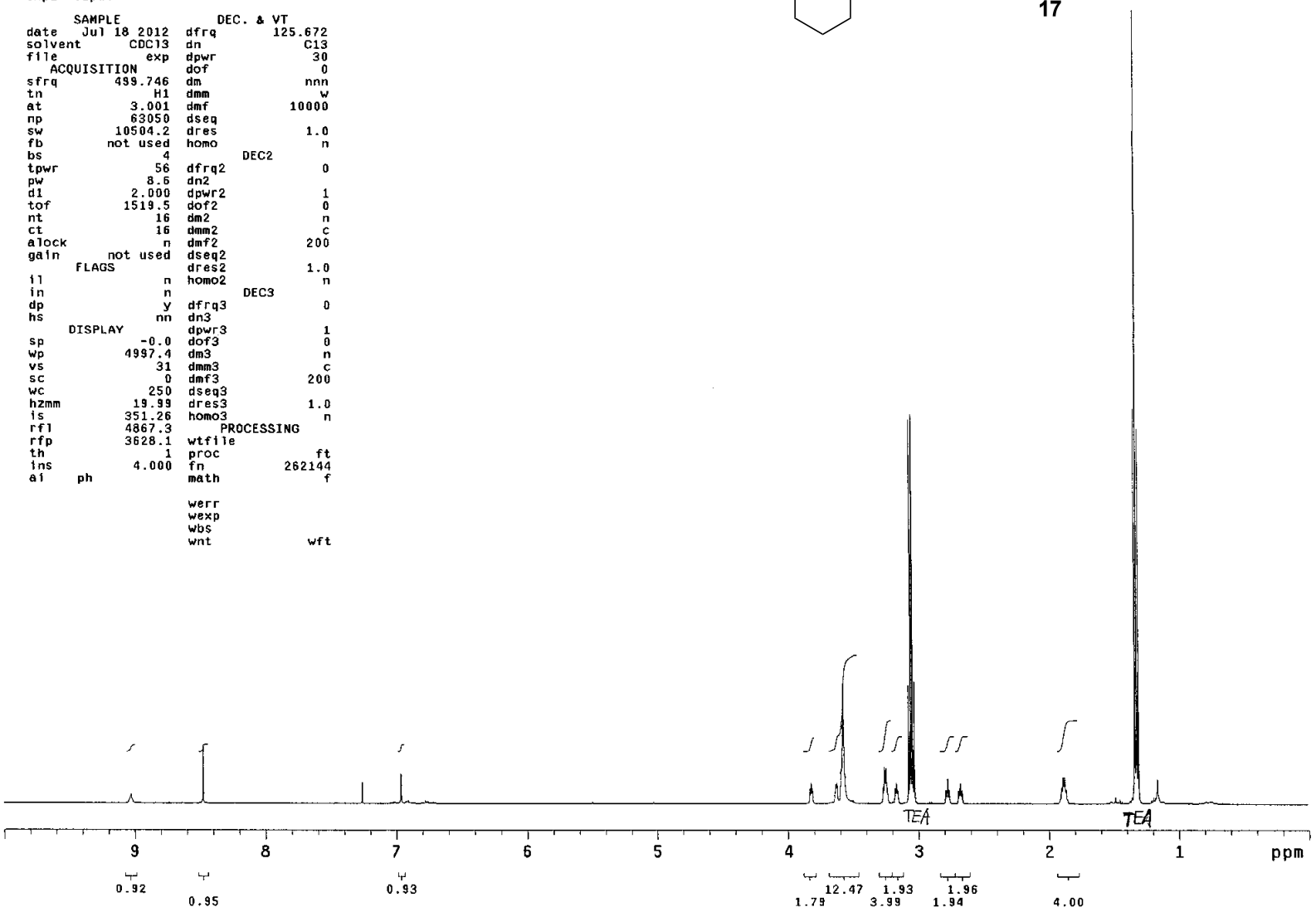
07182012_coumarin_PEG-NH2_with_TEA

exp1 s2pul

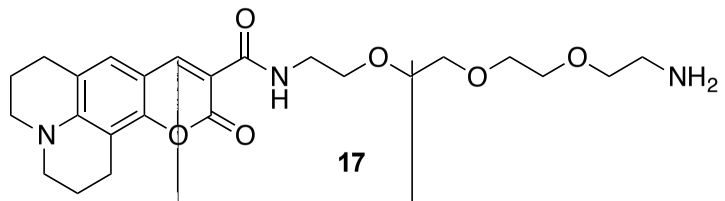
SAMPLE		DEC. & VT	
date	Jul 18 2012	dfrq	125.672
solvent	CDCl3	dn	C13
file	exp	dpwr	30
ACQUISITION		dof	0
sfrq	499.746	dm	nnn
tn	H1	dmm	w
at	3.001	dmf	10000
np	63050	dseq	
sw	10504.2	dres	1.0
fb	not used	homo	n
bs	4	DEC2	
tpwr	56	dfrq2	0
pw	8.6	dn2	
d1	2.000	dpwr2	1
tof	1519.5	dof2	0
nt	16	dm2	n
ct	16	dmm2	c
alock	n	dmf2	200
gain	not used	dseq2	
FLAGS		dres2	1.0
il	n	homo2	n
in	n	DEC3	
dp	y	dfrq3	0
hs	nn	dn3	
DISPLAY		dpwr3	1
sp	-0.0	dof3	0
wp	4997.4	dm3	n
vs	31	dmm3	c
sc	0	dmf3	200
wc	250	dseq3	
hzmm	19.98	dres3	1.0
is	351.26	homo3	n
rfl	4867.3	PROCESSING	
rfp	3628.1	wtfile	
th	1	proc	ft
ins	4.000	fn	262144
ai ph		math	f
		werr	
		wexp	
		wbs	
		wnt	wft



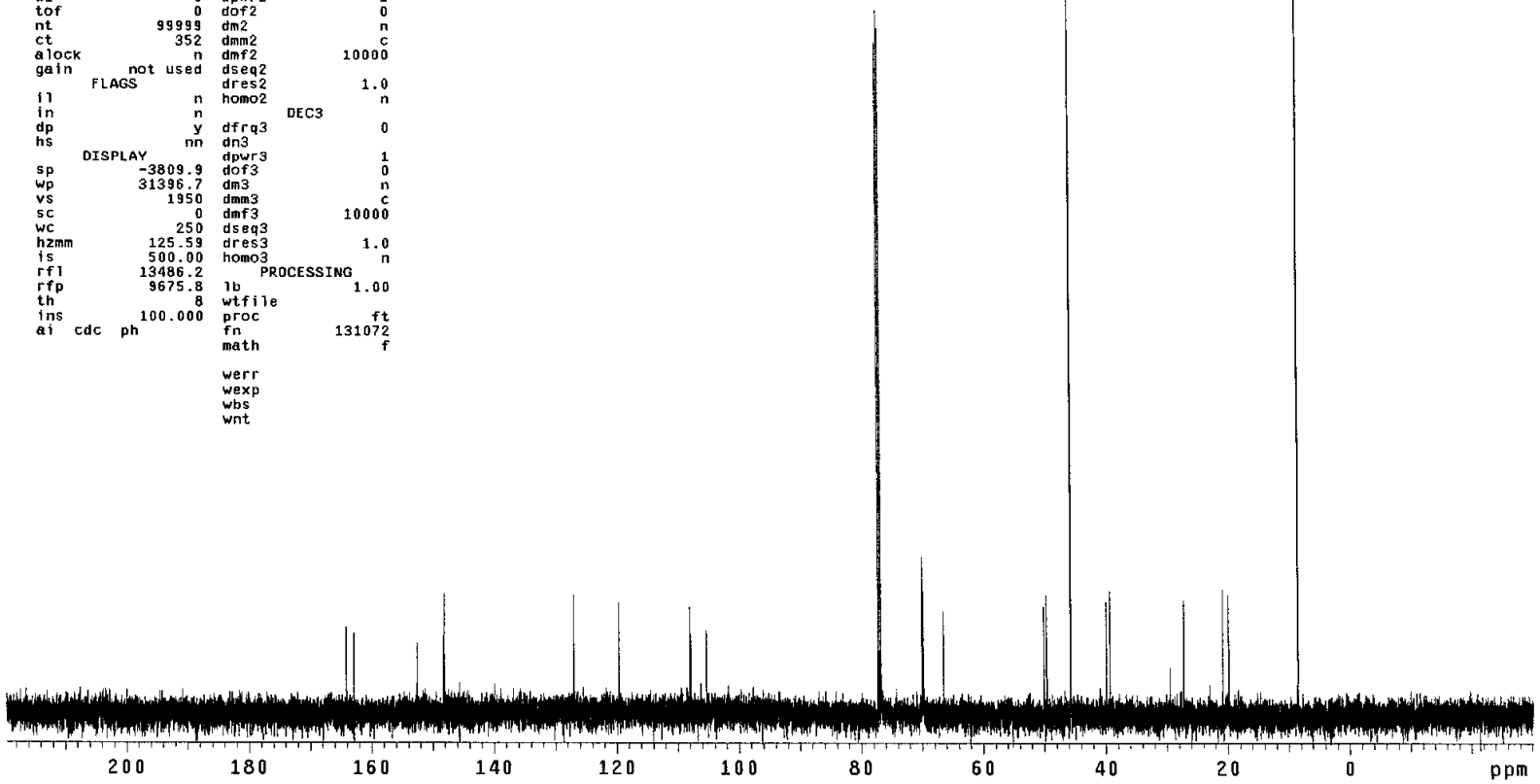
07182012_coumarin-PEG-NH2_13C_with_TEA

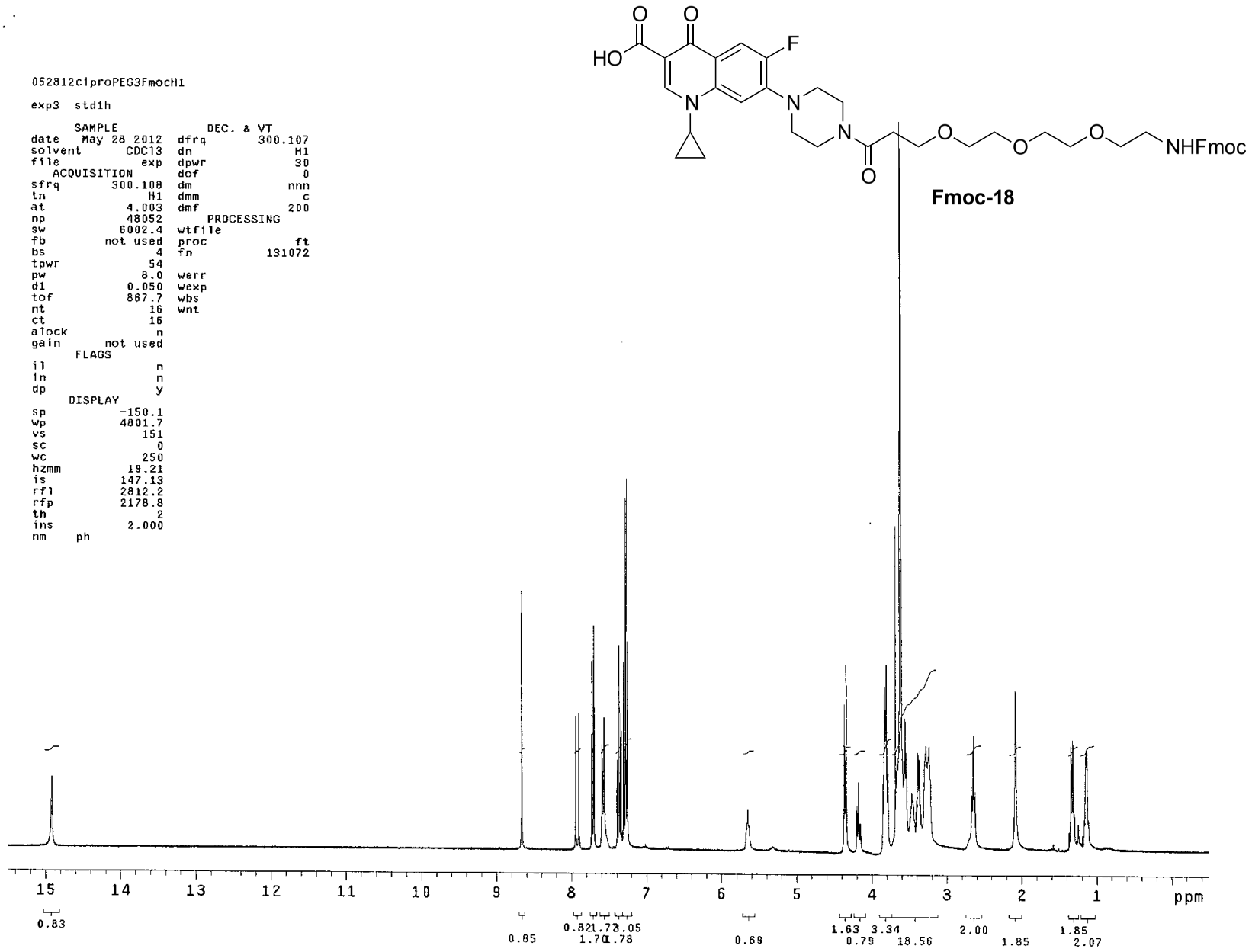
exp3 s2pul

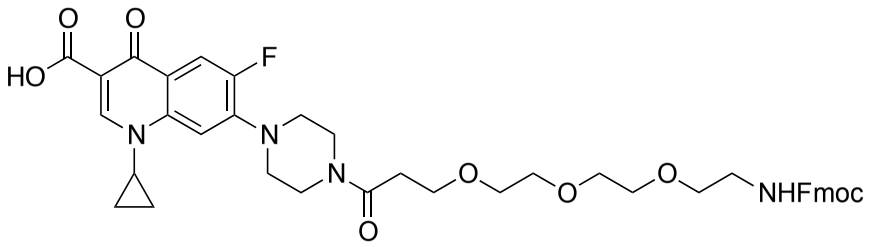
SAMPLE		DEC. & VT	
date	Jul 18 2012	dfrq	499.744
solvent	CDCl3	dn	H1
file	exp	dpwr	34
ACQUISITION		dof	0
sfrq	125.672	dm	yyy
tn	C13	dmm	w
at	2.000	dmf	10400
np	125588	dseq	
sw	31397.2	dres	1.0
fb	not used	homo	n
bs	16	DEC2	
tpwr	59	dfrq2	0
pw	6.7	dn2	
d1	0	dpwr2	1
tof	0	dof2	0
nt	99999	dm2	n
ct	352	dmm2	c
alock	n	dmf2	10000
gain	not used	dseq2	
FLAGS		dres2	1.0
il	n	homo2	n
in	n	DEC3	
dp	y	dfrq3	0
hs	nn	dn3	
DISPLAY		dpwr3	1
sp	-3809.9	dof3	0
wp	31396.7	dm3	n
vs	1950	dmm3	c
sc	0	dmf3	10000
wc	250	dseq3	
hzmm	125.59	dres3	1.0
is	500.00	homo3	n
rfl	13486.2	PROCESSING	
rfp	9675.8	lb	1.00
th	8	wtfile	
ins	100.000	proc	ft
ai cdc ph		fn	131072
		math	f
		werr	
		wexp	
		wbs	
		wnt	



17

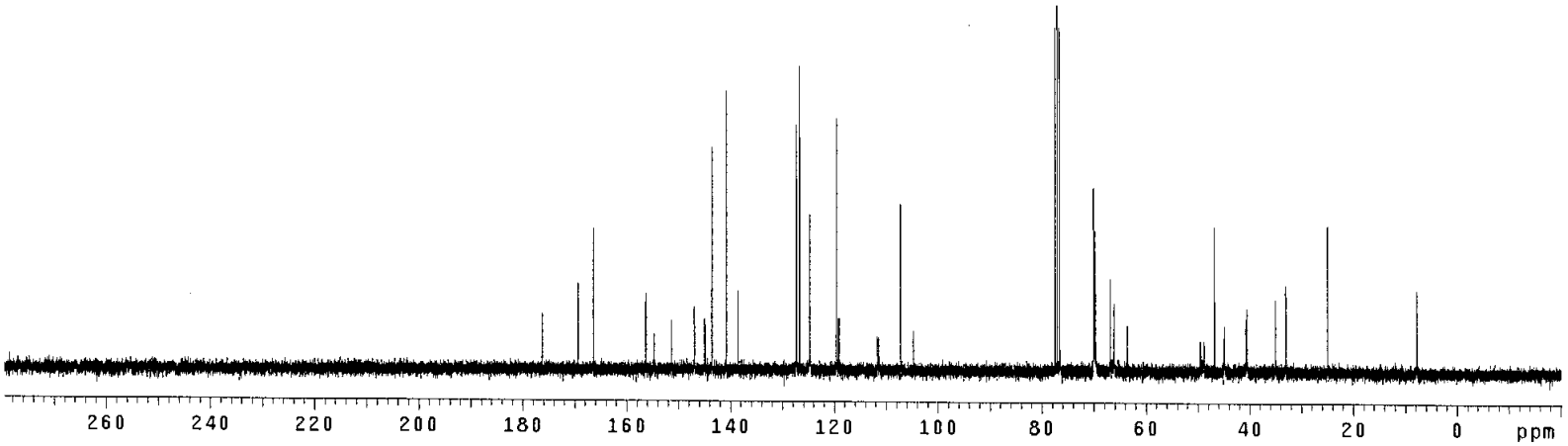



052812ciproPEG3FmocH1
exp3 std1h
SAMPLE
date May 28 2012
solvent CDCl3
file exp
ACQUISITION
sfrq 300.108
tn H1
at 4.003
np 48052
sw 6002.4
fb not used
bs 4
tpwr 54
pw 8.0
d1 0.050
tof 867.7
nt 16
ct 16
alock n
gain not used
FLAGS
il n
in n
dp y
DISPLAY
sp -150.1
wp 4801.7
vs 151
sc 0
wc 250
hzmm 19.21
is 147.13
rfl 2812.2
rfp 2178.8
th 2
ins 2.000
nm ph
DEC. & VT
dfrq 300.107
dn H1
dpwr 30
dof 0
dm nnn
dmm c
dmf 200
PROCESSING
wtfile
proc ft
fn 131072
werr
wexp
wbs
wnt
HO
O
O
F
N
N
N
O
O
O
O
O
NHFmoc
Fmoc-18
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm
0.83
0.85
0.82
1.70
1.73
1.78
3.05
0.69
1.63
0.79
3.34
18.56
2.00
1.85
1.85
2.07



Fmoc-18

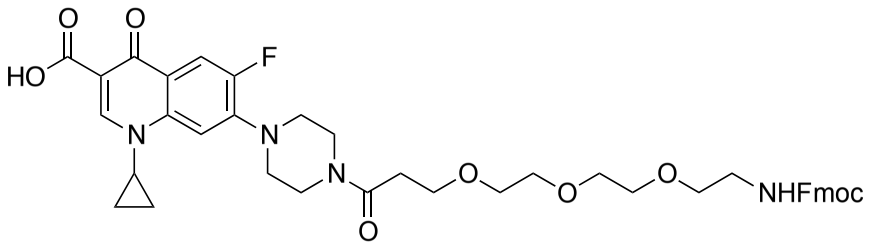
```
05222012ciproPEG3FmocC13

exp3  std13c

      SAMPLE                DEC. & VT
date    May 22 2012   dfrq        300.107
solvent       CDCl3   dn              H1
file            exp   dpwr            40
   ACQUISITION        dof              0
sfrq         75.471   dm             yyy
tn              C13   dmm              w
at            1.500   dmf           9800
np            67874       PROCESSING
sw          22624.4   sb          -1.500
fb            12400   sbs         -1.500
bs               16   wtfile
tpwr             55   proc            ft
pw             10.0   fn          262144
d1                0
tof           2264.0   werr
nt             99999   wexp
ct               480   wbs
alock              n   wnt
gain       not used
      FLAGS
il                 n
in                 n
dp                 y
     DISPLAY
sp          -1527.7
wp          22624.3
vs               55
sc                0
wc              250
hzmm          90.50
is           500.00
rfl          7338.4
rfp          5810.5
th                5
ins          100.000
ai  no  ph
```



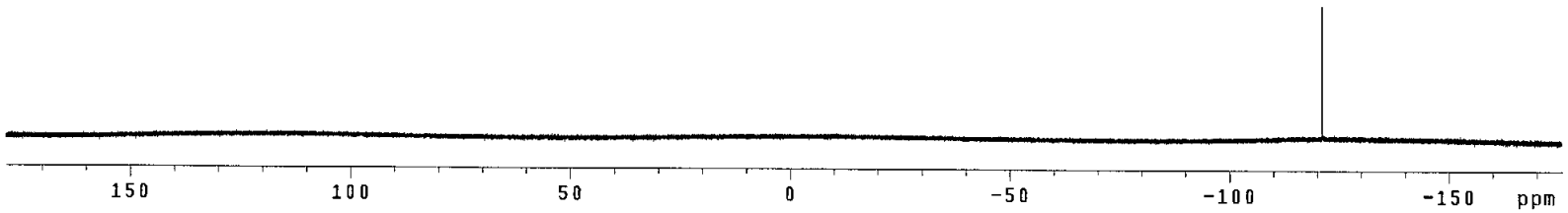
052812ciproPEG3FmocF19

exp3 s2pul

SAMPLE		DEC. & VT	
date	May 28 2012	dfrq	300.107
solvent	CDCl3	dn	H1
file	exp	dpwr	30
ACQUISITION		dof	0
sfrq	282.382	dm	nnn
tn	F19	dmm	c
at	0.300	dmf	200
np	59906	PROCESSING	
sw	100000.0	lb	0.30
fb	55000	wtfile	
bs	16	proc	ft
tpwr	56	fn	262144
pw	11.0		
d1	4.000	werr	
tof	29637.2	wexp	
nt	64	wbs	
ct	64	wnt	
alock	n		
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
DISPLAY			
sp	-49681.9		
wp	99999.2		
vs	22		
sc	0		
wc	250		
hzmm	50.46		
is	500.00		
rfl	49682.6		
rfp	0		
th	12		
ins	100.000		
nm	ph		



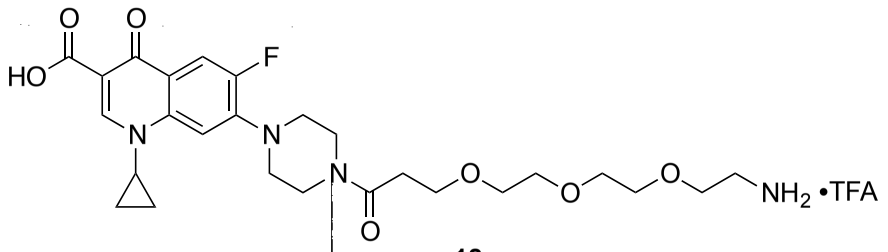
Fmoc-18



STANDARD PROTON PARAMETERS

expl s2pul

SAMPLE		DEC. & VT	
date	Jul 12 2012	dfrq	125.672
solvent	CDCl3	dn	C13
file	/data/export/~	dpwr	30
	home/nolan/NOtzhe/~	dof	0
	bullwinkle/071212C~	dm	nnn
	ipro-PEG-NH2.fid	dmm	w
ACQUISITION		dmf	10000
sfrq	499.746	dseq	
tn	H1	dres	1.0
at	3.001	homo	n
np	63050	PROCESSING	
sw	10504.2	wtfile	
fb	not used	proc	ft
bs	8	fn	262144
tpwr	56	math	f
pw	8.6		
d1	2.000	werr	
tof	1519.5	wexp	
nt	1	wbs	
ct	1	wnt	wft
alock	n		
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
hs	nn		
DISPLAY			
sp	-354.9		
wp	6777.3		
vs	171		
sc	0		
wc	250		
hzmm	27.11		
is	219.52		
rfl	4866.5		
rfp	3628.1		
th	5		
ins	1.000		
ai	ph		



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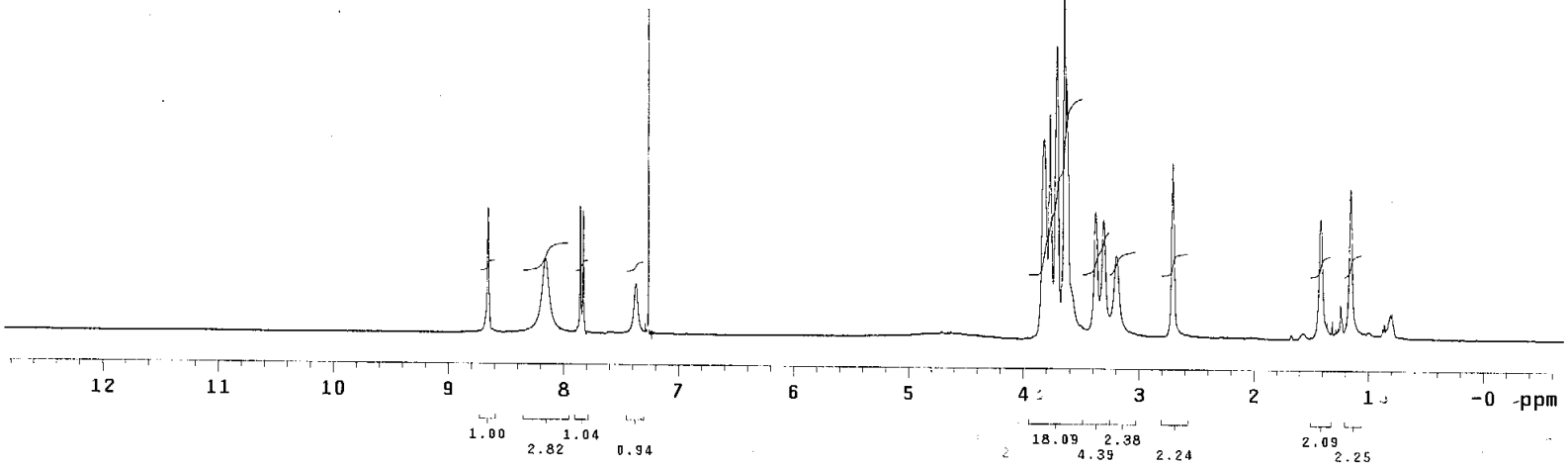
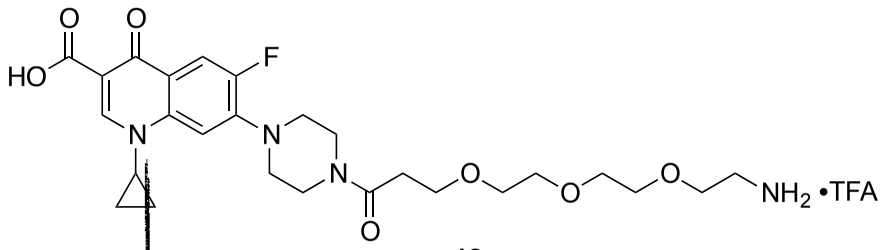


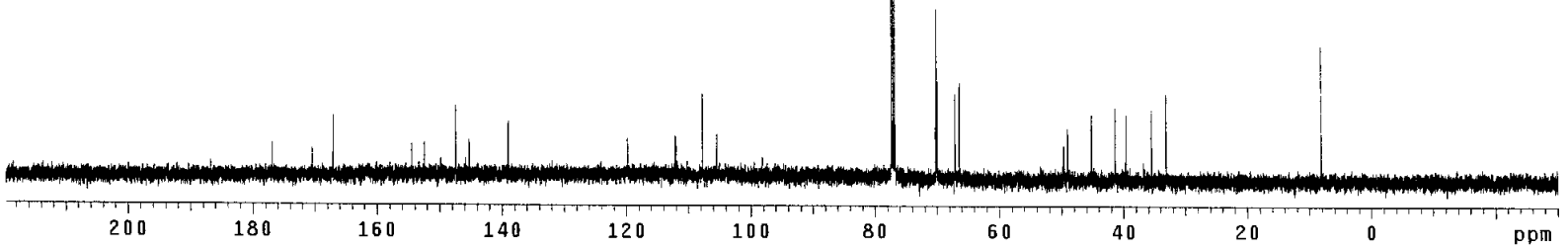
STANDARD CARBON PARAMETERS

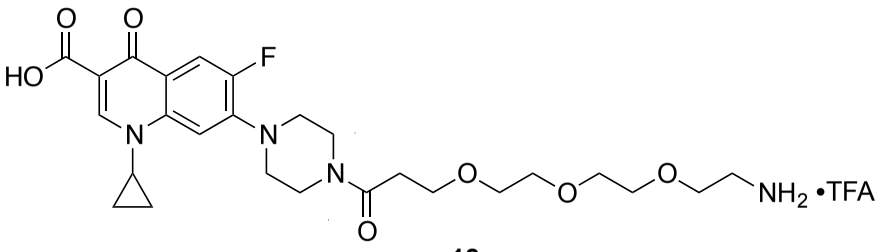
exp1 s2pul

SAMPLE		DEC. & VT	
date	Jul 12 2012	dfrq	499.744
solvent	CDCl3	dn	H1
file	exp	dpwr	34
ACQUISITION		dof	0
sfrq	125.672	dm	yyy
tn	C13	dmm	w
at	2.000	dmf	10400
np	125588	dseq	
sw	31397.2	dres	1.0
fb	not used	homo	n
bs	16	DEC2	
tpwr	59	dfrq2	0
pw	6.7	dn2	
d1	0	dpwr2	1
tof	0	dof2	0
nt	9999	dm2	n
ct	1952	dmm2	c
alock	n	dmf2	10000
gain	not used	dseq2	
FLAGS		dres2	1.0
il	n	homo2	n
in	n	DEC3	
dp	y	dfrq3	0
hs	nn	dn3	
DISPLAY		dpwr3	1
sp	-3796.0	dof3	0
wp	31396.7	dm3	n
vs	2287	dmm3	c
sc	0	dmf3	10000
wc	250	dseq3	
hzmm	125.59	dres3	1.0
is	500.00	homo3	n
rfl	13472.3	PROCESSING	
rfp	9675.8	lb	1.00
th	12	wtfile	
ins	100.000	proc	ft
ai cdc ph		fn	131072
		math	f
		werr	
		wexp	
		wbs	
		wnt	



18



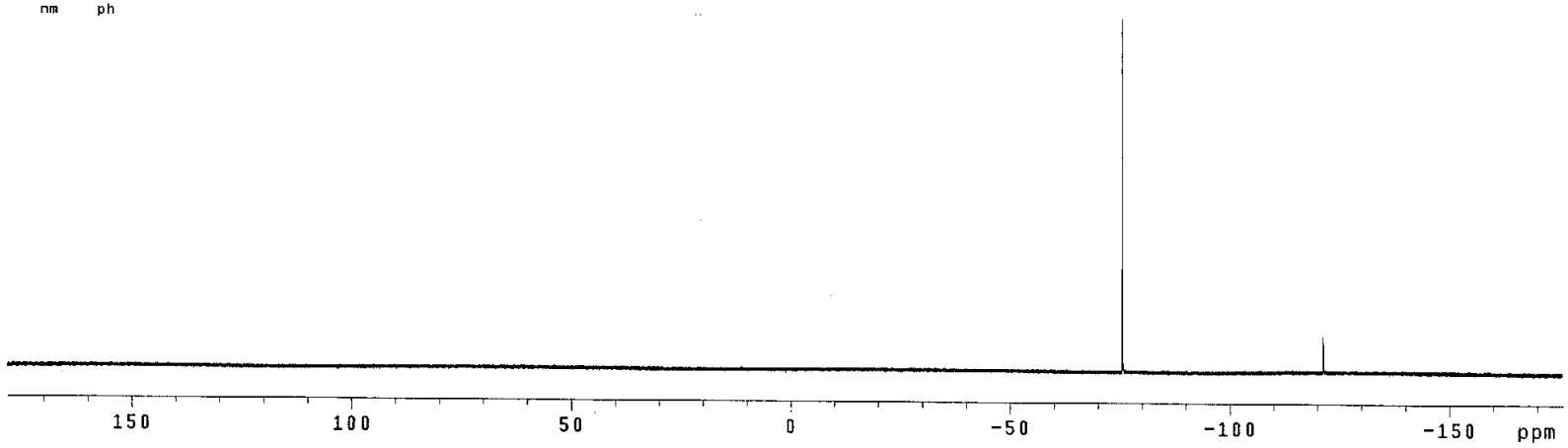


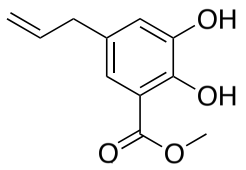
18

```
19F OBSERVE
STANDARD PARAMETERS

exp3  s2pul

      SAMPLE                DEC. & VT
date    Jul 12 2012   dfrq          300.107
solvent       CDCl3   dn                Hl
file /data/nolan/N~   dpwr              30
Otzhe/071212cipro--~  dof                0
   PEG-NH2-F19.fid    dm               nnn
   ACQUISITION        dmm                c
sfrq         282.382   dmf              200
tn               F19       PROCESSING
at             0.300   lb              0.30
np             59906   wtfile
sw         100000.0    proc               ft
fb             55000   fn             262144
bs                 4
tpwr              56   werr
pw              11.0   wexp
d1             4.000   wbs
tof         29637.2    wnt
nt                16
ct                16
alock              n
gain       not used
      FLAGS
il                 n
in                 n
dp                 y
     DISPLAY
sp          -49680.3
wp           99999.2
vs                56
sc                 0
wc               250
hzmm          400.00
is            500.00
rfl          49681.1
rfp                0
th                10
ins           100.000
nm    ph
```





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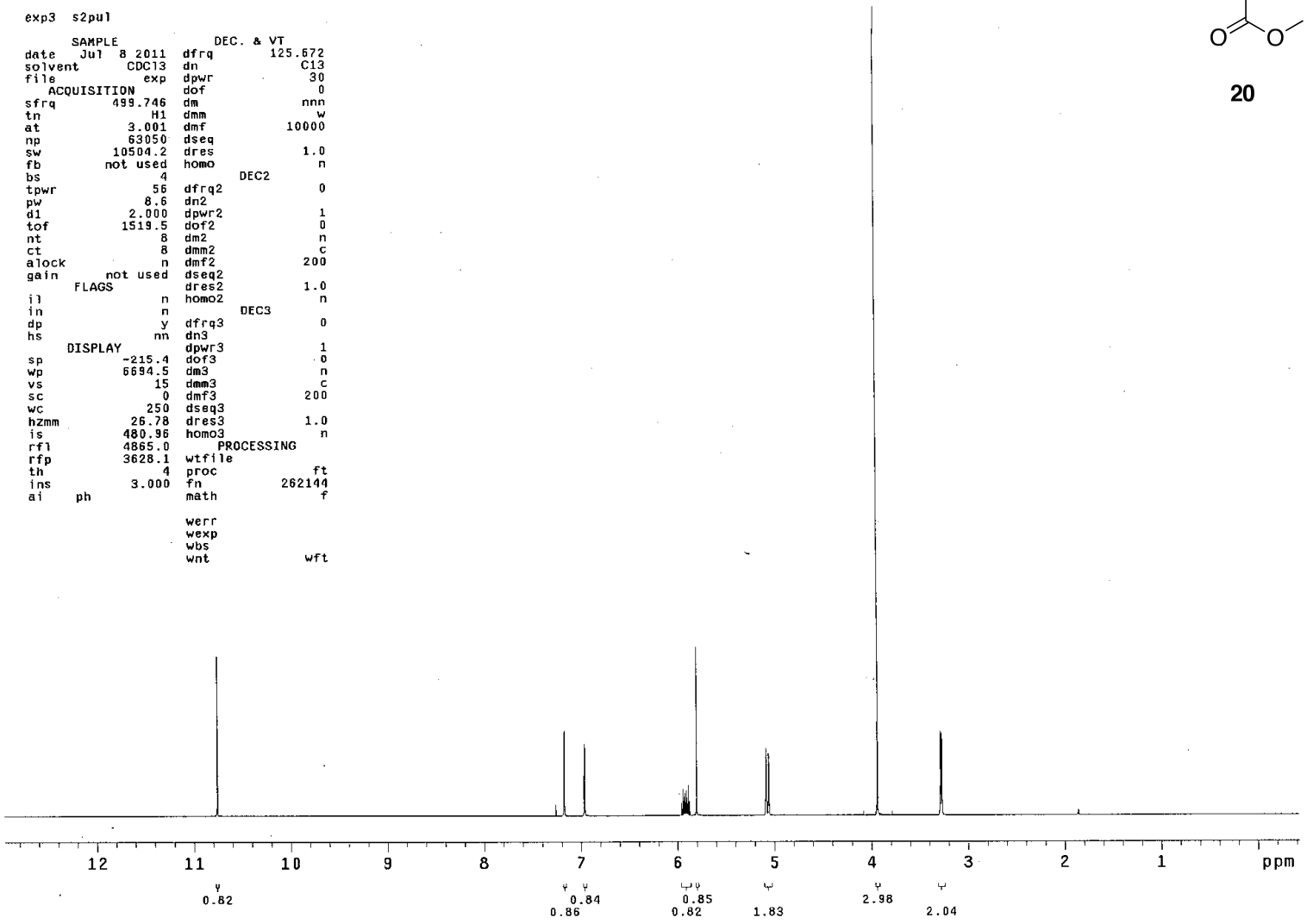
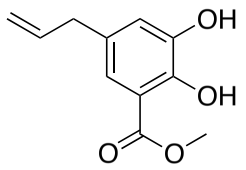
STANDARD PROTON PARAMETERS

exp3 s2pul

SAMPLE		DEC. & VT	
date	Jul 8 2011	dfrq	125.672
solvent	CDCl3	dn	C13
file	exp	dpwr	30
ACQUISITION		dof	0
sfrq	499.746	dm	nnn
tn	H1	dmm	w
at	3.001	dmf	10000
np	63050	dseq	
sw	10504.2	dres	1.0
fb	not used	homo	n
bs	4	DEC2	
tpwr	56	dfrq2	0
pw	8.6	dn2	
d1	2.000	dpwr2	1
tof	1519.5	dof2	0
nt	8	dm2	n
ct	8	dmm2	c
alock	n	dmf2	200
gain	not used	dseq2	
FLAGS		dres2	1.0
il	n	homo2	n
in	n	DEC3	
dp	y	dfrq3	0
hs	nn	dn3	
DISPLAY		dpwr3	1
sp	-215.4	dof3	0
wp	6694.5	dm3	n
vs	15	dmm3	c
sc	0	dmf3	200
wc	250	dseq3	
hzmm	26.78	dres3	1.0
is	480.96	homo3	n
rfl	4865.0	PROCESSING	
rfp	3628.1	wtfile	
th	4	proc	ft
ins	3.000	fn	262144
ai ph		math	f
		werr	
		wexp	
		wbs	
		wnt	wft





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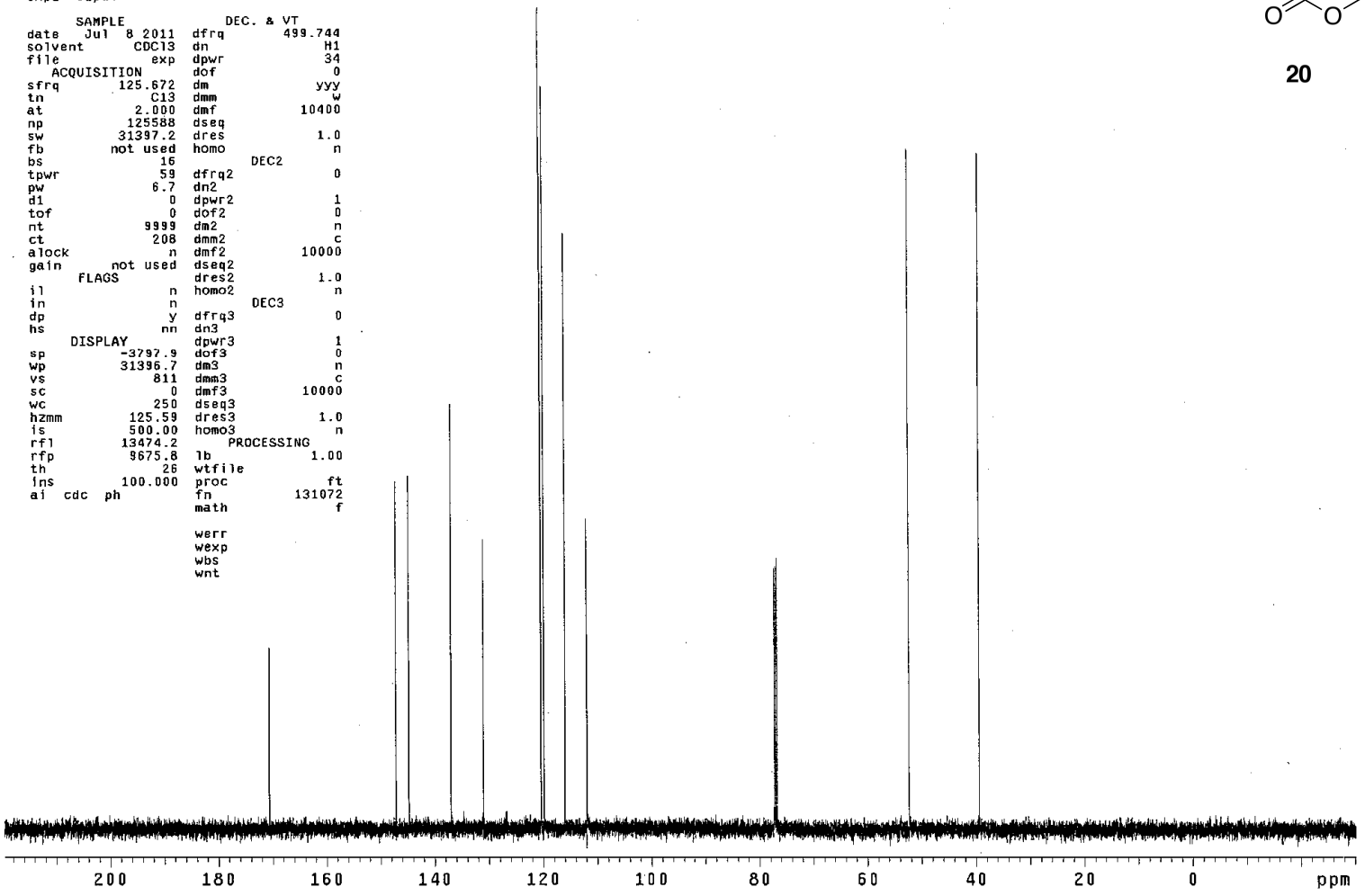
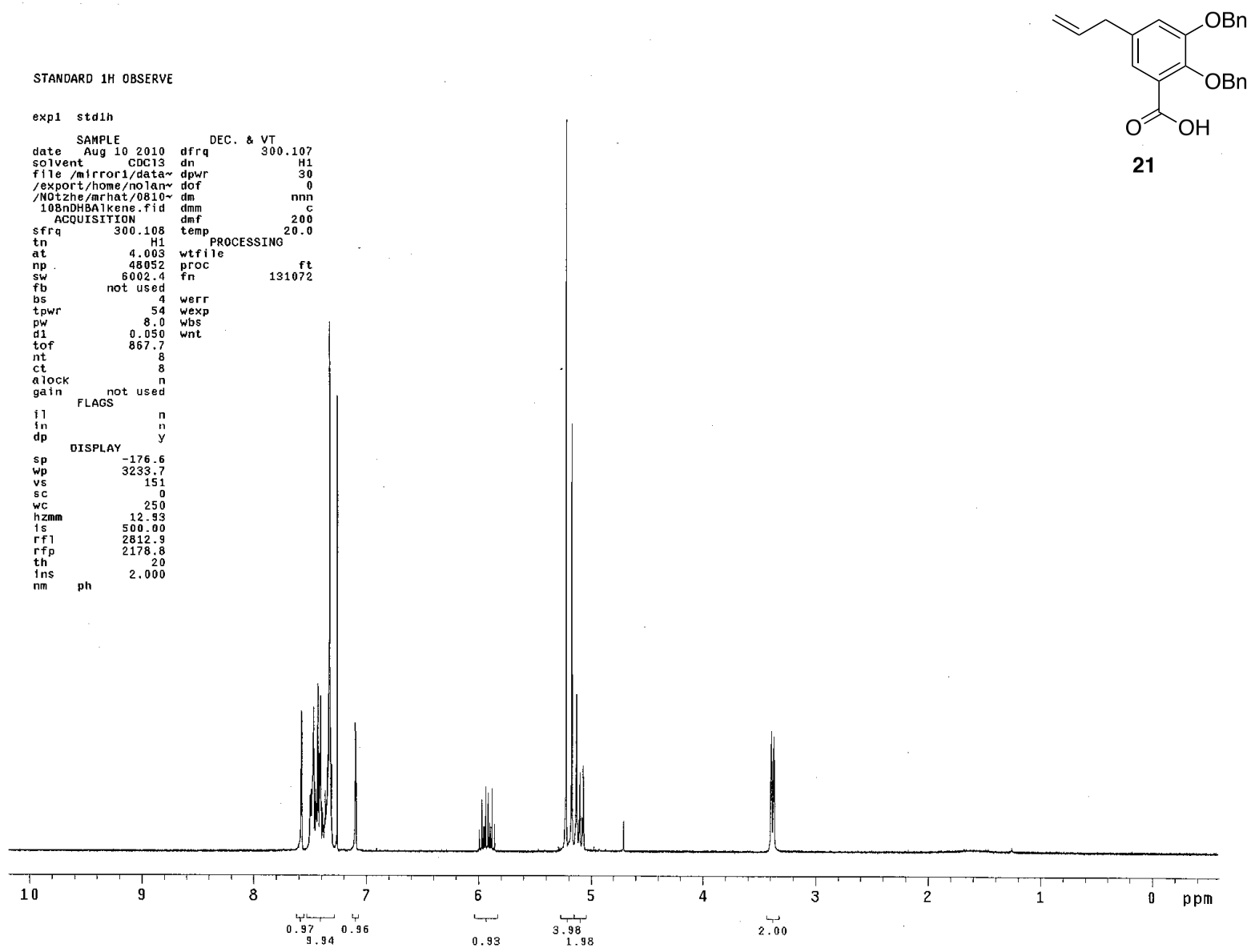
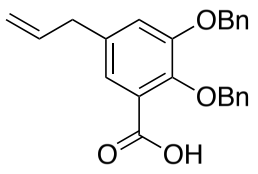
STANDARD CARBON PARAMETERS

exp2 s2pul

SAMPLE		DEC. & VT	
date	Jul 8 2011	dfrq	499.744
solvent	CDCl3	dn	H1
file	exp	dpwr	34
ACQUISITION		dof	0
sfrq	125.672	dm	yyy
tn	C13	dmm	w
at	2.000	dmf	10400
np	125588	dseq	
sw	31397.2	dres	1.0
fb	not used	homo	n
bs	16	DEC2	
tpwr	59	dfrq2	0
pw	6.7	dn2	
d1	0	dpwr2	1
tof	0	dof2	0
nt	9999	dm2	n
ct	208	dmm2	c
alock	n	dmf2	10000
gain	not used	dseq2	
FLAGS		dres2	1.0
il	n	homo2	n
in	n	DEC3	
dp	y	dfrq3	0
hs	nn	dn3	
DISPLAY		dpwr3	1
sp	-3797.9	dof3	0
wp	31396.7	dm3	n
vs	811	dmm3	c
sc	0	dmf3	10000
wc	250	dseq3	
hzmm	125.59	dres3	1.0
is	500.00	homo3	n
rfl	13474.2	PROCESSING	
rfp	9675.8	lb	1.00
th	26	wtfile	
ins	100.000	proc	ft
ai cdc ph		fn	131072
		math	f
		werr	
		wexp	
		wbs	
		wnt	




STANDARD 1H OBSERVE
exp1 stdlh
SAMPLE
date Aug 10 2010
solvent CDCl3
file /mirror1/data~
/export/home/nolan~
/NOtzhe/mrhat/0810~
10BnDHBAlkene.fid
ACQUISITION
sfrq 300.108
tn H1
at 4.003
np 48052
sw 6002.4
fb not used
bs 4
tpwr 54
pw 8.0
d1 0.050
tof 867.7
nt 8
ct 8
alock n
gain not used
FLAGS
il n
in n
dp y
DISPLAY
sp -176.6
wp 3233.7
vs 151
sc 0
wc 250
hzmm 12.93
is 500.00
rfl 2812.9
rfp 2178.8
th 20
ins 2.000
nm ph
DEC. & VT
dfrq 300.107
dn H1
dpwr 30
dof 0
dm nnn
dmm c
dmf 200
temp 20.0
PROCESSING
wtfile
proc ft
fn 131072
werr
wexp
wbs
wnt
OBn
OBn
O
OH
21
10
9
8
7
6
5
4
3
2
1
0
ppm
0.97
9.94
0.96
0.93
3.98
1.98
2.00



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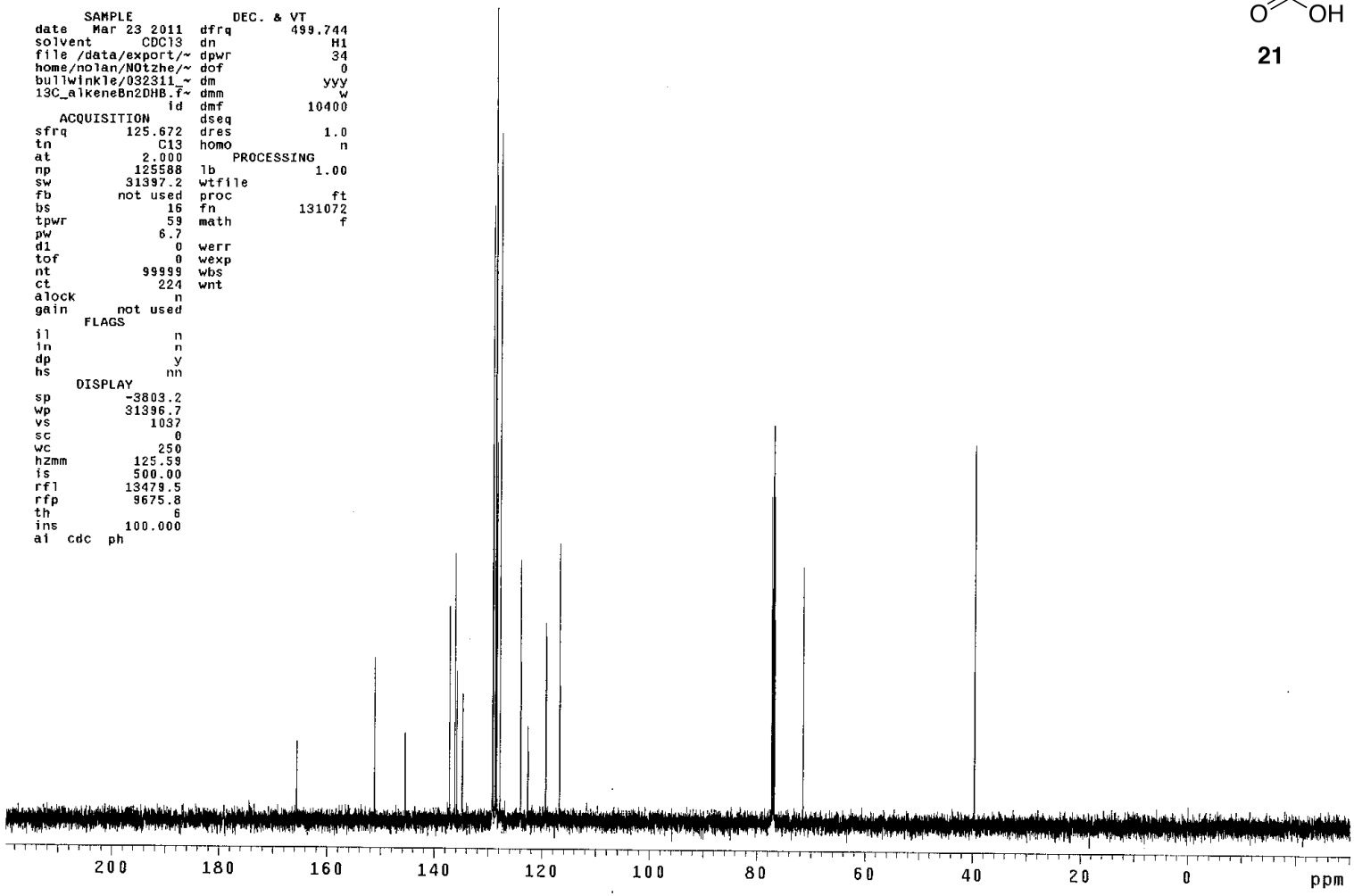
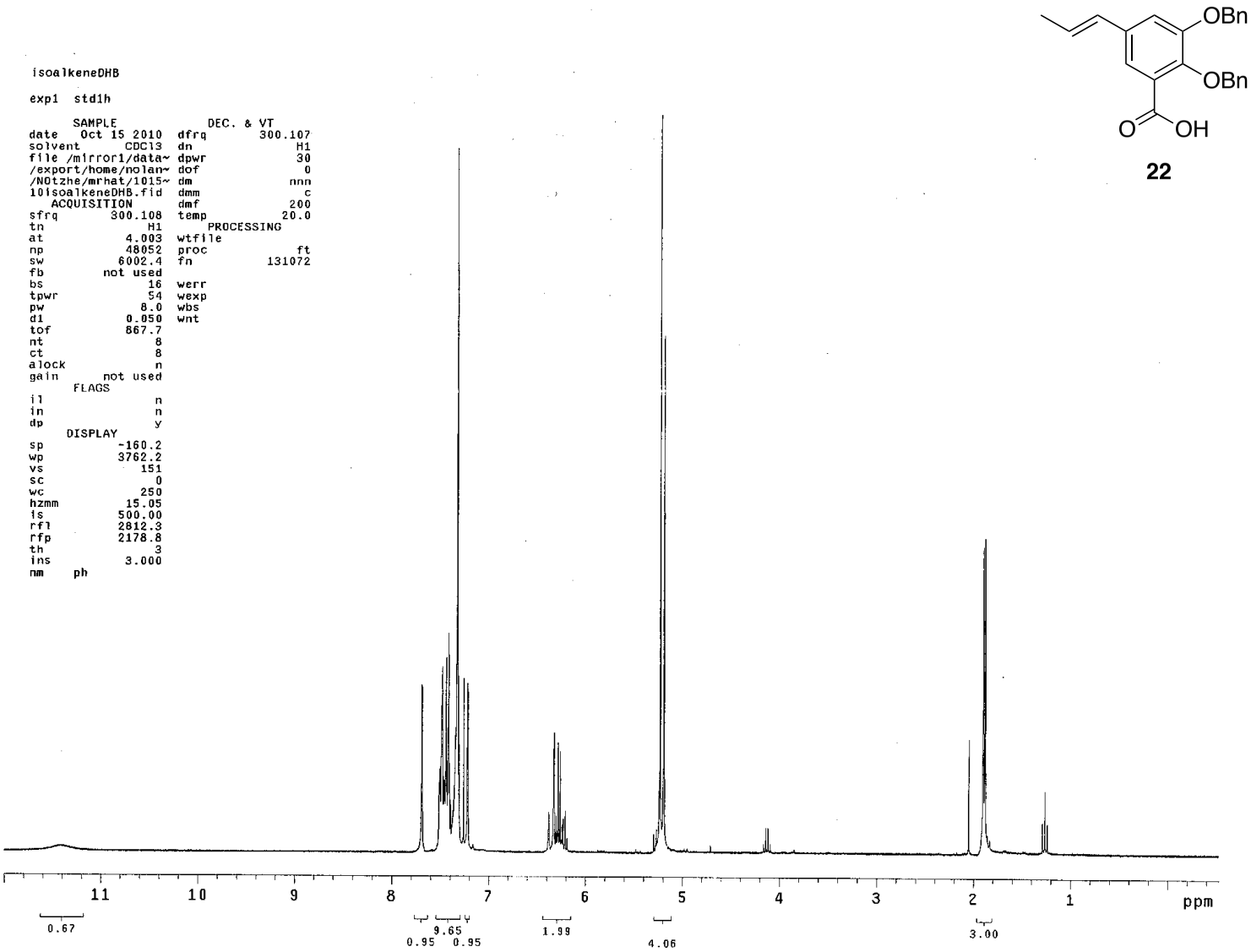
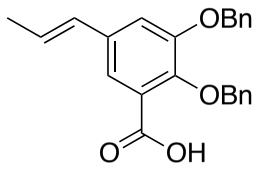
```
032311_13C_alkeneBn2DHB

exp1  s2pul

      SAMPLE            DEC. & VT
date   Mar 23 2011  dfrq        499.744
solvent      CDCl3  dn             H1
file /data/export/~ dpwr            34
home/nolan/NOtzhe/~ dof              0
bullwinkle/032311_~ dm             yyy
13C_alkeneBn2DHB.f~ dmm              w
                id  dmf          10400
   ACQUISITION      dseq
sfrq       125.672  dres          1.0
tn             C13  homo            n
at           2.000      PROCESSING
np          125588  lb            1.00
sw         31397.2  wtfile
fb       not used  proc            ft
bs             16  fn          131072
tpwr           59  math             f
pw            6.7
d1               0  werr
tof              0  wexp
nt           99999  wbs
ct             224  wnt
alock            n
gain     not used
      FLAGS
il               n
in               n
dp               y
hs              nn
     DISPLAY
sp         -3803.2
wp         31396.7
vs            1037
sc               0
wc             250
hzmm        125.59
is          500.00
rfl        13479.5
rfp         9675.8
th               6
ins        100.000
ai  cdc  ph
```




isoalkeneDHB
exp1 std1h
SAMPLE
date Oct 15 2010
solvent CDCl3
file /mirror1/data~
/export/home/nolan~
/NOtzhe/mrhat/1015~
10isoalkeneDHB.fid
ACQUISITION
sfrq 300.108
tn H1
at 4.003
np 48052
sw 6002.4
fb not used
bs 16
tpwr 54
pw 8.0
d1 0.050
tof 867.7
nt 8
ct 8
alock n
gain not used
FLAGS
il n
in n
dp y
DISPLAY
sp -160.2
wp 3762.2
vs 151
sc 0
wc 250
hzmm 15.05
is 500.00
rfl 2812.3
rfp 2178.8
th 3
ins 3.000
nm ph
DEC. & VT
dfrq 300.107
dn H1
dpwr 30
dof 0
dm nnn
dmm c
dmf 200
temp 20.0
PROCESSING
wtfile
proc ft
fn 131072
werr
wexp
wbs
wnt
11
10
9
8
7
6
5
4
3
2
1
ppm
0.67
0.95
9.65
0.95
1.99
4.06
3.00

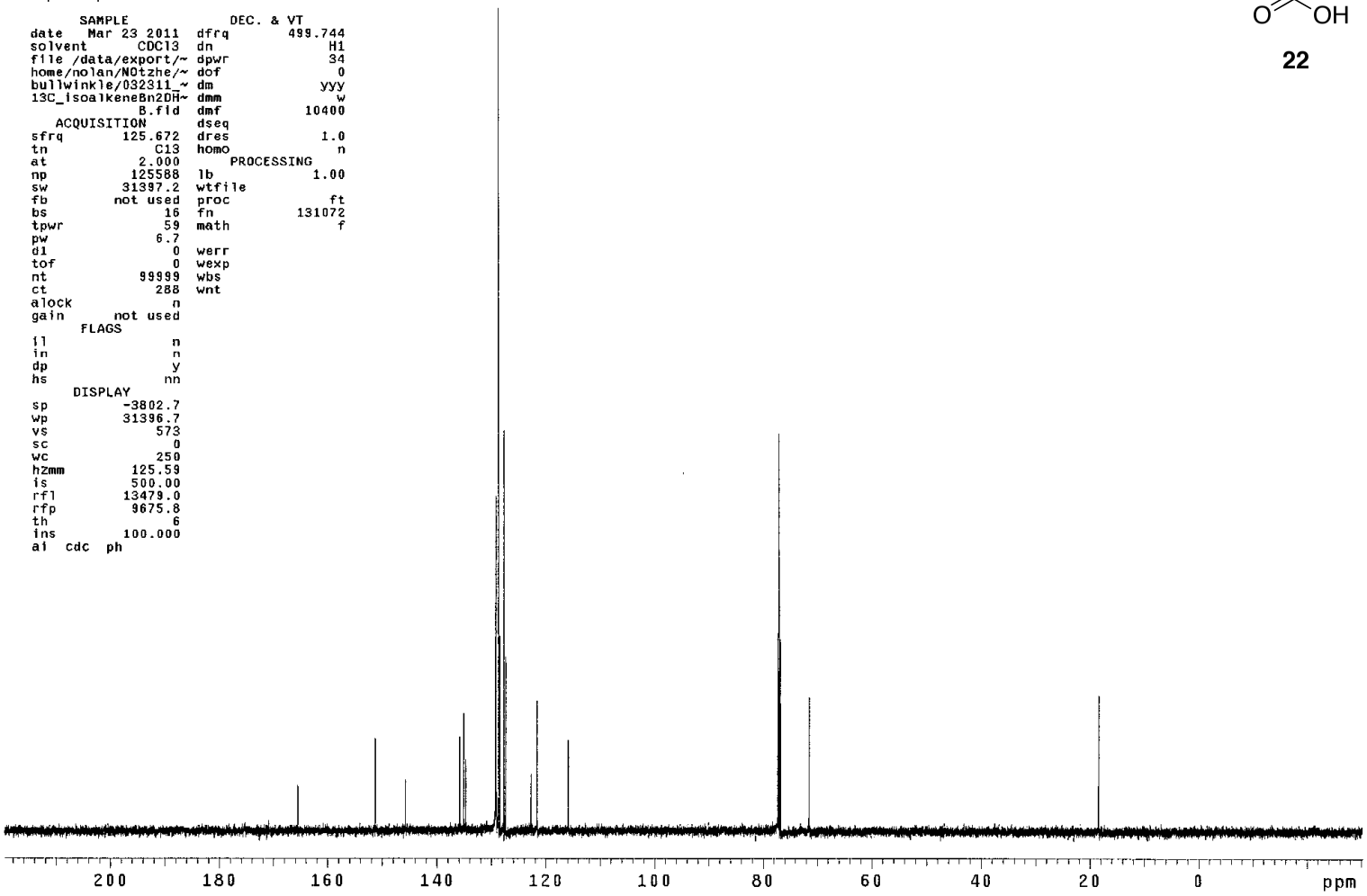
OBn
OBn
O
OH

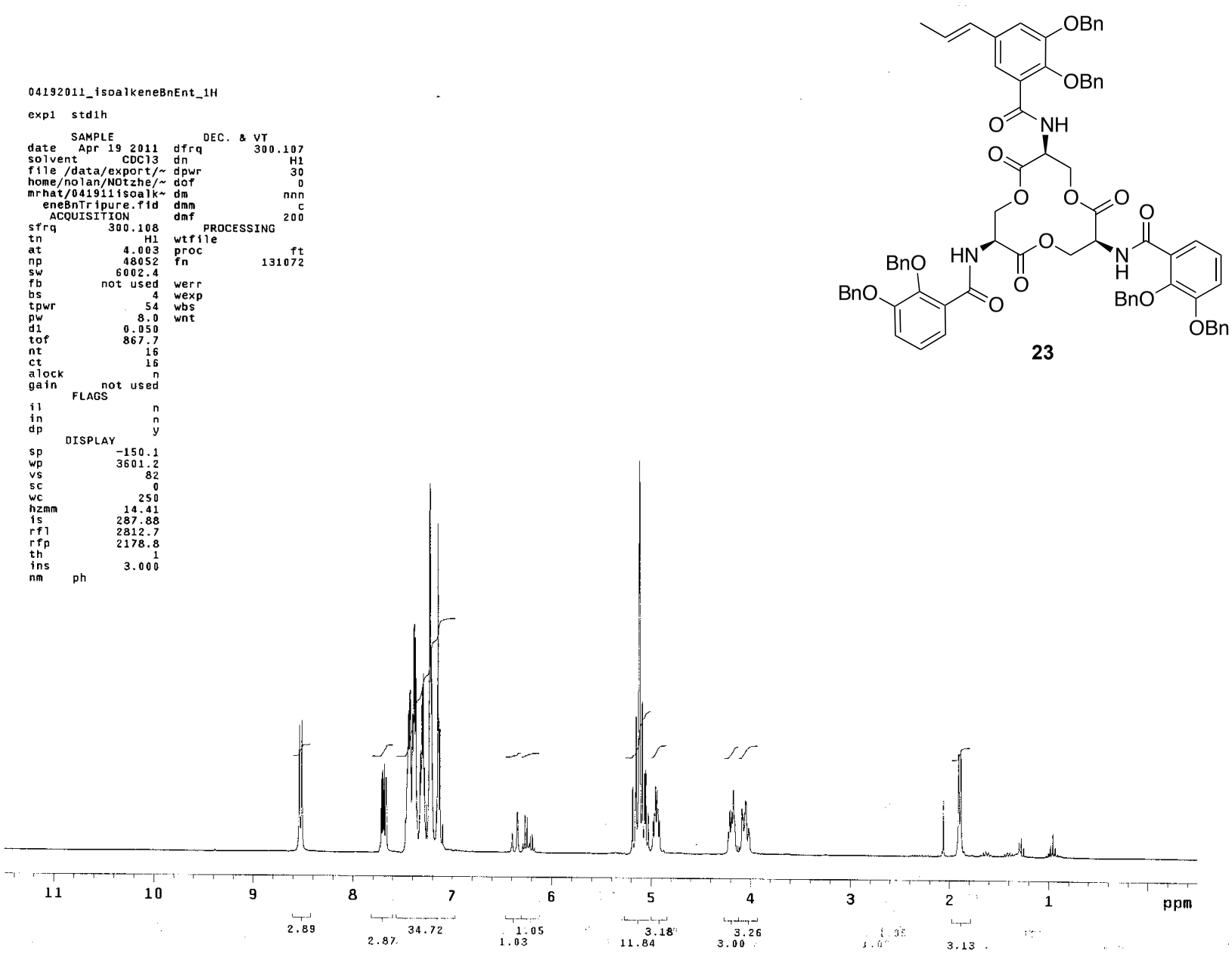
22



22

```
032311_13C_isoalkeneBn2DHB

exp1  s2pul

      SAMPLE            DEC. & VT
date   Mar 23 2011  dfrq        499.744
solvent      CDCl3  dn              H1
file /data/export/~ dpwr            34
home/nolan/NOtzhe/~ dof              0
bullwinkle/032311_~ dm             yyy
13C_isoalkeneBn2DH~ dmm              w
                B.fid  dmf         10400
   ACQUISITION       dseq
sfrq        125.672  dres          1.0
tn              C13  homo           n
at            2.000       PROCESSING
np           125588  lb            1.00
sw         31397.2  wtfile
fb        not used  proc            ft
bs              16  fn          131072
tpwr            59  math             f
pw             6.7
d1               0  werr
tof              0  wexp
nt           99999  wbs
ct             288  wnt
alock            n
gain      not used
      FLAGS
il               n
in               n
dp               y
hs              nn
     DISPLAY
sp         -3802.7
wp         31396.7
vs             573
sc               0
wc             250
hzmm         125.59
is           500.00
rfl        13479.0
rfp         9675.8
th               6
ins          100.000
ai  cdc  ph
```

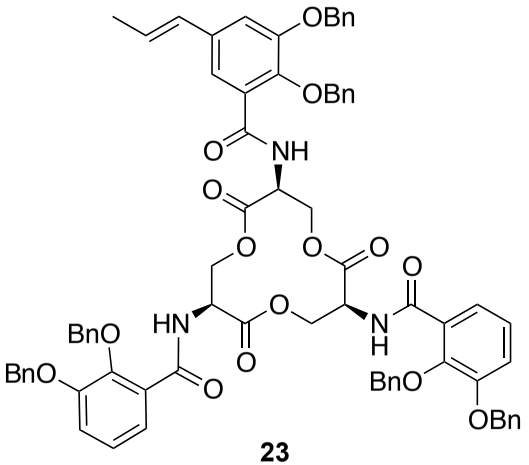



04192011_isoalkeneBnEnt_1H
expl stdlh
SAMPLE
date Apr 19 2011
solvent CDCl3
file /data/export/~
home/nolan/NOtzhe/~
mrhat/041911isoalk~
eneBnTripure.fid
ACQUISITION
sfrq 300.108
tn H1
at 4.003
np 48052
sw 6002.4
fb not used
bs 4
tpwr 54
pw 8.0
d1 0.050
tof 867.7
nt 16
ct 16
alock n
gain not used
FLAGS
il n
in n
dp y
DISPLAY
sp -150.1
wp 3601.2
vs 82
sc 0
wc 250
hzmm 14.41
is 287.88
rfl 2812.7
rfp 2178.8
th 1
ins 3.000
nm ph
DEC. & VT
dfrq 300.107
dn H1
dpwr 30
dof 0
dm nnn
dmm c
dmf 200
PROCESSING
wtfile
proc ft
fn 131072
werr
wexp
wbs
wnt
OBn
OBn
NH
HN
BnO
BnO
BnO
OBn
23
11
10
9
8
7
6
5
4
3
2
1
ppm
2.89
34.72
1.05
3.18
3.26
2.87
1.03
11.84
3.00
3.13

STANDARD CARBON PARAMETERS

exp2 s2pul

SAMPLE		DEC. & VT	
date	Jun 4 2011	dfrq	499.744
solvent	CDCl3	dn	H1
file	/data/nolan/N~	dpwr	34
	Otzhe/060411isoalk~	dof	0
	eneBn6Trilactone.f~	dm	yyy
	id	dmm	w
ACQUISITION		dmf	10400
sfrq	125.672	dseq	
tn	C13	dres	1.0
at	2.000	homo	n
np	125588	DEC2	
sw	31397.2	dfrq2	0
fb	not used	dn2	
bs	16	dpwr2	1
tpwr	59	dof2	0
pw	6.7	dm2	n
d1	0	dmm2	c
tof	0	dmf2	10000
nt	99999	dseq2	
ct	208	dres2	1.0
alock	n	homo2	n
gain	not used	DEC3	
FLAGS		dfrq3	0
il	n	dn3	
in	n	dpwr3	1
dp	y	dof3	0
hs	nn	dm3	n
DISPLAY		dmm3	c
sp	-3830.0	dmf3	10000
wp	31396.7	dseq3	
vs	581	dres3	1.0
sc	0	homo3	n
wc	250	PROCESSING	
hzmm	125.59	lb	1.00
is	500.00	wtfile	
rfl	13506.3	proc	ft
rfp	9675.8	fn	131072
th	6	math	f
ins	100.000		
ai cdc ph		werr	
		wexp	
		wbs	
		wnt	



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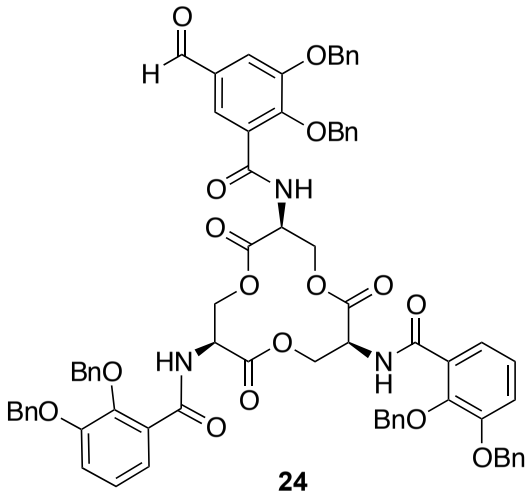
STANDARD PROTON PARAMETERS

exp2 s2pul

SAMPLE		DEC. & VT	
date	Jun 4 2011	dfrq	125.672
solvent	CDCl3	dn	C13
file	exp	dpwr	30
ACQUISITION		dof	0
sfrq	499.746	dm	nnn
tn	H1	dmm	w
at	3.001	dmf	10000
np	63050	dseq	
sw	10504.2	dres	1.0
fb	not used	homo	n
bs	8	DEC2	
tpwr	56	dfrq2	0
pw	8.6	dn2	
d1	2.000	dpwr2	1
tof	1519.5	dof2	0
nt	4	dm2	n
ct	4	dmm2	c
alock	n	dmf2	200
gain	not used	dseq2	
FLAGS		dres2	1.0
il	n	homo2	n
in	n	DEC3	
dp	y	dfrq3	0
hs	nn	dn3	
DISPLAY		dpwr3	1
sp	-462.6	dof3	0
wp	6763.5	dm3	n
vs	243	dmm3	c
sc	0	dmf3	200
wc	250	dseq3	
hzmm	27.05	dres3	1.0
is	356.84	homo3	n
rfl	2260.1	PROCESSING	
rfp	1024.5	wtfile	
th	7	proc	ft
ins	3.000	fn	262144
ai ph		math	f
		werr	
		wexp	
		wbs	
		wnt	wft



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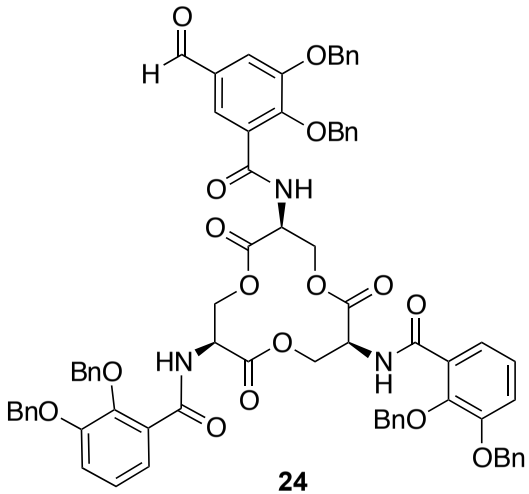
STANDARD CARBON PARAMETERS

exp3 s2pul

SAMPLE		DEC. & VT	
date	Jun 4 2011	dfrq	499.744
solvent	CDCl3	dn	H1
file	exp	dpwr	34
ACQUISITION		dof	0
sfrq	125.672	dm	yyy
tn	C13	dmm	w
at	2.000	dmf	10400
np	125588	dseq	
sw	31397.2	dres	1.0
fb	not used	homo	n
bs	16	DEC2	
tpwr	59	dfrq2	0
pw	6.7	dn2	
d1	0	dpwr2	1
tof	0	dof2	0
nt	9999	dm2	n
ct	480	dmm2	c
alock	n	dmf2	10000
gain	not used	dseq2	
FLAGS		dres2	1.0
il	n	homo2	n
in	n	DEC3	
dp	y	dfrq3	0
hs	nn	dn3	
DISPLAY		dpwr3	1
sp	-3811.8	dof3	0
wp	31396.7	dm3	n
vs	1411	dmm3	c
sc	0	dmf3	10000
wc	250	dseq3	
hzmm	125.59	dres3	1.0
is	500.00	homo3	n
rfl	13488.1	PROCESSING	
rfp	9675.8	lb	1.00
th	9	wtfile	
ins	100.000	proc	ft
ai cdc ph		fn	131072
		math	f
		werr	
		wexp	
		wbs	
		wnt	



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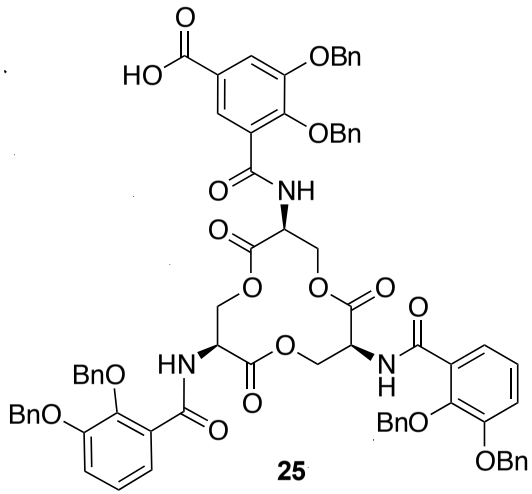
STANDARD PROTON PARAMETERS

exp1 s2pul

SAMPLE		DEC. & VT	
date	Jul 6 2011	dfrq	125.672
solvent	CDCl3	dn	C13
file	/data/export/~	dpwr	30
home/nolan/NOtzhe/~		dof	0
bullwinkle/070611B~		dm	nnn
n6EntAcidH1.fid		dmm	w
ACQUISITION		dmf	10000
sfrq	499.746	dseq	
tn	H1	dres	1.0
at	3.001	homo	n
np	63050	PROCESSING	
sw	10504.2	wtfile	
fb	not used	proc	ft
bs	4	fn	262144
tpwr	56	math	f
pw	8.6		
d1	2.000	werr	
tof	1519.5	wexp	
nt	16	wbs	
ct	16	wnt	wft
alock	n		
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
hs	nn		
DISPLAY			
sp	-167.7		
wp	6266.5		
vs	199		
sc	0		
wc	250		
hzmm	25.07		
is	287.18		
rfl	4858.7		
rfp	3628.1		
th	2		
ins	3.000		
ai ph			



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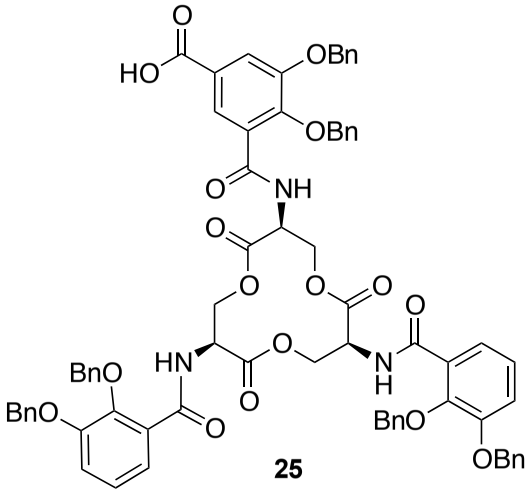
STANDARD CARBON PARAMETERS

exp2 s2pul

SAMPLE		DEC. & VT	
date	Jul 6 2011	dfrq	499.744
solvent	CDCl3	dn	H1
file	exp	dpwr	34
ACQUISITION		dof	0
sfrq	125.672	dm	yyy
tn	C13	dmm	w
at	2.000	dmf	10400
np	125588	dseq	
sw	31397.2	dres	1.0
fb	not used	homo	n
bs	16	DEC2	
tpwr	59	dfrq2	0
pw	6.7	dn2	
d1	0	dpwr2	1
tof	0	dof2	0
nt	99999	dm2	n
ct	448	dmm2	c
alock	n	dmf2	10000
gain	not used	dseq2	
FLAGS		dres2	1.0
il	n	homo2	n
in	n	DEC3	
dp	y	dfrq3	0
hs	nn	dn3	
DISPLAY		dpwr3	1
sp	-3810.3	dof3	0
wp	31396.7	dm3	n
vs	1307	dmm3	c
sc	0	dmf3	10000
wc	250	dseq3	
hzmm	125.59	dres3	1.0
is	500.00	homo3	n
rfl	13486.7	PROCESSING	
rfp	9675.8	lb	1.00
th	4	wtfile	
ins	100.000	proc	ft
ai cdc ph		fn	131072
		math	f
		werr	
		wexp	
		wbs	
		wnt	

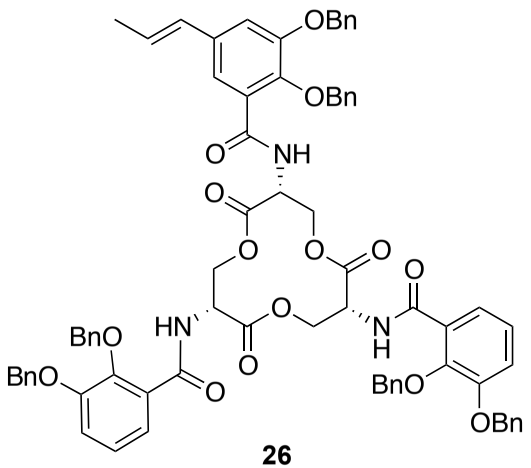


25

STANDARD PROTON PARAMETERS

expl s2pul

SAMPLE		DEC. & VT	
date	Feb 10 2012	dfrq	125.672
solvent	CDCl3	dn	C13
file	/data/export/~	dpwr	30
home/nolan/NOjbul/~		dof	0
bullwinkle/021012m~		dm	nnn
	dhbitlr.fid	dmm	w
ACQUISITION		dmf	10000
sfrq	499.746	dseq	
tn	H1	dres	1.0
at	3.001	homo	n
np	63050	PROCESSING	
sw	10504.2	wtfile	
fb	not used	proc	ft
bs	4	fn	262144
tpwr	56	math	f
pw	8.6		
d1	2.000	werr	
tof	1519.5	wexp	
nt	16	wbs	
ct	16	wnt	wft
alock	n		
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
hs	nn		
DISPLAY			
sp	-267.6		
wp	6487.4		
vs	111		
sc	0		
wc	250		
hzmm	25.95		
is	33.57		
rfl	1233.8		
rfp	0		
th	7		
ins	3.000		
ai cdc ph			



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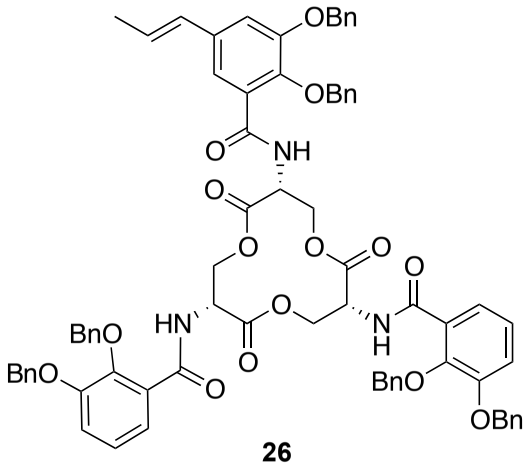
STANDARD CARBON PARAMETERS

expl s2pul

SAMPLE		DEC. & VT	
date	Feb 10 2012	dfrq	499.744
solvent	CDCl3	dn	H1
file	/data/export/~	dpwr	34
	home/nolan/NOjbul/~	dof	0
	bullwinkle/021012c~	dm	yyy
	arlmdhbtlr.fid	dmm	w
ACQUISITION		dmf	10400
sfrq	125.672	dseq	
tn	C13	dres	1.0
at	2.000	homo	n
np	125588	PROCESSING	
sw	31397.2	lb	1.00
fb	not used	wtfile	
bs	16	proc	ft
tpwr	59	fn	131072
pw	6.7	math	f
d1	0		
tof	0	werr	
nt	99999	wexp	
ct	2272	wbs	
alock	n	wnt	
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
hs	nn		
DISPLAY			
sp	-3794.5		
wp	31396.7		
vs	2322		
sc	0		
wc	250		
hzmm	125.59		
is	500.00		
rfl	13470.9		
rfp	9675.8		
th	3		
ins	100.000		

ai cdc ph



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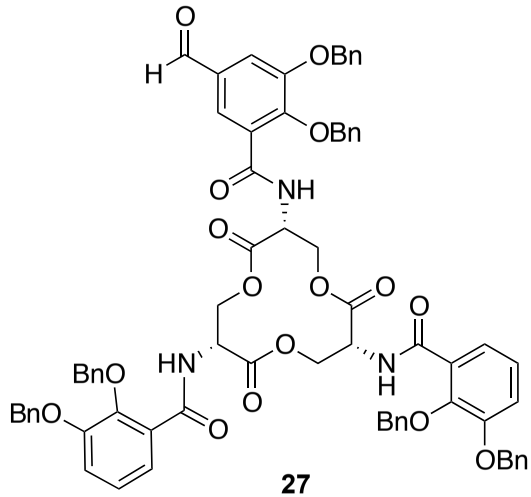
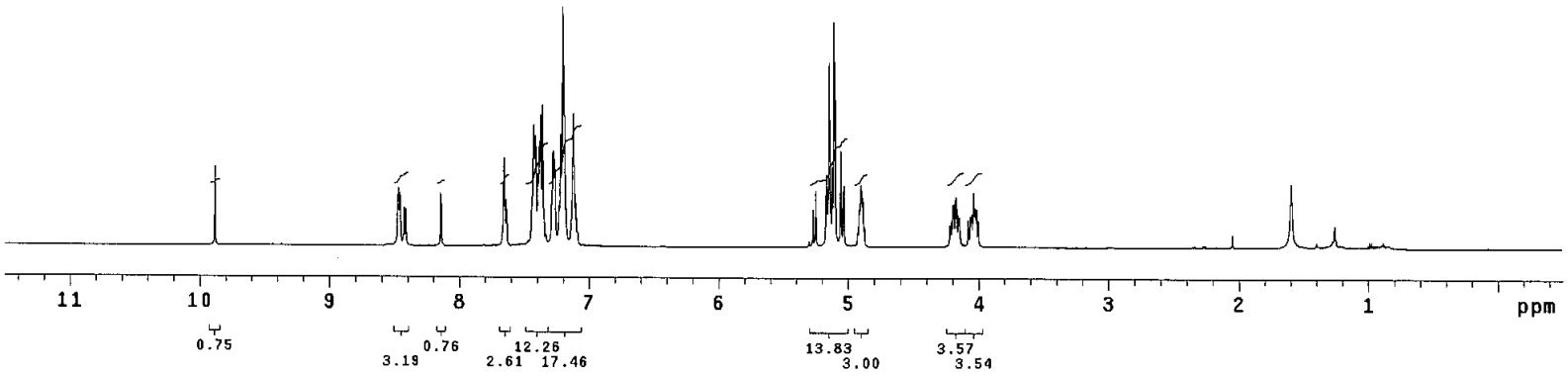
Bn_Ent_aldehyde_1_1H

exp2 s2pul

SAMPLE		DEC. & VT	
date	Aug 1 2012	dfrq	500.176
solvent	CDCl3	dn	H1
file	exp	dpwr	32
ACQUISITION		dof	0
sfrq	500.176	dm	nnn
tn	H1	dmm	c
at	2.048	dmf	8770
np	32768	dseq	
sw	8000.0	dres	1.0
fb	4000	homo	n
bs	4	temp	23.0
ss	2	PROCESSING	
tpwr	58	lb	0.50
pw	5.0	wtfile	
d1	0	proc	ft
tof	0	fn	not used
nt	32	math	f
ct	32		
alock	n	werr	
gain	not used	wexp	
FLAGS		wbs	
il	n	wnt	wft
in	n		
dp	y		
hs	nn		
DISPLAY			
sp	-250.1		
wp	6002.0		
vs	38		
sc	0		
wc	250		
hzmm	24.01		
is	88.64		
rfl	5130.9		
rfp	3631.3		
th	2		
ins	3.000		
nm	ph		



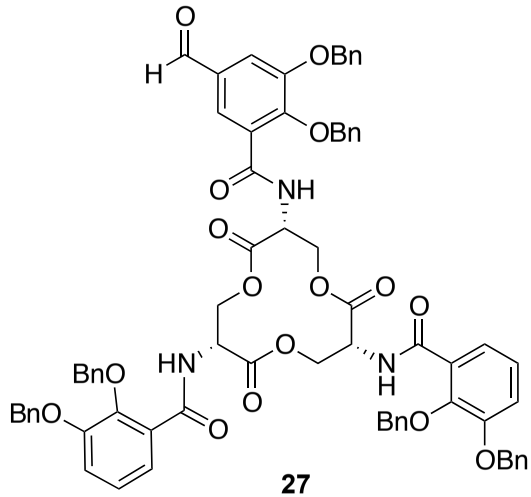
27



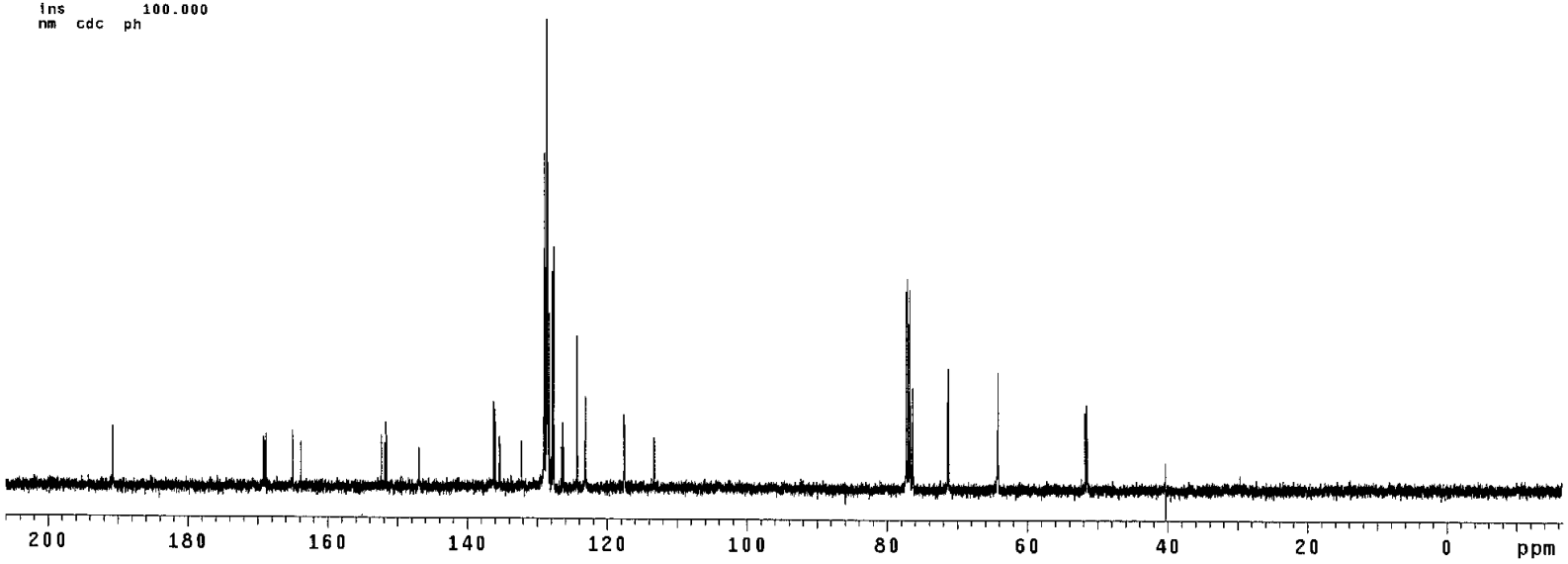
Bn_Ent_Aldehyde_1D_13C

exp3 s2pul

SAMPLE		DEC. & VT	
date	Aug 1 2012	dfrq	500.176
solvent	CDCl3	dn	H1
file	exp	dpwr	38
ACQUISITION		dof	0
sfrq	125.781	dm	yyy
tn	C13	dmm	w
at	1.170	dmf	15370
np	65536	dseq	
sw	28001.4	dres	1.0
fb	15000	homo	n
bs	16	temp	23.0
tpwr	57	PROCESSING	
pw	8.0	lb	1.00
d1	0	wtfile	
tof	0	proc	ft
nt	99999	fn	not used
ct	2064	math	f
alock	n		
gain	56	werr	
FLAGS		wexp	
il	n	wbs	
in	n	wnt	
dp	y		
hs	nn		
DISPLAY			
sp	-2089.6		
wp	28000.5		
vs	75		
sc	0		
wc	250		
hzmm	112.00		
is	500.00		
rfl	11774.6		
rfp	9684.2		
th	4		
ins	100.000		
nm cdc ph			



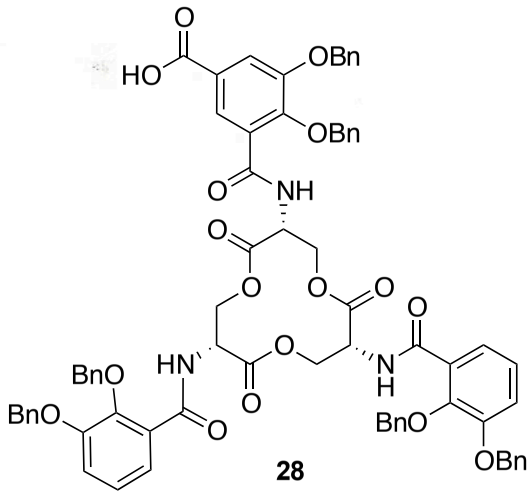
27



STANDARD PROTON PARAMETERS

expl s2pul

SAMPLE		DEC. & VT	
date	Aug 15 2012	dfrq	125.672
solvent	CDCl3	dn	C13
file	/data/export/~	dpwr	30
	home/nolan/NOtzhe/~	dof	0
	bullwinkle/081512D~	dm	nnn
	~BnEnt-COOH.fid	dmm	w
ACQUISITION		dmf	10000
sfrq	499.746	dseq	
tn	H1	dres	1.0
at	3.001	homo	n
np	63050	PROCESSING	
sw	10504.2	wtfile	
fb	not used	proc	ft
bs	4	fn	262144
tpwr	56	math	f
pw	8.6		
d1	2.000	werr	
tof	1519.5	wexp	
nt	16	wbs	
ct	16	wnt	wft
alock	n		
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
hs	nn		
DISPLAY			
sp	-561.9		
wp	7011.9		
vs	226		
sc	0		
wc	250		
hzmm	28.05		
is	158.28		
rfl	4866.4		
rfp	3628.1		
th	5		
ins	3.000		
ai	ph		



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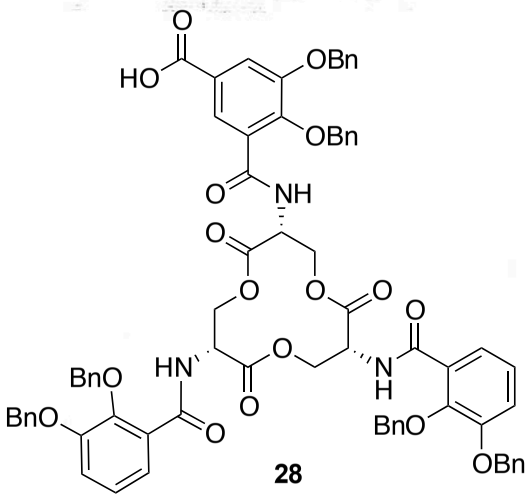
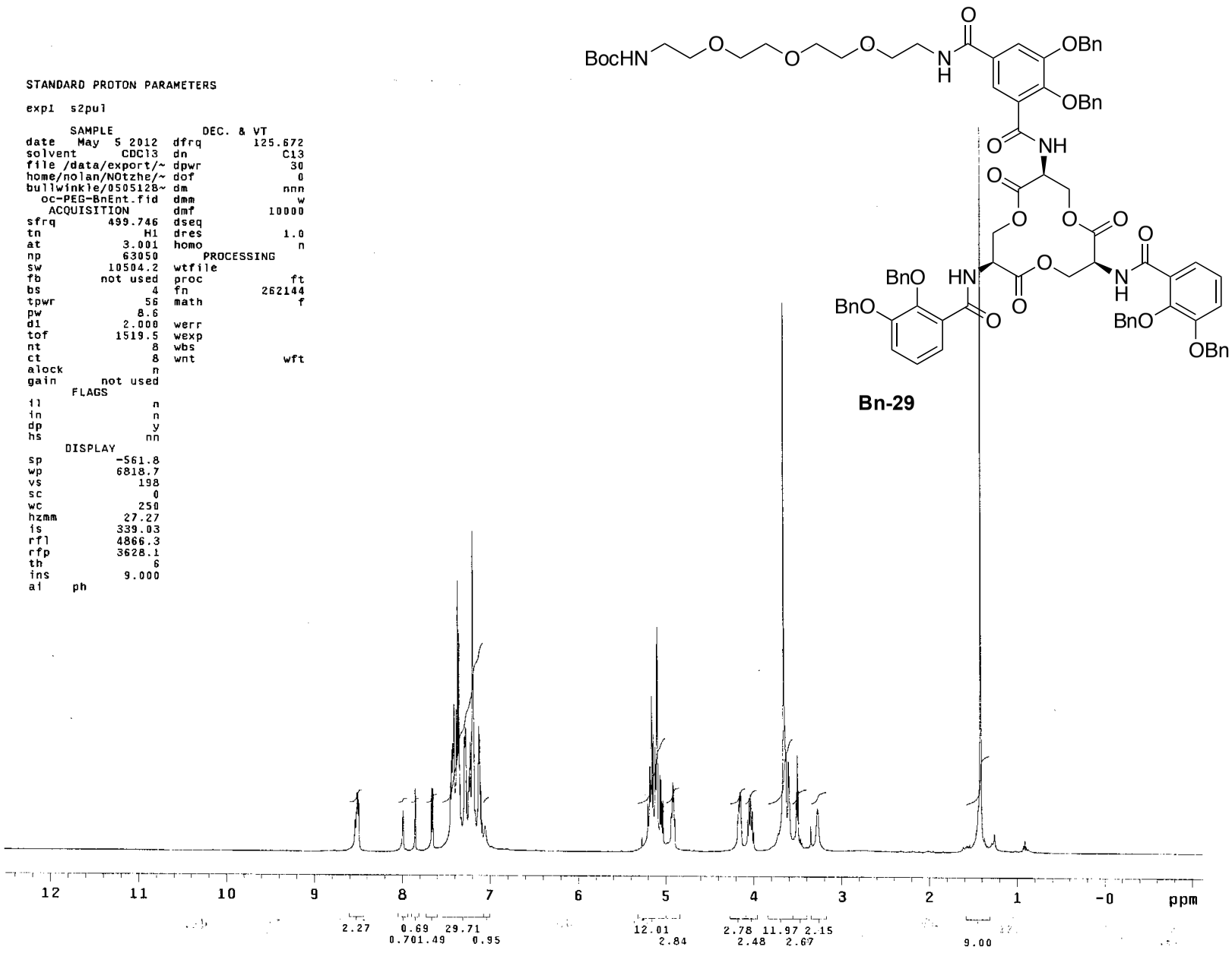
STANDARD CARBON PARAMETERS

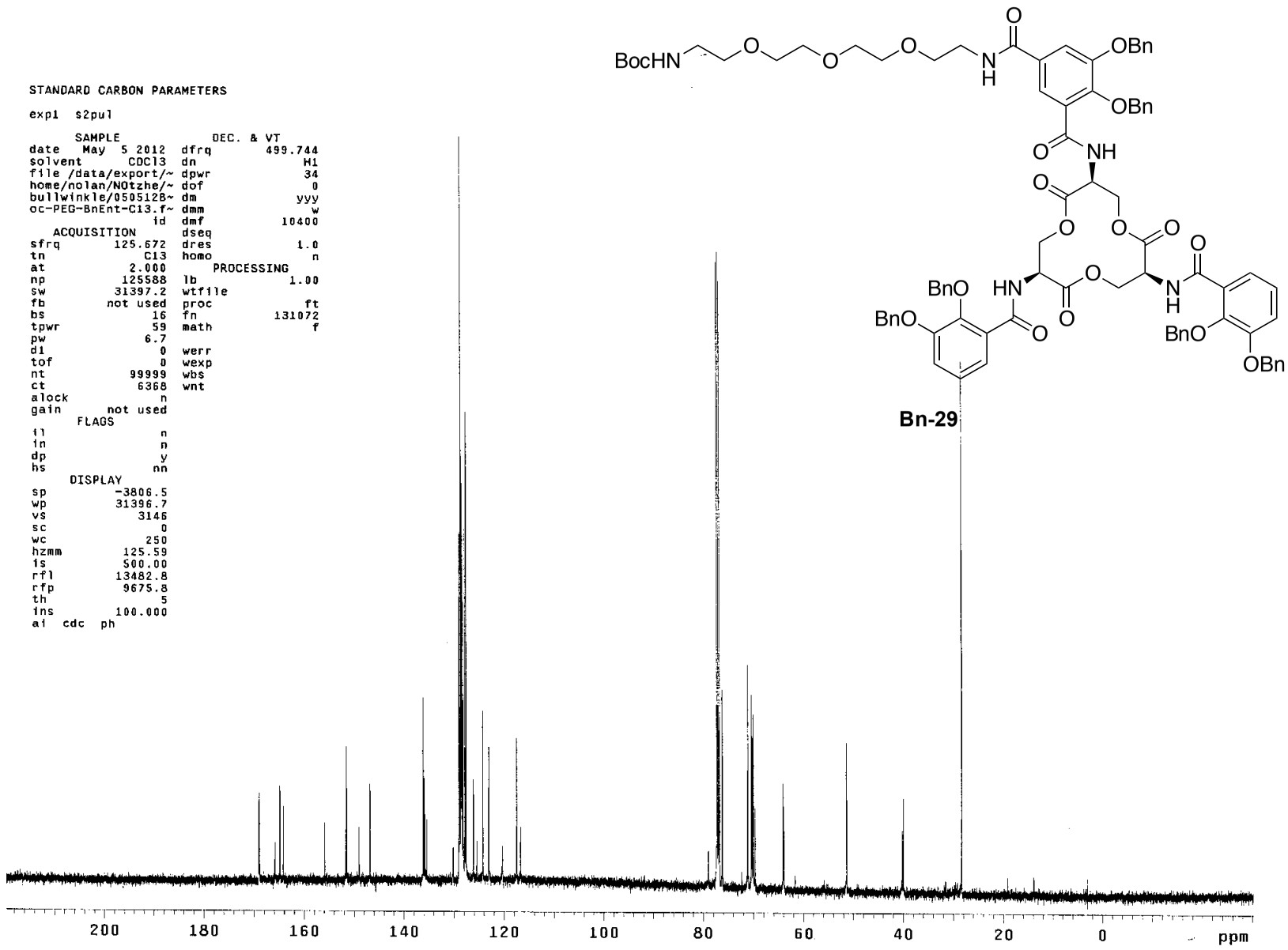
exp1 s2pul

SAMPLE		DEC. & VT	
date	Aug 15 2012	dfrq	499.744
solvent	CDCl3	dn	H1
file	/data/export/~	dpwr	34
home/nolan/NOtzhe/~		dof	0
bullwinkle/081512D~		dm	yyy
-BnEnt-COOH-C13.fi~		dmm	w
	d	dmf	10400
ACQUISITION		dseq	
sfrq	125.672	dres	1.0
tn	C13	homo	n
at	2.000	PROCESSING	
np	125588	lb	1.00
sw	31397.2	wtfile	
fb	not used	proc	ft
bs	16	fn	131072
tpwr	59	math	f
pw	6.7		
d1	0	werr	
tof	0	wexp	
nt	99999	wbs	
ct	19120	wnt	
alock	n		
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
hs	nn		
DISPLAY			
sp	-3796.0		
wp	31396.7		
vs	3706		
sc	0		
wc	250		
hzmm	125.59		
is	500.00		
rfl	13472.3		
rfp	9675.8		
th	1		
ins	100.000		
ai cdc ph			



28

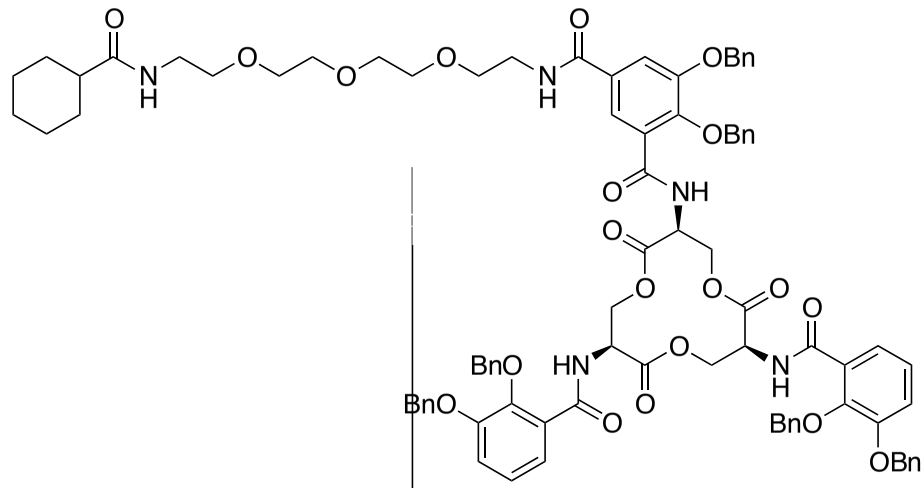

STANDARD PROTON PARAMETERS
expl s2pul
SAMPLE
date May 5 2012
solvent CDCl3
file /data/export/~
home/nolan/NOtzhe/~
bullwinkle/0505128~
oc-PEG-BnEnt.fid
ACQUISITION
sfrq 499.746
tn H1
at 3.001
np 63050
sw 10504.2
fb not used
bs 4
tpwr 56
pw 8.6
d1 2.000
tof 1519.5
nt 8
ct 8
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -561.8
wp 6818.7
vs 198
sc 0
wc 250
hzmm 27.27
is 339.03
rfl 4866.3
rfp 3628.1
th 6
ins 9.000
ai ph
DEC. & VT
dfrq 125.672
dn C13
dpwr 30
dof 0
dm nnn
dmm w
dmf 10000
dseq
dres 1.0
homo n
PROCESSING
wtfile
proc ft
fn 262144
math f
werr
wexp
wbs
wnt wft
BocHN
OBn
NH
BnO
HN
Bn-29
12
11
10
9
8
7
6
5
4
3
2
1
-0
ppm
2.27
0.69
29.71
0.701.49
0.95
12.01
2.84
2.78
11.97
2.15
2.48
2.67
9.00


STANDARD CARBON PARAMETERS
expl s2pul
SAMPLE
date May 5 2012
solvent CDCl3
file /data/export/~
home/nolan/NOtzhe/~
bullwinkle/050512B~
oc-PEG-BnEnt-C13.f~
id
ACQUISITION
sfrq 125.672
tn C13
at 2.000
np 125588
sw 31397.2
fb not used
bs 16
tpwr 59
pw 6.7
d1 0
tof 0
nt 99999
ct 6368
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -3806.5
wp 31396.7
vs 3146
sc 0
wc 250
hzmm 125.59
is 500.00
rfl 13482.8
rfp 9675.8
th 5
ins 100.000
ai cdc ph
DEC. & VT
dfrq 499.744
dn H1
dpwr 34
dof 0
dm yyy
dmm w
dmf 10400
dseq
dres 1.0
homo n
PROCESSING
lb 1.00
wtfile
proc ft
fn 131072
math f
werr
wexp
wbs
wnt
BocHN
OBn
NH
BnO
HN
Bn-29
200
180
160
140
120
100
80
60
40
20
0
ppm

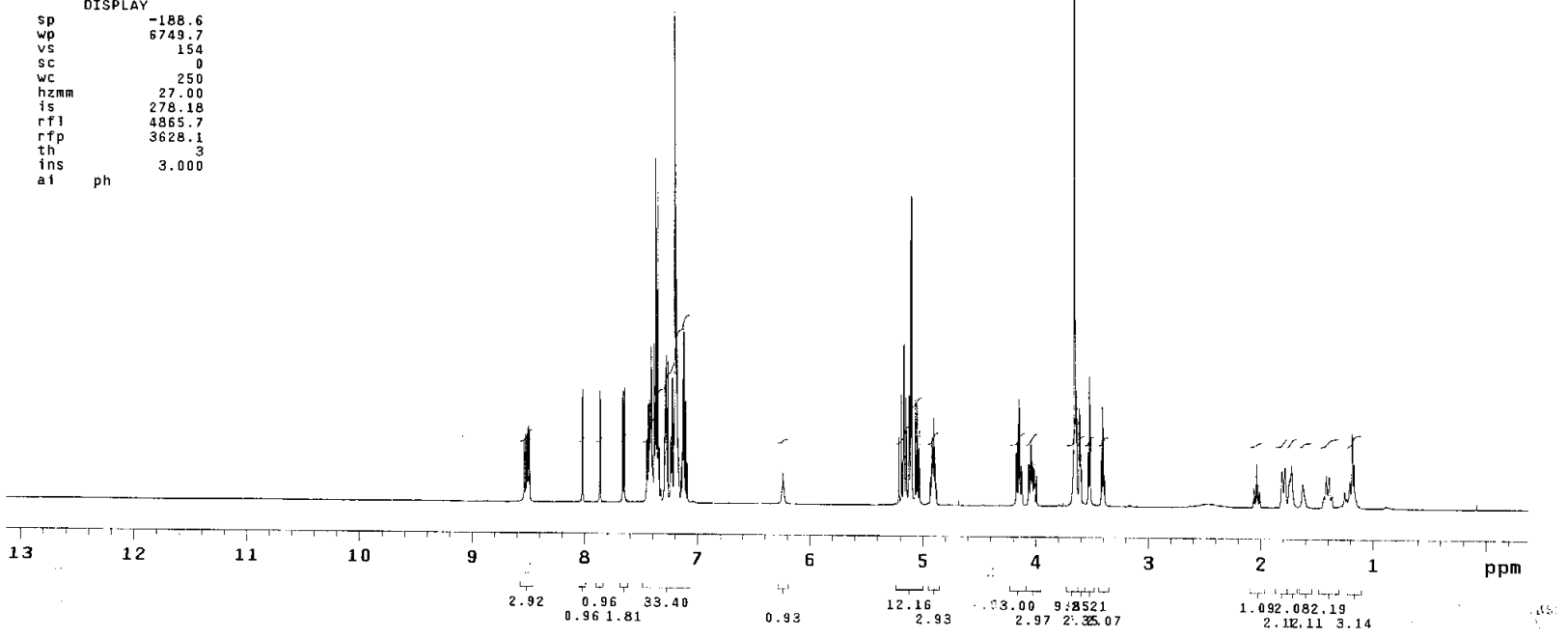
STANDARD PROTON PARAMETERS

expl s2pul

SAMPLE		DEC. & VT	
date	May 17 2012	dfrq	125.672
solvent	CDCl3	dn	C13
file	/data/export/~	dpwr	30
	home/nolan/NOtzhe/~	dof	0
	bullwinkle/051712c~	dm	nnn
	cyclohex-PEG3-BnEnt~	dmm	w
	.fid	dmf	10000
ACQUISITION		dseq	
sfrq	499.746	dres	1.0
tn	H1	homo	n
at	3.001	PROCESSING	
np	63050	wtfile	
sw	10504.2	proc	ft
fb	not used	fn	262144
bs	4	math	f
tpwr	56	werr	
pw	8.6	wexp	
dl	2.000	wbs	
tof	1519.5	wnt	wft
nt	8		
ct	8		
alock	n		
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
hs	nn		
DISPLAY			
sp	-188.6		
wp	6749.7		
vs	154		
sc	0		
wc	250		
hzmm	27.00		
is	278.18		
rfl	4865.7		
rfp	3628.1		
th	3		
ins	3.000		
ai	ph		



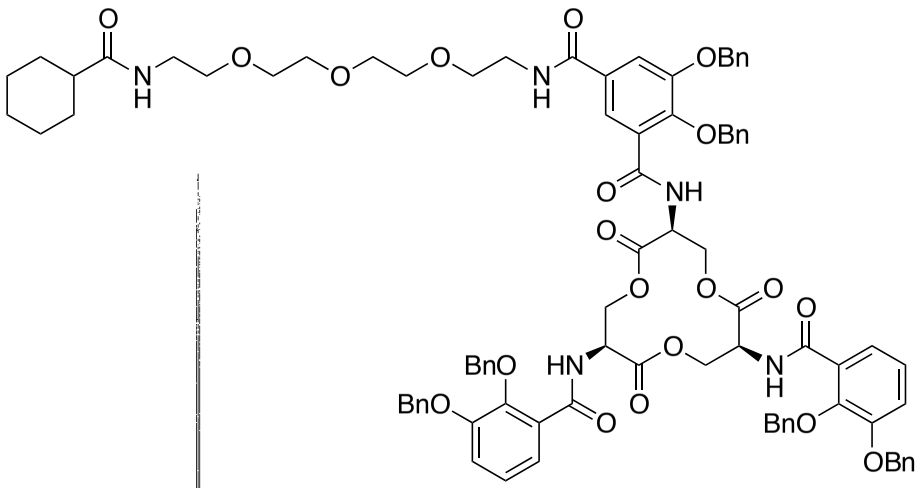
Bn-30



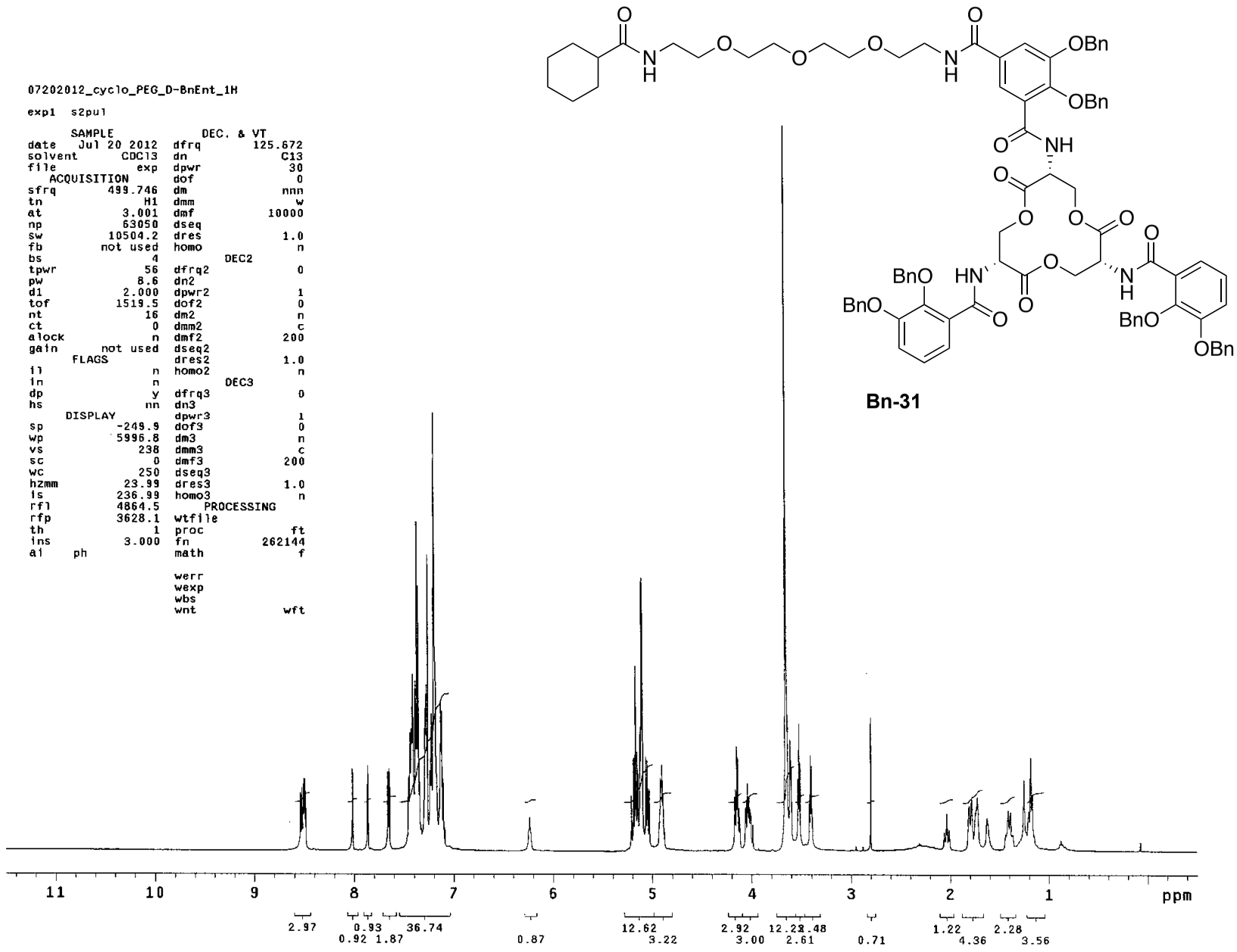
STANDARD CARBON PARAMETERS

expl s2pul

SAMPLE		DEC. & VT	
date	May 17 2012	dfrq	499.744
solvent	CDCl3	dn	H1
file	/data/export/~	dpwr	34
	home/nolan/NOtzhe/~	dof	0
	bullwinkle/051712c~	dm	yyy
	yclohex-PEG3-BnEnt~	dmm	w
	-C13.fid	dmf	10400
ACQUISITION		dseq	
sfrq	125.672	dres	1.0
tn	C13	homo	n
at	2.000	PROCESSING	
np	125588	lb	1.00
sw	31397.2	wtfile	
fb	not used	proc	ft
bs	16	fn	131072
tpwr	59	math	f
pw	6.7		
d1	0	werr	
tof	0	wexp	
nt	99999	wbs	
ct	5728	wnt	
alock	n		
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
hs	nn		
DISPLAY			
sp	-3802.2		
wp	31396.7		
vs	2822		
sc	0		
wc	250		
hzmm	3.14		
is	500.00		
rfl	13478.5		
rfp	9675.8		
th	7		
ins	100.000		
ai cdc ph			



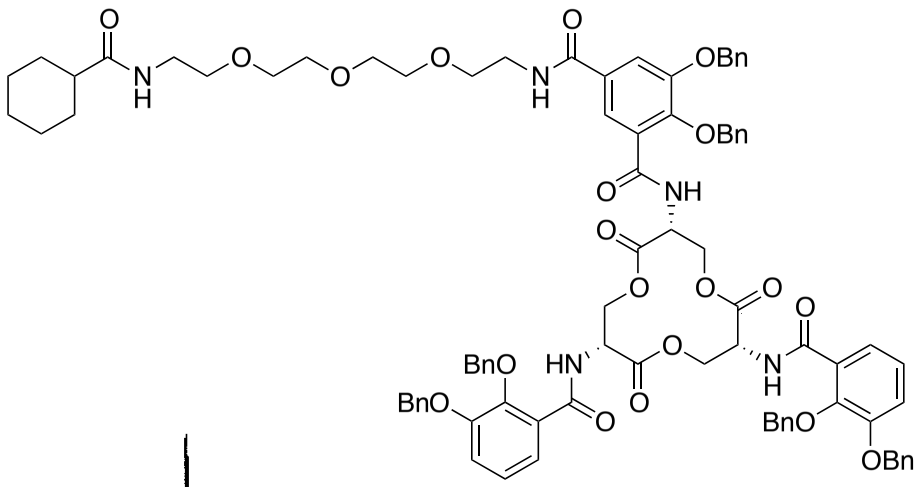
Bn-30


07202012_cyclo_PEG_D-BnEnt_1H
exp1 s2pul
SAMPLE
date Jul 20 2012
solvent CDCl3
file exp
ACQUISITION
sfrq 499.746
tn H1
at 3.001
np 63050
sw 10504.2
fb not used
bs 4
tpwr 56
pw 8.6
d1 2.000
tof 1519.5
nt 16
ct 0
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -249.9
wp 5996.8
vs 238
sc 0
wc 250
hzmm 23.99
is 236.99
rfl 4864.5
rfp 3628.1
th 1
ins 3.000
ai ph
DEC. & VT
dfrq 125.672
dn C13
dpwr 30
dof 0
dm nnn
dmm w
dmf 10000
dseq
dres 1.0
homo n
DEC2
dfrq2 0
dn2
dpwr2 1
dof2 0
dm2 n
dmm2 c
dmf2 200
dseq2
dres2 1.0
homo2 n
DEC3
dfrq3 0
dn3
dpwr3 1
dof3 0
dm3 n
dmm3 c
dmf3 200
dseq3
dres3 1.0
homo3 n
PROCESSING
wtfile
proc ft
fn 262144
math f
werr
wexp
wbs
wnt wft
OBn
OBn
NH
BnO
HN
BnO
BnO
OBn
Bn-31
11
10
9
8
7
6
5
4
3
2
1
ppm
2.97
0.92
0.93
1.87
36.74
0.87
12.62
3.22
2.92
3.00
12.23
2.61
3.48
0.71
1.22
4.36
2.28
3.56

07202012_cyclo_PEG_D-BnEnt_13C

exp3 s2pul

SAMPLE		DEC. & VT	
date	Jul 20 2012	dfrq	499.744
solvent	CDCl3	dn	H1
file	exp	dpwr	34
ACQUISITION		dof	0
sfrq	125.672	dm	yyy
tn	C13	dmm	w
at	2.000	dmf	10400
np	125588	dseq	
sw	31397.2	dres	1.0
fb	not used	homo	n
bs	16	DEC2	
tpwr	59	dfrq2	0
pw	6.7	dn2	
d1	0	dpwr2	1
tof	0	dof2	0
nt	99999	dm2	n
ct	0	dmm2	c
alock	n	dmf2	10000
gain	not used	dseq2	
FLAGS		dres2	1.0
il	n	homo2	n
in	n	DEC3	
dp	y	dfrq3	0
hs	nn	dn3	
DISPLAY		dpwr3	1
sp	-3797.4	dof3	0
wp	31396.7	dm3	n
vs	2683	dmm3	c
sc	0	dmf3	10000
wc	250	dseq3	
hzmm	156.98	dres3	1.0
is	500.00	homo3	n
rfl	13473.7	PROCESSING	
rfp	9675.8	lb	1.00
th	4	wtfile	
ins	100.000	proc	ft
ai cdc ph		fn	131072
		math	f
		werr	
		wexp	
		wbs	
		wnt	



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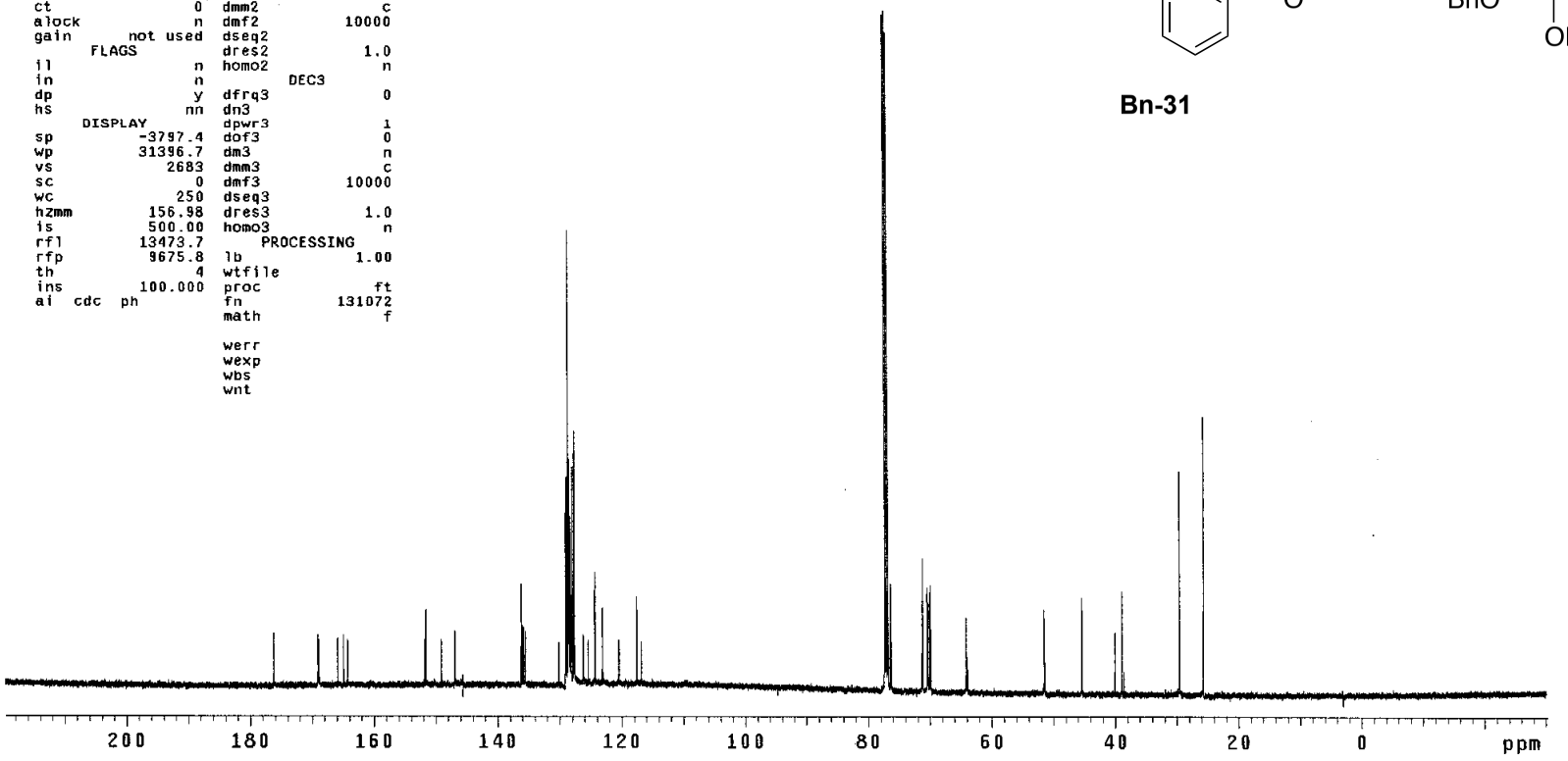
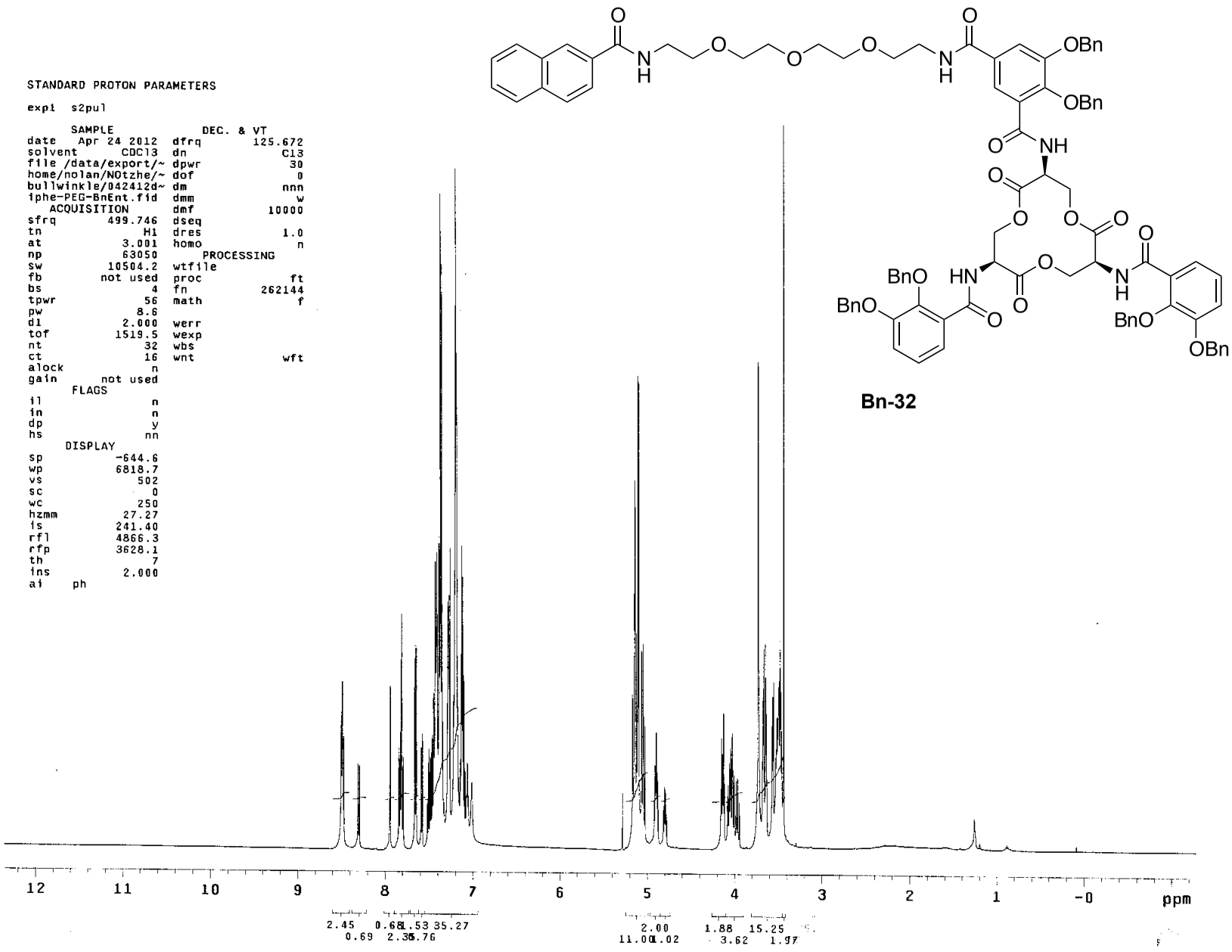
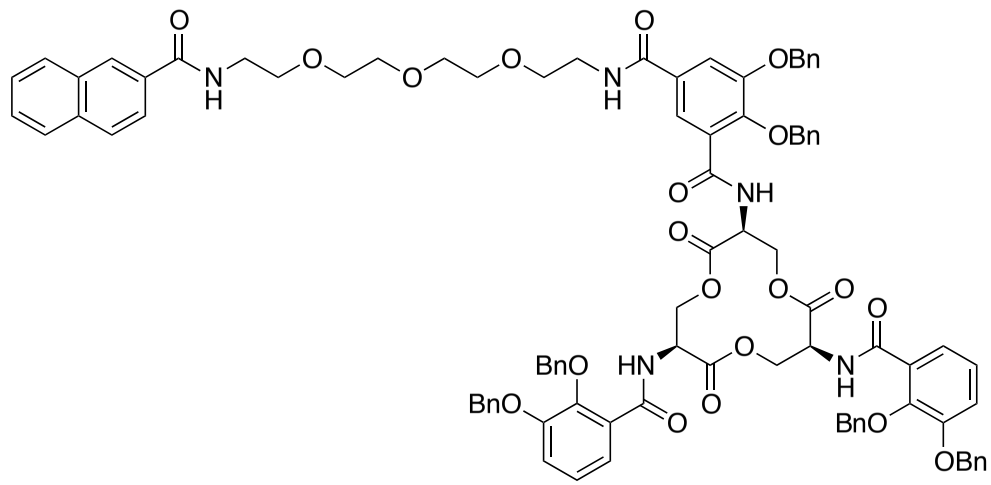



STANDARD PROTON PARAMETERS
expt s2pul
SAMPLE
date Apr 24 2012
solvent CDCl3
file /data/export/~
home/nolan/NOtzhe/~
bullwinkle/042412d~
iphe-PEG-BnEnt.fid
ACQUISITION
sfrq 499.746
tn H1
at 3.001
np 63050
sw 10504.2
fb not used
bs 4
tpwr 56
pw 8.6
d1 2.000
tof 1519.5
nt 32
ct 16
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -644.6
wp 6818.7
vs 502
sc 0
wc 250
hzmm 27.27
is 241.40
rfl 4866.3
rfp 3628.1
th 7
ins 2.000
ai ph
DEC. & VT
dfrq 125.672
dn C13
dpwr 30
dof 0
dm nnn
dmm w
dmf 10000
dseq
dres 1.0
homo n
PROCESSING
wtfile
proc ft
fn 262144
math f
werr
wexp
wbs
wnt wft
OBn
OBn
NH
BnO
HN
BnO
NH
BnO
OBn
Bn-32
12
11
10
9
8
7
6
5
4
3
2
1
-0
ppm
2.45
0.68
1.53
35.27
0.69
2.35
6.76
2.00
11.00
1.02
1.88
15.25
3.62
1.97

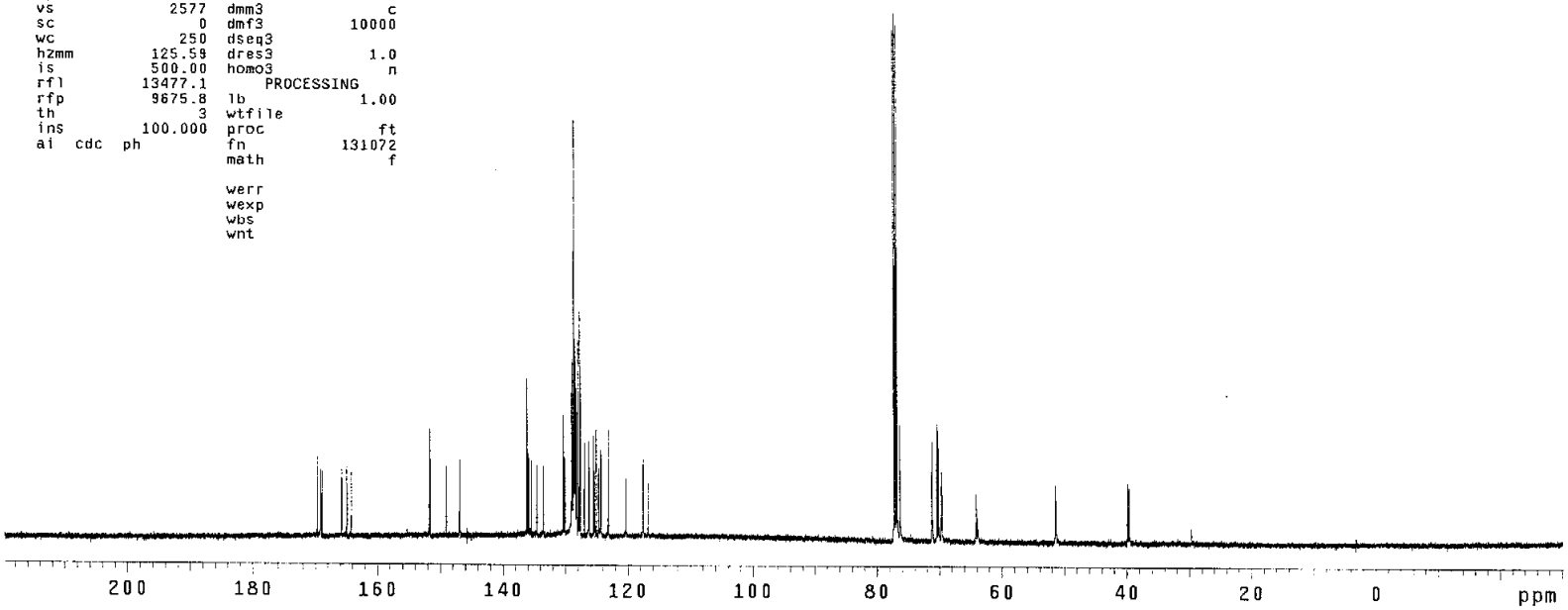
042912diphe-PEG-BnEnt_C13

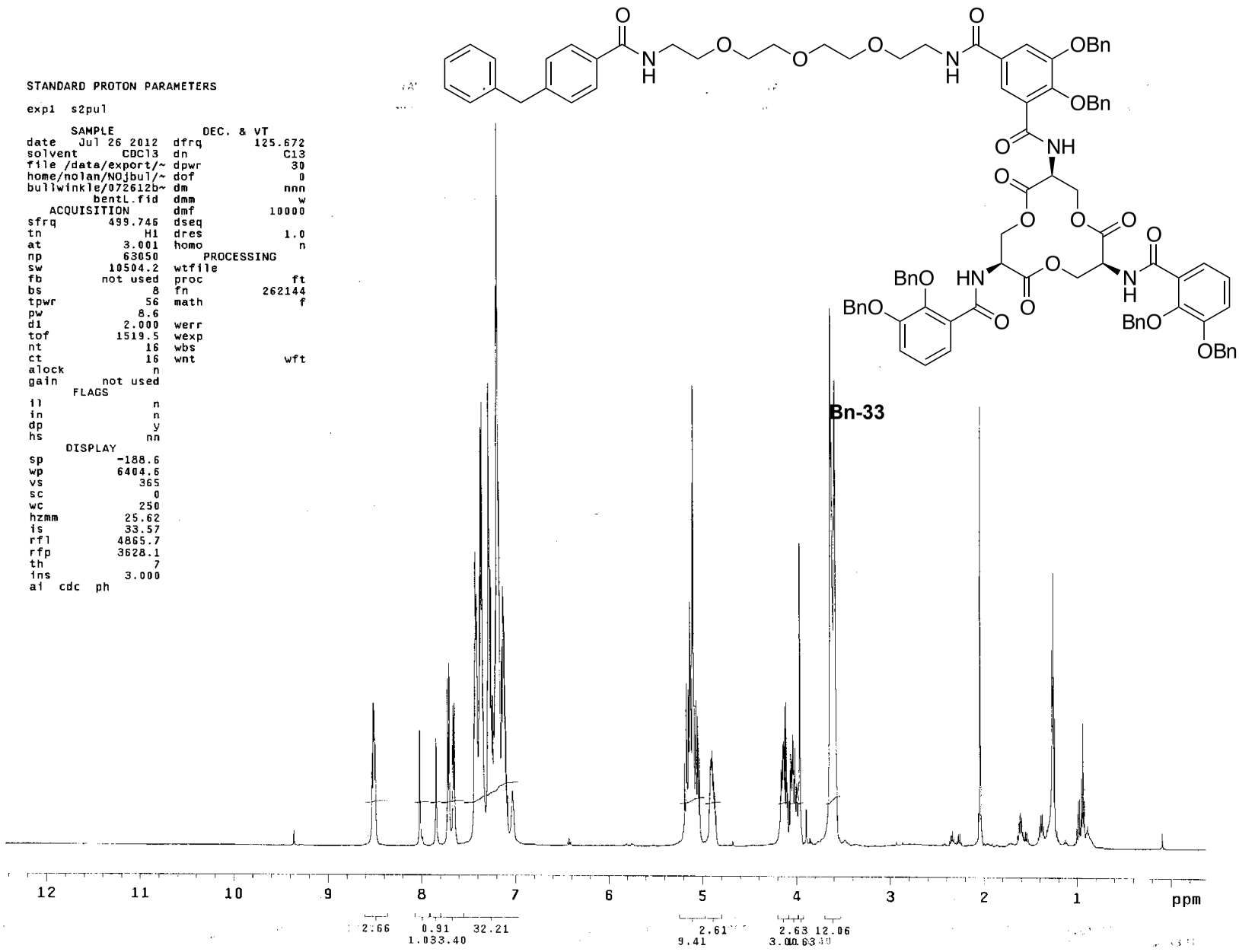
exp3 s2pul

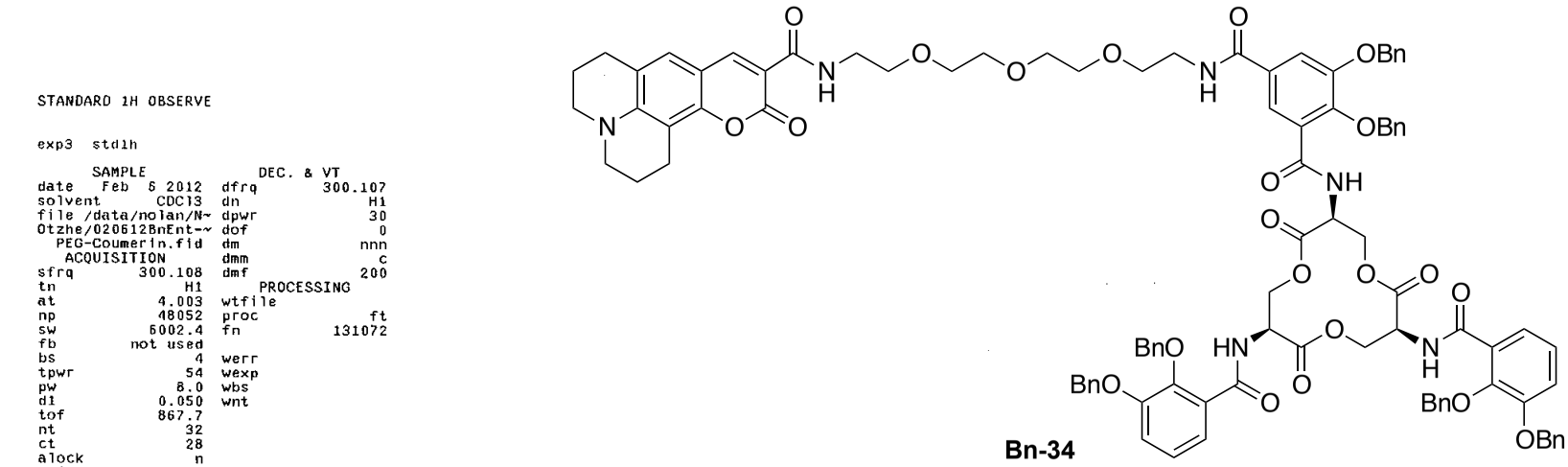
SAMPLE		DEC. & VT	
date	Apr 28 2012	dfrq	499.744
solvent	CDCl3	dn	H1
file	exp	dpwr	34
ACQUISITION		dof	0
sfrq	125.672	dm	yyy
tn	C13	dmm	w
at	2.000	dmf	10400
np	125588	dseq	
sw	31397.2	dres	1.0
fb	not used	homo	n
bs	16	DEC2	
tpwr	59	dfrq2	0
pw	6.7	dn2	
d1	0	dpwr2	1
tof	0	dof2	0
nt	1e+07	dm2	n
ct	21024	dmm2	c
alock	n	dmf2	10000
gain	not used	dseq2	
FLAGS		dres2	1.0
il	n	homo2	n
in	n	DEC3	
dp	y	dfrq3	0
hs	nn	dn3	
DISPLAY		dpwr3	1
sp	-3800.8	dof3	0
wp	31396.7	dm3	n
vs	2577	dmm3	c
sc	0	dmf3	10000
wc	250	dseq3	
hzmm	125.59	dres3	1.0
is	500.00	homo3	n
rfl	13477.1	PROCESSING	
rfp	9675.8	lb	1.00
th	3	wtfile	
ins	100.000	proc	ft
ai cdc ph		fn	131072
		math	f
		werr	
		wexp	
		wbs	
		wnt	



Bn-32




STANDARD PROTON PARAMETERS
expl s2pul
SAMPLE
date Jul 26 2012
solvent CDCl3
file /data/export/~
home/nolan/NOjbul/~
bullwinkle/072612b~
bentL.fid
ACQUISITION
sfrq 499.746
tn H1
at 3.001
np 63050
sw 10504.2
fb not used
bs 8
tpwr 56
pw 8.6
d1 2.000
tof 1519.5
nt 16
ct 16
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -188.6
wp 6404.6
vs 365
sc 0
wc 250
hzmm 25.62
is 33.57
rfl 4865.7
rfp 3628.1
th 7
ins 3.000
ai cdc ph
DEC. & VT
dfrq 125.672
dn C13
dpwr 30
dof 0
dm nnn
dmm w
dmf 10000
dseq
dres 1.0
homo n
PROCESSING
wtfile
proc ft
fn 262144
math f
werr
wexp
wbs
wnt wft
Bn-33
12
11
10
9
8
7
6
5
4
3
2
1
ppm
2.66
0.91
32.21
1.033.40
2.61
9.41
2.63
12.06

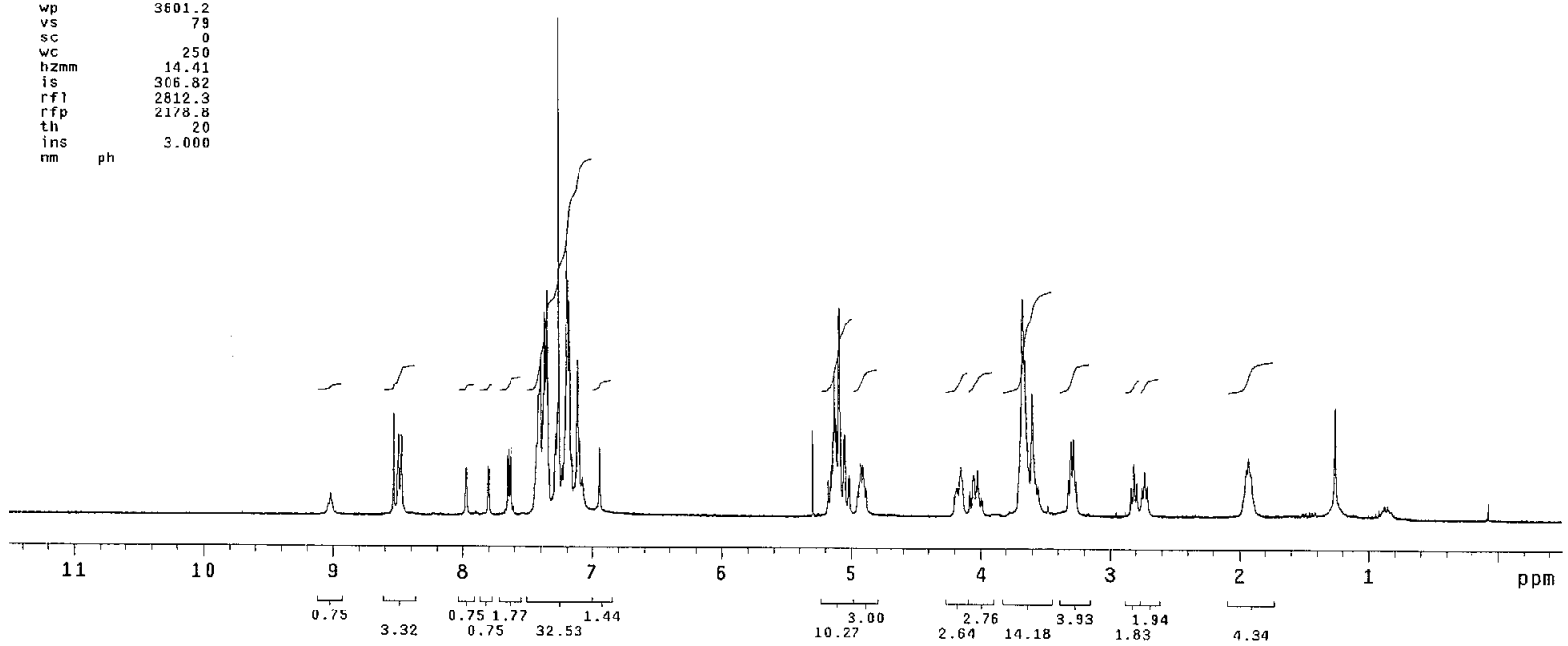


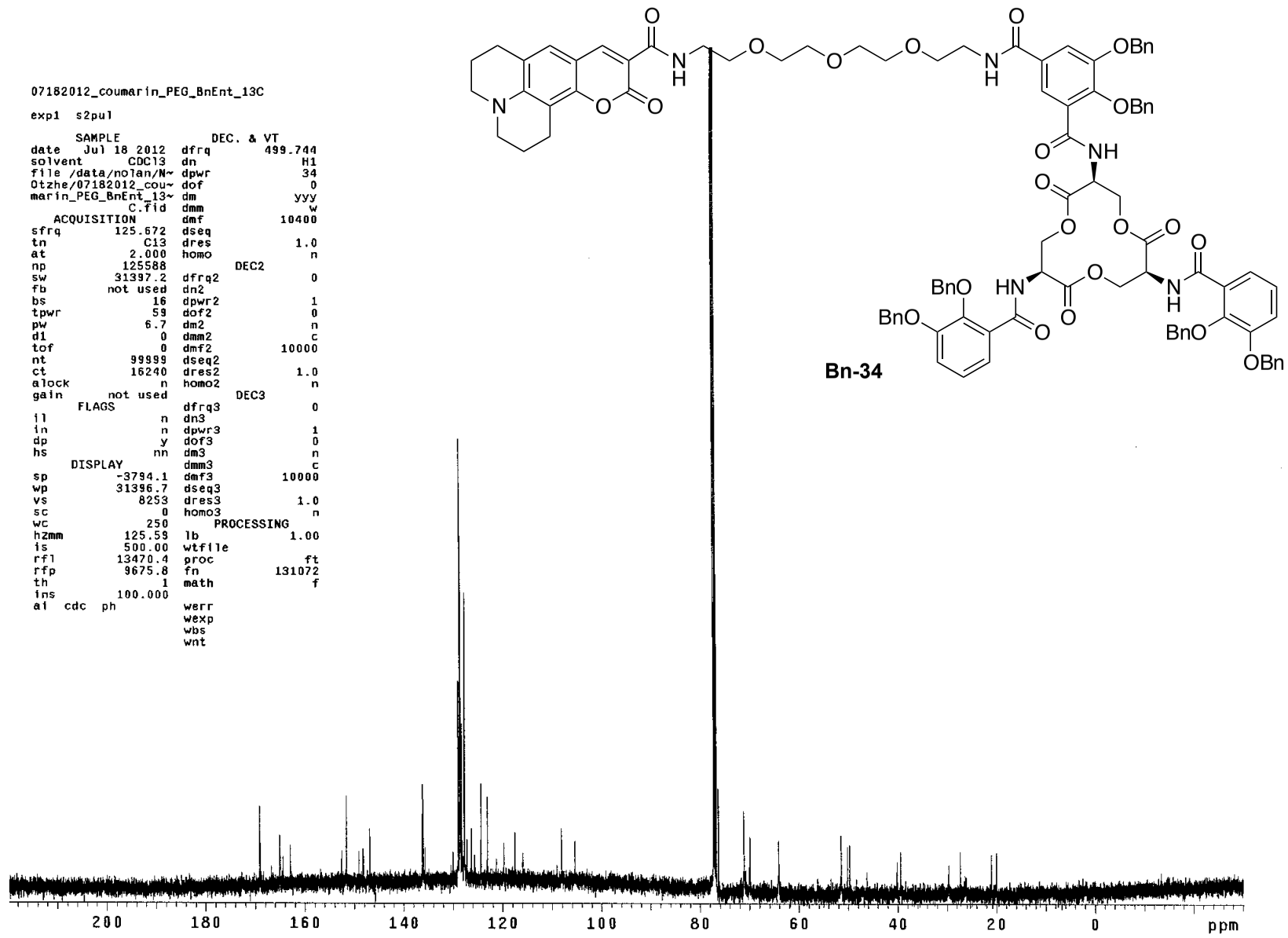
Bn-34

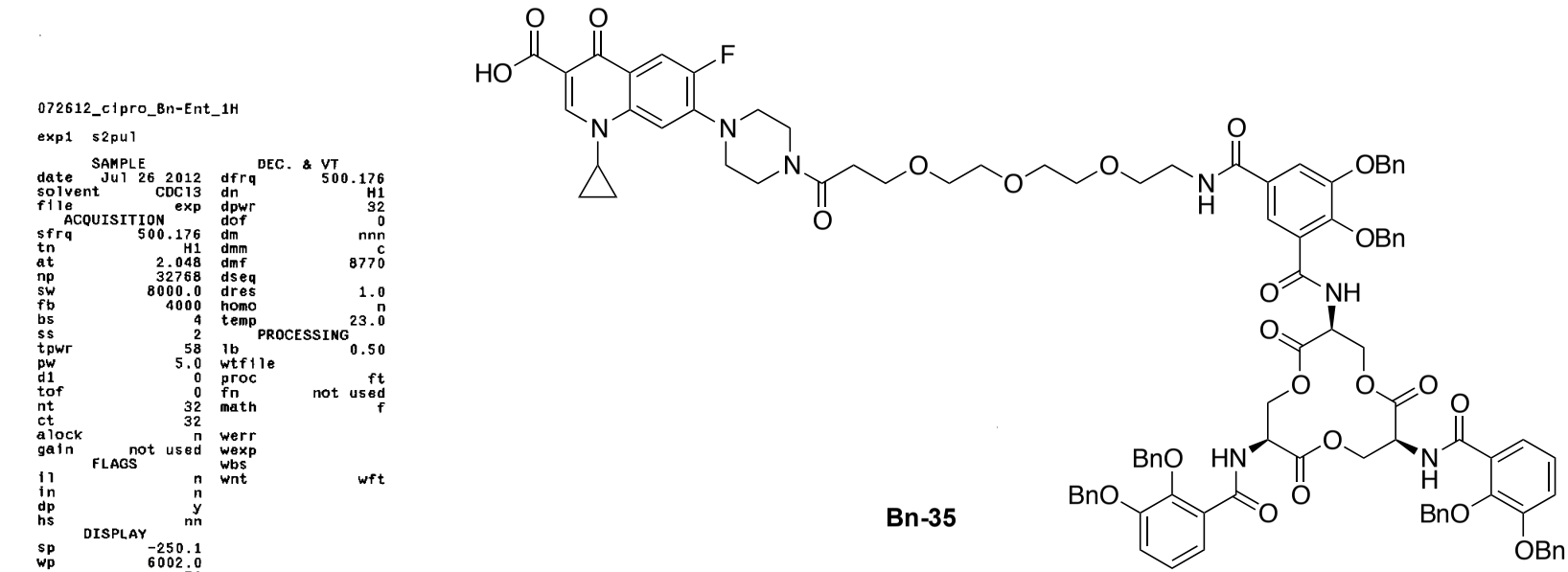
```
STANDARD 1H OBSERVE

exp3  std1h

      SAMPLE              DEC. & VT
date    Feb  6 2012  dfrq        300.107
solvent      CDCl3   dn              H1
file /data/nolan/N~  dpwr            30
Otzhe/020612BnEnt-~  dof              0
  PEG-Coumerin.fid   dm             nnn
   ACQUISITION       dmm              c
sfrq        300.108  dmf            200
tn               H1       PROCESSING
at             4.003  wtfile
np             48052  proc            ft
sw            6002.4  fn          131072
fb         not used
bs                4
tpwr             54
pw              8.0
d1            0.050
tof           867.7
nt               32
ct               28
alock             n
gain       not used
      FLAGS
ii                n
in                n
dp                y
     DISPLAY
sp           -150.1
wp           3601.2
vs               79
sc                0
wc              250
hzmm          14.41
is           306.82
rf1          2812.3
rfp          2178.8
th               20
ins           3.000
nm    ph
```




07182012_coumarin_PEG_BnEnt_13C
exp1 s2pul
SAMPLE
date Jul 18 2012
solvent CDCl3
file /data/nolan/N~
Otzhe/07182012_cou~
marin_PEG_BnEnt_13~
C.fid
ACQUISITION
sfrq 125.672
tn C13
at 2.000
np 125588
sw 31397.2
fb not used
bs 16
tpwr 59
pw 6.7
d1 0
tof 0
nt 99999
ct 16240
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -3794.1
wp 31396.7
vs 8253
sc 0
wc 250
hzmm 125.59
is 500.00
rfl 13470.4
rfp 9675.8
th 1
ins 100.000
ai cdc ph
DEC. & VT
dfrq 499.744
dn H1
dpwr 34
dof 0
dm yyy
dmm w
dmf 10400
dseq
dres 1.0
homo n
DEC2
dfrq2 0
dn2
dpwr2 1
dof2 0
dm2 n
dmm2 c
dmf2 10000
dseq2
dres2 1.0
homo2 n
DEC3
dfrq3 0
dn3
dpwr3 1
dof3 0
dm3 n
dmm3 c
dmf3 10000
dseq3
dres3 1.0
homo3 n
PROCESSING
lb 1.00
wtfile
proc ft
fn 131072
math f
werr
wexp
wbs
wnt
Bn-34
200
180
160
140
120
100
80
60
40
20
0
ppm

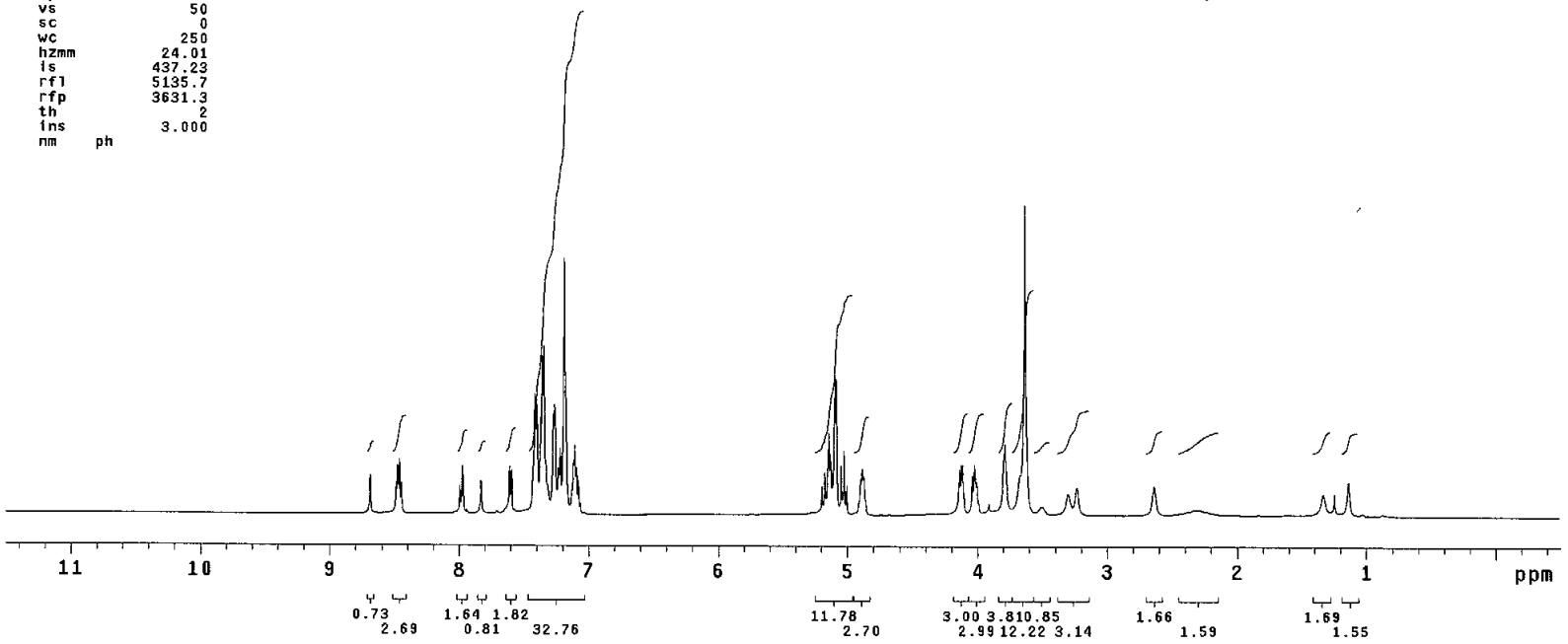


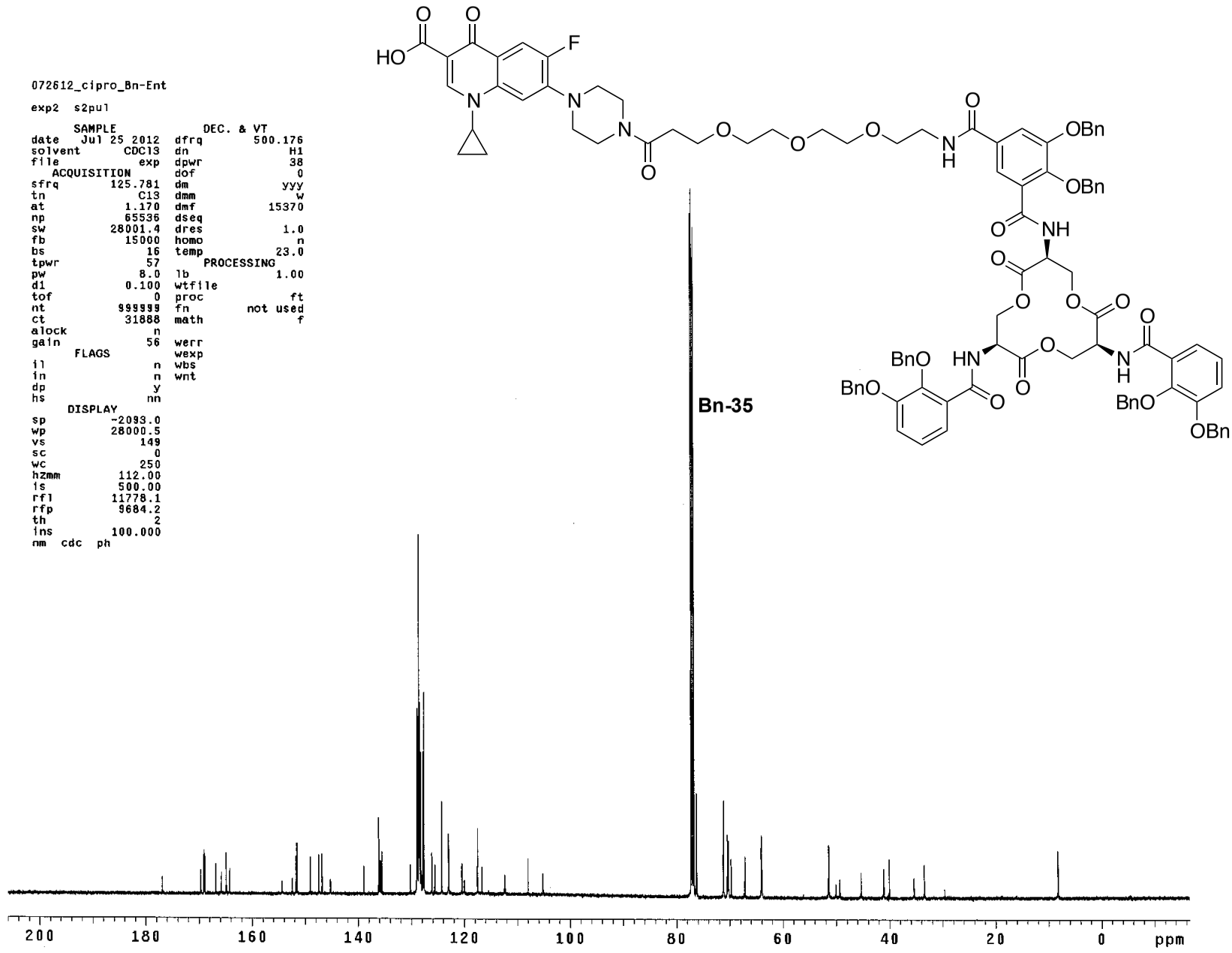
Bn-35

```
072612_cipro_Bn-Ent_1H

exp1  s2pul

      SAMPLE              DEC. & VT
date   Jul 26 2012  dfrq        500.176
solvent      CDCl3  dn               H1
file           exp  dpwr             32
   ACQUISITION      dof               0
sfrq       500.176  dm              nnn
tn              H1  dmm               c
at           2.048  dmf            8770
np           32768  dseq
sw          8000.0  dres            1.0
fb            4000  homo              n
bs               4  temp           23.0
ss               2      PROCESSING
tpwr            58  lb             0.50
pw             5.0  wtfile
d1               0  proc             ft
tof              0  fn         not used
nt              32  math              f
ct              32
alock            n  werr
gain     not used   wexp
      FLAGS         wbs
il               n  wnt             wft
in               n
dp               y
hs              nn
     DISPLAY
sp          -250.1
wp          6002.0
vs              50
sc               0
wc             250
hzmm         24.01
is          437.23
rfl         5135.7
rfp         3631.3
th               2
ins          3.000
nm    ph
```

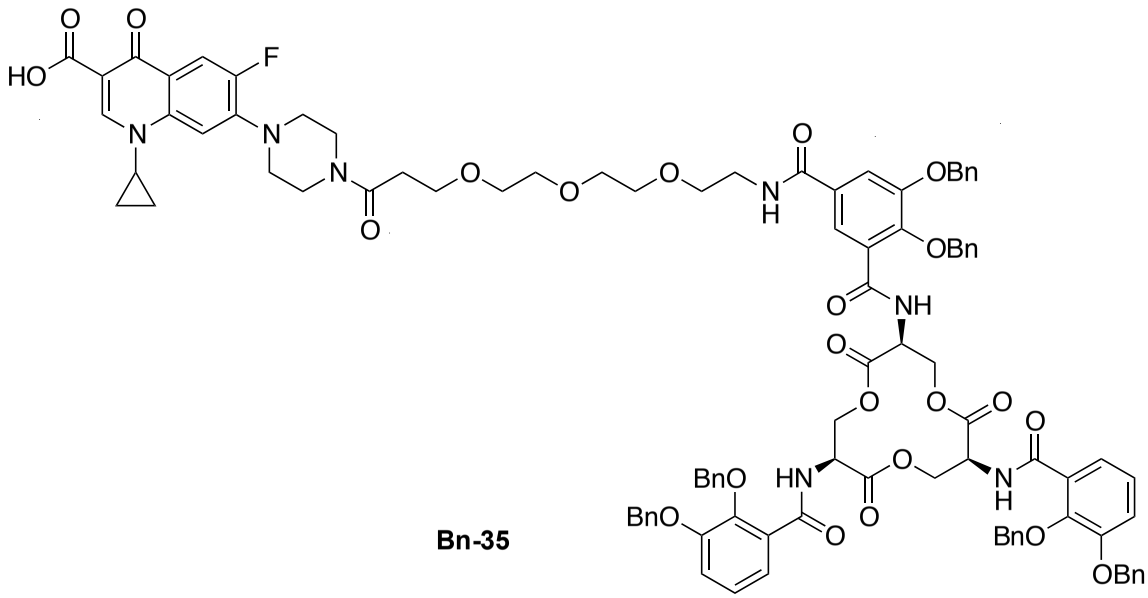



072612_cipro_Bn-Ent
exp2 s2pul
SAMPLE
date Jul 25 2012
solvent CDCl3
file exp
ACQUISITION
sfrq 125.781
tn C13
at 1.170
np 65536
sw 28001.4
fb 15000
bs 16
tpwr 57
pw 8.0
d1 0.100
tof 0
nt 999999
ct 31888
alock n
gain 56
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -2093.0
wp 28000.5
vs 149
sc 0
wc 250
hzmm 112.00
is 500.00
rfl 11778.1
rfp 9684.2
th 2
ins 100.000
nm cdc ph
DEC. & VT
dfrq 500.176
dn H1
dpwr 38
dof 0
dm yyy
dmm w
dmf 15370
dseq
dres 1.0
homo n
temp 23.0
PROCESSING
lb 1.00
wtfile
proc ft
fn not used
math f
werr
wexp
wbs
wnt
Bn-35
200
180
160
140
120
100
80
60
40
20
0
ppm

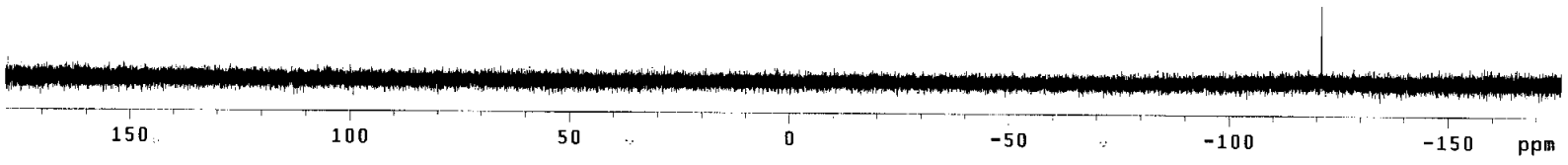
```
19F OBSERVE
STANDARD PARAMETERS

expl  s2pul

      SAMPLE              DEC. & VT
date    Jul 20 2012  dfrq        300.107
solvent      CDCl3   dn              H1
file /data/export/~  dpwr            30
home/nolan/NOtzhe/~  dof              0
mrhat/072012Cipro-~  dm             nnn
 PEG-BnEnt-F19.fid   dmm              c
   ACQUISITION       dmf            200
sfrq         282.382      PROCESSING
tn               F19   lb           0.30
at             0.300   wtfile
np             59906   proc           ft
sw        100000.0     fn         262144
fb             55000
bs                 4   werr
tpwr              56   wexp
pw              11.0   wbs
d1             4.000   wnt
tof         29637.2
nt                32
ct                32
alock              n
gain       not used
      FLAGS
il                 n
in                 n
dp                 y
     DISPLAY
sp          -49675.8
wp          99999.2
vs                12
sc                 0
wc               250
hzmm          400.00
is            500.00
rfl         49676.5
rfp                0
th                20
ins           100.000
nm    ph
```



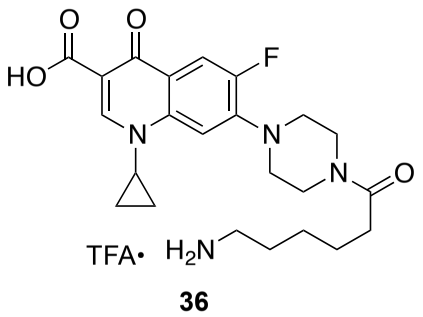
Bn-35



STANDARD 1H OBSERVE

```
expl  std1h

      SAMPLE              DEC. & VT
date   Aug  5 2011  dfrq        300.108
solvent      CD3OD  dn              H1
file /data/export/~ dpwr            30
home/nolan/NOjbul/~ dof              0
mrhat/080511ciproC~ dm             nnn
        5amine.fid  dmm              c
   ACQUISITION      dmf            200
sfrq       300.109       PROCESSING
tn              H1  wtfile
at           4.003  proc            ft
np           48052  fn          131072
sw          6002.4
fb        not used  werr
bs               4  wexp
tpwr            54  wbs
pw             8.0  wnt
d1           0.050
tof          867.7
nt              32
ct              28
alock            n
gain      not used
      FLAGS
il               n
in               n
dp               y
     DISPLAY
sp          -150.1
wp          3001.0
vs             151
sc               0
wc             250
hzmm         12.00
is          277.00
rfl         1625.3
rfp          993.4
th              20
ins          2.000
nm    ph
```



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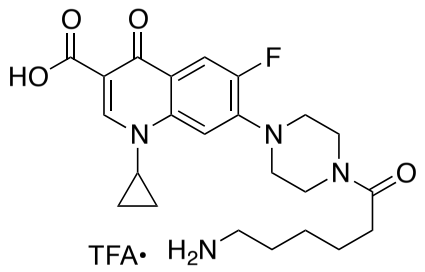
STANDARD CARBON PARAMETERS

expl s2pul

SAMPLE		DEC. & VT	
date	Oct 28 2011	dfrq	499.744
solvent	CDCl3	dn	H1
file	/data/export/~	dpwr	34
	home/nolan/NOtzhe/~	dof	0
	bullwinkle/102811C~	dm	yyy
	13_CiproC5_CCl3D_C~	dmm	w
	D3OD.fid	dmf	10400
ACQUISITION		dseq	
sfrq	125.672	dres	1.0
tn	C13	homo	n
at	2.000	PROCESSING	
np	125588	lb	1.00
sw	31397.2	wtfile	
fb	not used	proc	ft
bs	16	fn	131072
tpwr	59	math	f
pw	6.7		
d1	0	werr	
tof	0	wexp	
nt	9999	wbs	
ct	0	wnt	
alock	n		
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
hs	nn		
DISPLAY			
sp	-3812.3		
wp	31396.7		
vs	979		
sc	0		
wc	250		
hzmm	125.59		
is	500.00		
rfl	13488.6		
rfp	9675.8		
th	4		
ins	100.000		
ai cdc ph			



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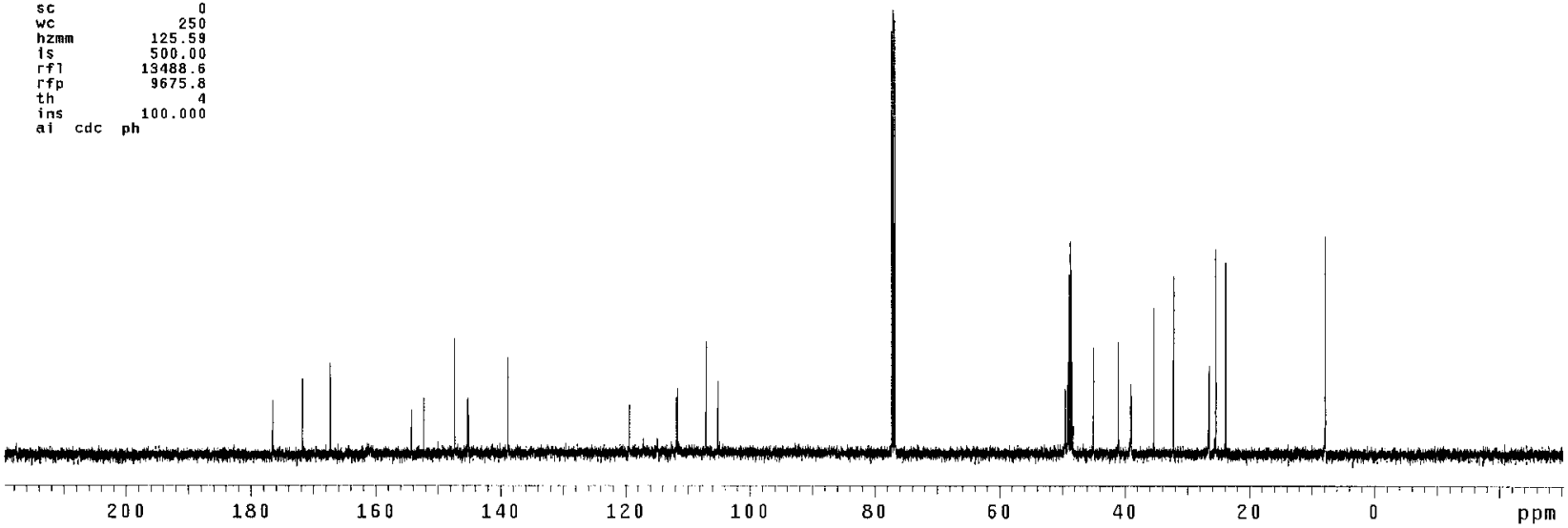
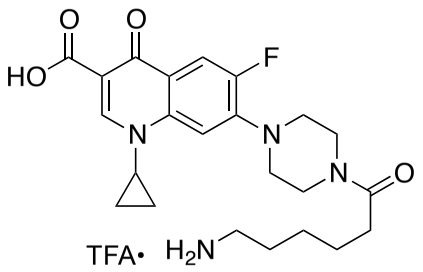


19F OBSERVE
STANDARD PARAMETERS

expl s2pul

SAMPLE		DEC. & VT	
date	Oct 28 2011	dfrq	300.107
solvent	CDCl3	dn	H1
file	/data/export/~	dpwr	30
home/nolan/NOtzhe/~		dof	0
mrhat/102811F19Clp~		dm	nnn
	roCS.fid	dmm	c
ACQUISITION		dmf	200
sfrq	282.382	PROCESSING	
tn	F19	lb	0.30
at	0.300	wtfile	
np	59906	proc	ft
sw	100000.0	fn	262144
fb	55000		
bs	4	werr	
tpwr	56	wexp	
pw	11.0	wbs	
d1	4.000	wnt	
tof	29637.2		
nt	64		
ct	64		
alock	n		
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
DISPLAY			
sp	-49635.3		
wp	99999.2		
vs	101		
sc	0		
wc	250		
hzmm	400.00		
is	2.5e+08		
rfl	49636.1		
rfp	0		
th	6		
ins	1.000		
nm	ph		



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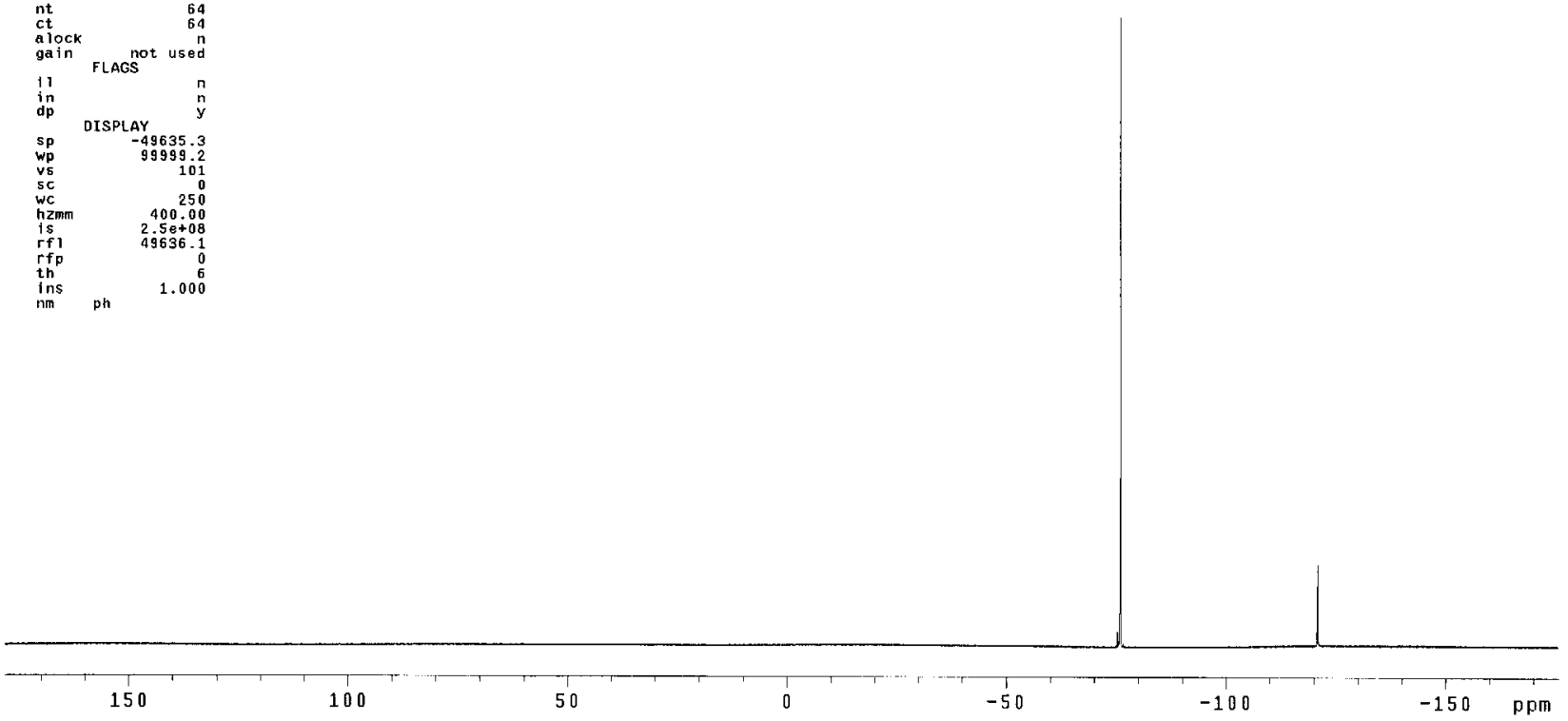
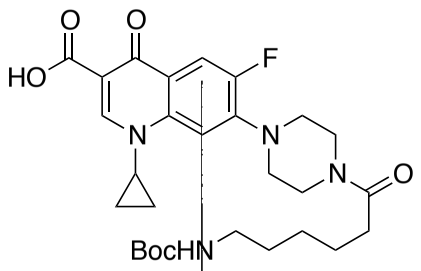


STANDARD 1H OBSERVE

exp1 stdih

SAMPLE		DEC. & VT	
date	Aug 1 2011	dfrq	300.107
solvent	CDCl3	dn	H1
file	/data/export/~	dpwr	30
home/nolan/NOtzhe/~		dof	0
mrhat/080111cipro-~		dm	nnn
stable-Boc.fid		dmm	c
ACQUISITION		dmf	200
sfrq	300.108	**PROCESSING**	
tn	H1	wtfile	
at	4.003	proc	ft
np	48052	fn	131072
sw	6002.4		
fb	not used	werr	
bs	4	wexp	
tpwr	54	wbs	
pw	8.0	wnt	
d1	0.050		
tof	867.7		
nt	16		
ct	16		
alock	n		
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
DISPLAY			
sp	-150.1		
wp	4801.7		
vs	151		
sc	0		
wc	250		
hzmm	19.21		
is	181.52		
rfl	2812.5		
rfp	2178.8		
th	20		
ins	2.000		
nm	ph		



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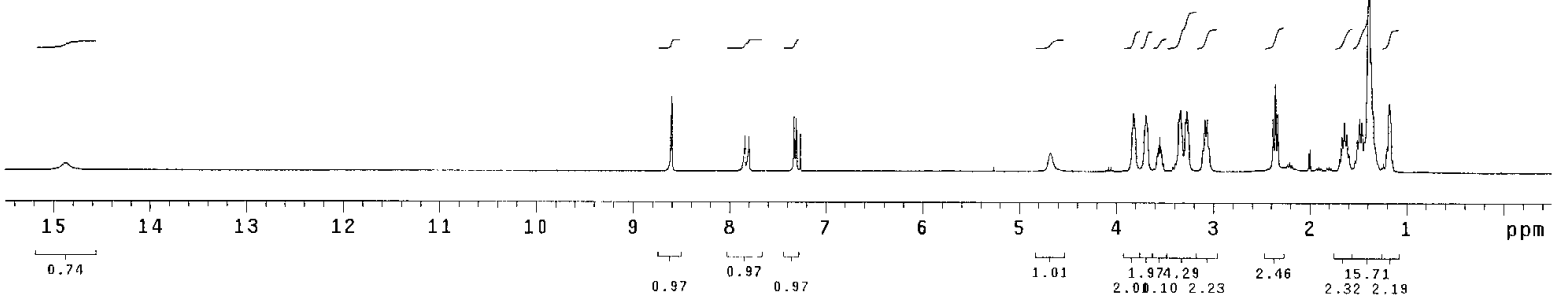
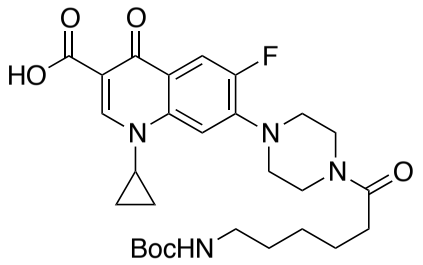


STANDARD CARBON PARAMETERS

exp1 s2pul

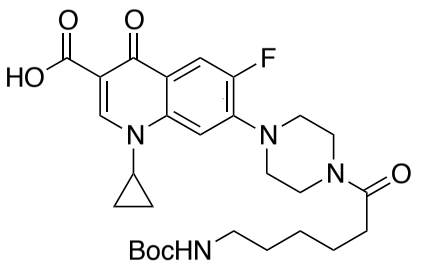
SAMPLE		DEC. & VT	
date	Oct 27 2011	dfrq	499.744
solvent	CDCl3	dn	H1
file	/data/export/~	dpwr	34
	home/nolan/NOtzhe/~	dof	0
	bullwinkle/102711C~	dm	yyy
	13CiproC5Boc.fid	dmm	w
ACQUISITION		dmf	10400
sfrq	125.672	dseq	
tn	C13	dres	1.0
at	2.000	homo	n
np	125588	PROCESSING	
sw	31397.2	lb	1.00
fb	not used	wtfile	
bs	16	proc	ft
tpwr	59	fn	131072
pw	6.7	math	f
d1	0		
tof	0	werr	
nt	9999	wexp	
ct	1216	wbs	
alock	n	wnt	
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
hs	nn		
DISPLAY			
sp	-3805.6		
wp	31396.7		
vs	1459		
sc	0		
wc	250		
hzmm	4.95		
is	500.00		
rfl	13481.9		
rfp	9675.8		
th	4		
ins	100.000		

ai cdc ph

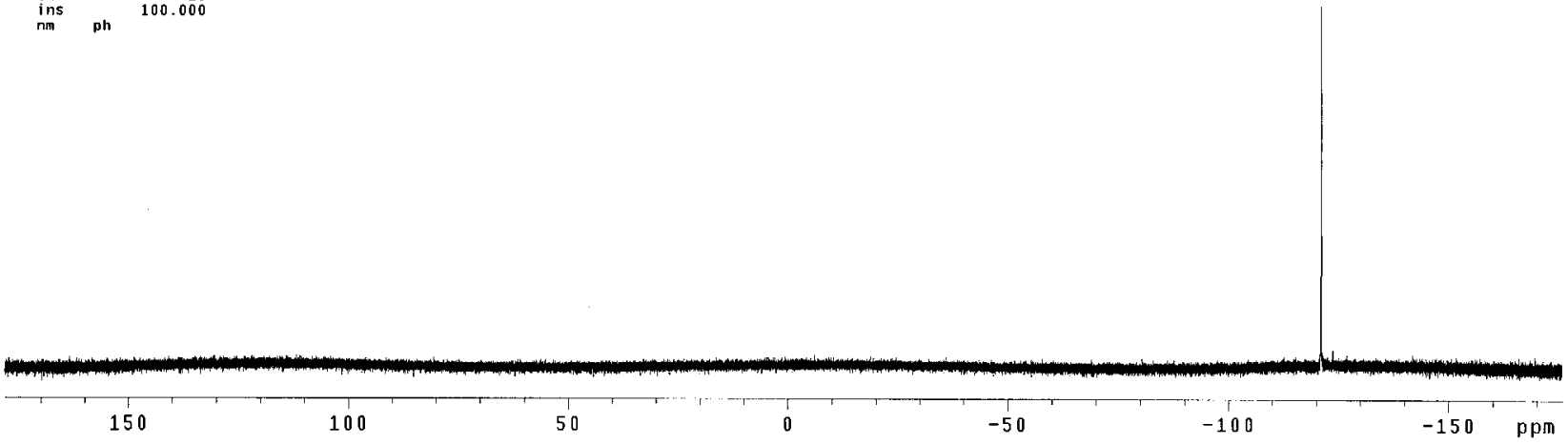


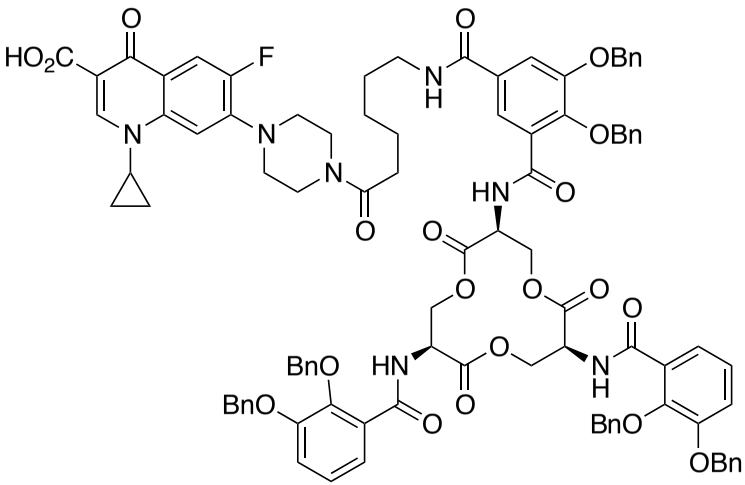
38

```
19F OBSERVE
STANDARD PARAMETERS

exp1  s2pul

      SAMPLE            DEC. & VT
date   Oct 27 2011  dfrq        300.107
solvent      CDCl3  dn               H1
file /data/export/~ dpwr             30
home/nolan/NOtzhe/~ dof               0
mrhat/102711F19cip~ dm              nnn
      roC5Boc.fid   dmm               c
   ACQUISITION      dmf             200
sfrq       282.382       PROCESSING
tn             F19   lb           0.30
at           0.300   wtfile
np           59906   proc            ft
sw        100000.0   fn          262144
fb           55000
bs               4   werr
tpwr            56   wexp
pw            11.0   wbs
d1           4.000   wnt
tof        29637.2
nt              16
ct              16
alock            n
gain      not used
      FLAGS
il               n
in               n
dp               y
     DISPLAY
sp        -49668.9
wp         99999.2
vs              58
sc               0
wc             250
hzmm         400.00
is           500.00
rfl         49669.6
rfp              0
th              20
ins         100.000
nm    ph
```



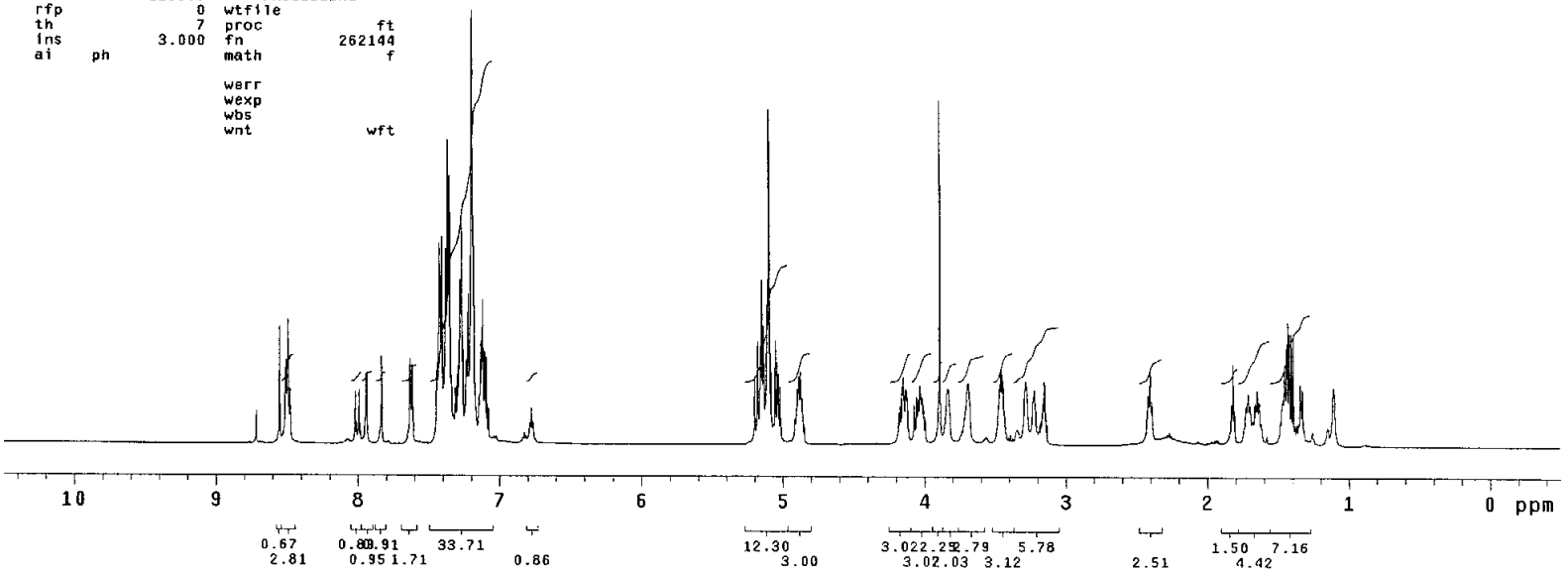
38

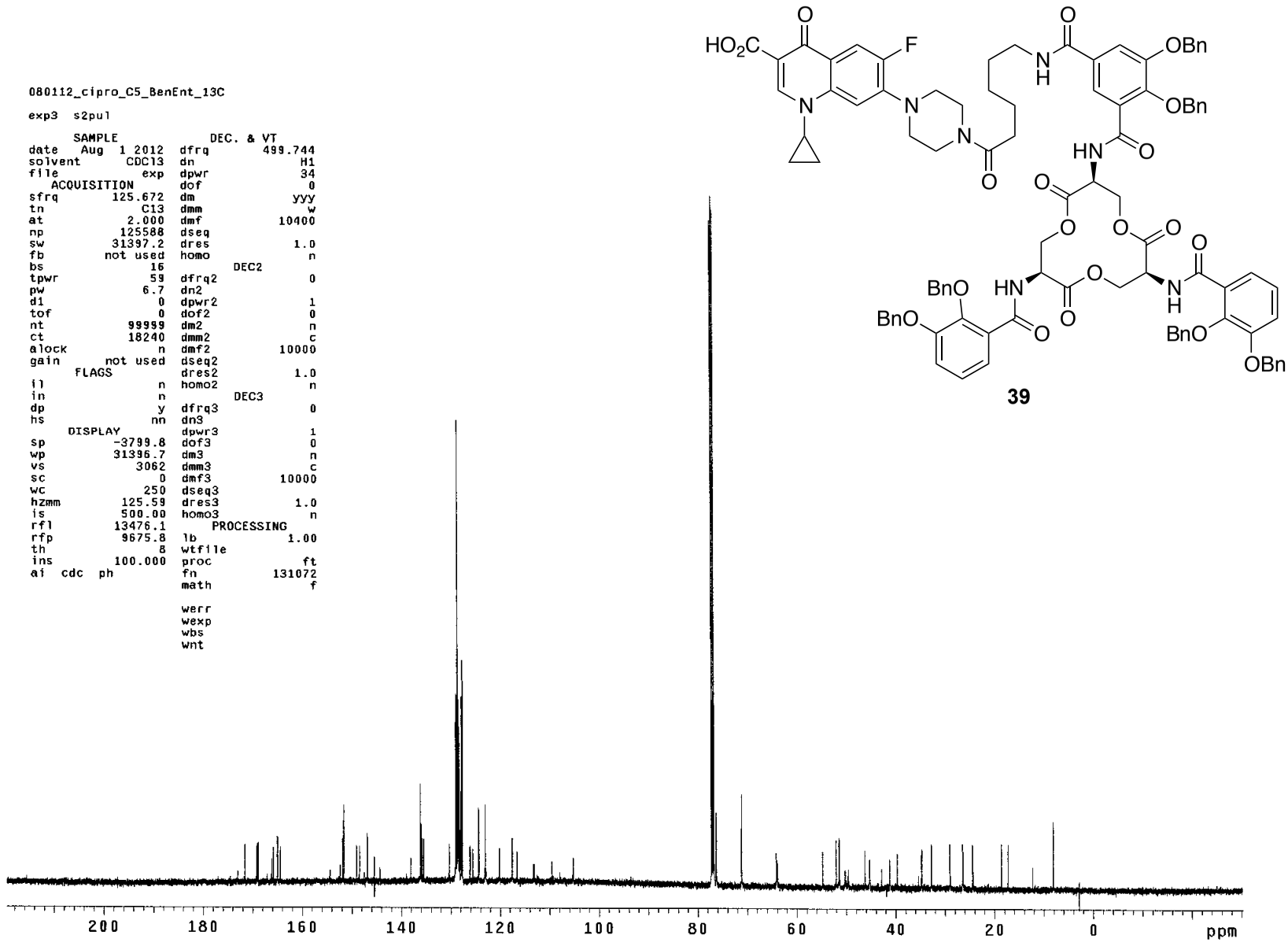




39

```
080112_cipro_C5_BenEnt_1H

exp1  s2pul

      SAMPLE           DEC. & VT
date    Aug  1 2012  dfrq        125.672
solvent      CDCl3   dn             C13
file           exp   dpwr            30
   ACQUISITION       dof             0
sfrq       499.746   dm            nnn
tn              H1   dmm             w
at           3.001   dmf         10000
np           63050   dseq
sw         10504.2   dres          1.0
fb       not used    homo            n
bs              4              DEC2
tpwr           56   dfrq2           0
pw            8.6   dn2
d1          2.000   dpwr2           1
tof        1519.5   dof2            0
nt             16   dm2             n
ct             16   dmm2            c
alock           n   dmf2          200
gain     not used   dseq2
      FLAGS         dres2         1.0
il              n   homo2           n
in              n              DEC3
dp              y   dfrq3           0
hs             nn   dn3
      DISPLAY       dpwr3           1
sp         -249.9   dof3            0
wp         5497.1   dm3             n
vs            175   dmm3            c
sc              0   dmf3          200
wc            250   dseq3
hzmm        21.99   dres3         1.0
is         504.45   homo3           n
rf1       1233.8         PROCESSING
rfp             0   wtfile
th              7   proc            ft
ins         3.000   fn          262144
ai     ph           math             f

                   werr
                   wexp
                   wbs
                   wnt            wft
```

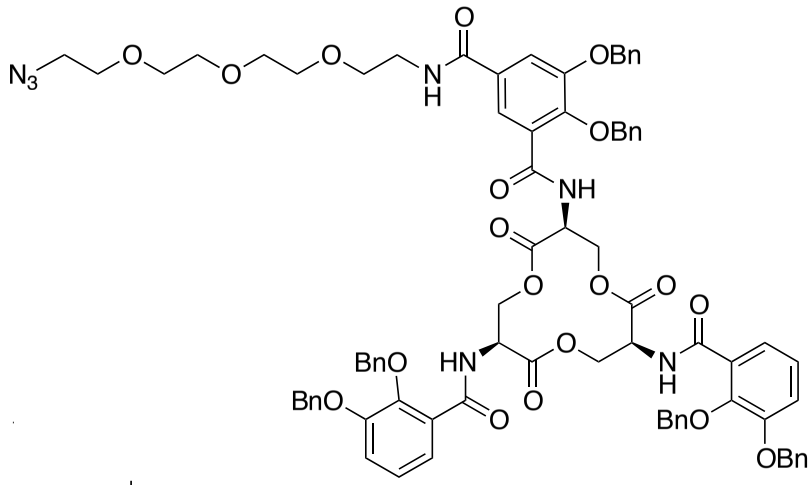



080112_cipro_C5_BenEnt_13C
exp3 s2pul
SAMPLE
date Aug 1 2012
solvent CDCl3
file exp
ACQUISITION
sfrq 125.672
tn C13
at 2.000
np 125588
sw 31397.2
fb not used
bs 16
tpwr 59
pw 6.7
d1 0
tof 0
nt 99999
ct 18240
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -3799.8
wp 31396.7
vs 3062
sc 0
wc 250
hzmm 125.59
is 500.00
rfl 13476.1
rfp 9675.8
th 8
ins 100.000
ai cdc ph
DEC. & VT
dfrq 499.744
dn H1
dpwr 34
dof 0
dm yyy
dmm w
dmf 10400
dseq
dres 1.0
homo n
DEC2
dfrq2 0
dn2
dpwr2 1
dof2 0
dm2 n
dmm2 c
dmf2 10000
dseq2
dres2 1.0
homo2 n
DEC3
dfrq3 0
dn3
dpwr3 1
dof3 0
dm3 n
dmm3 c
dmf3 10000
dseq3
dres3 1.0
homo3 n
PROCESSING
lb 1.00
wtfile
proc ft
fn 131072
math f
werr
wexp
wbs
wnt
HO2C
F
N
O
OBn
HN
BnO
39
200
180
160
140
120
100
80
60
40
20
0
ppm

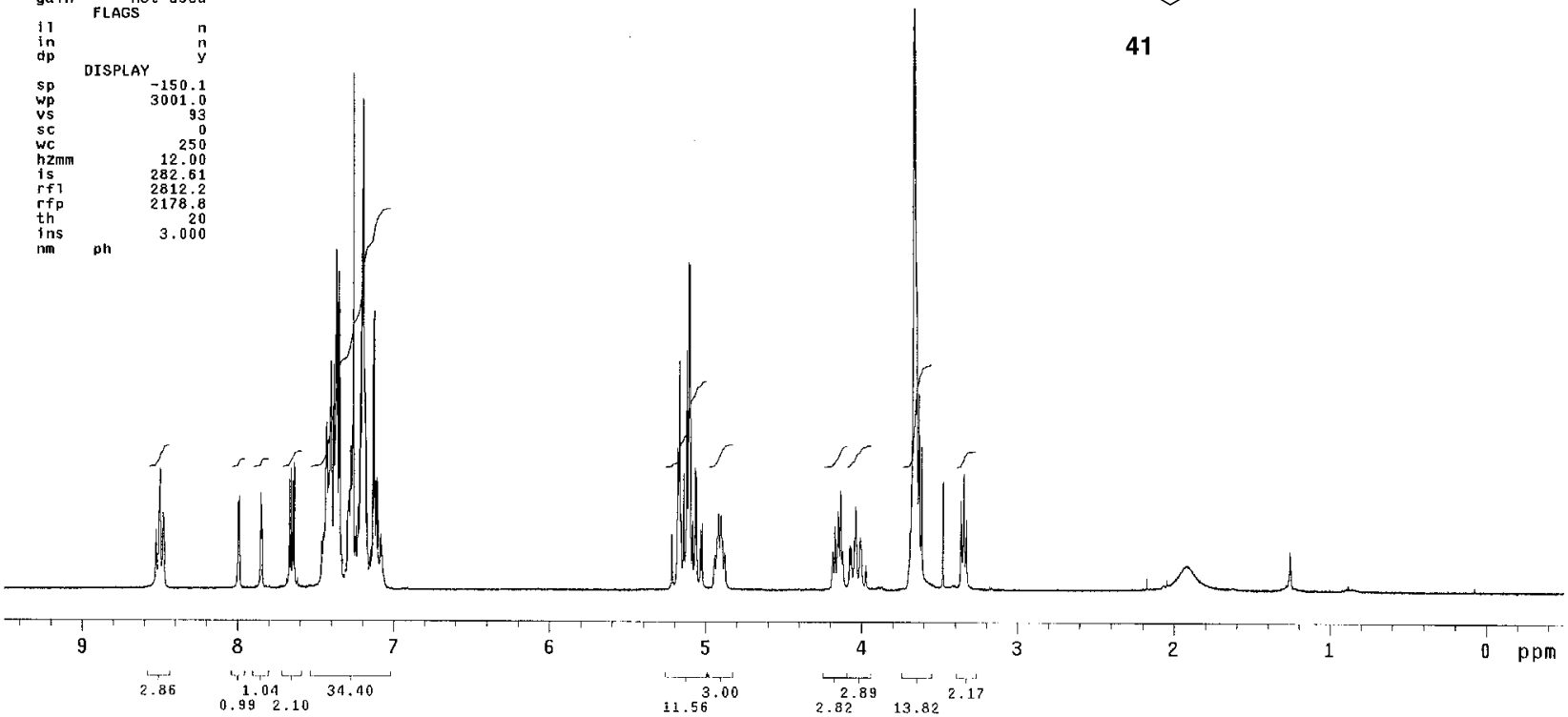
STANDARD 1H OBSERVE

exp1 std1h

SAMPLE		DEC. & VT	
date	Sep 29 2011	dfrq	300.107
solvent	CDCl3	dn	H1
file	/data/export/~	dpwr	30
	home/nolan/NOtzhe/~	dof	0
	mrhat/092911BnEntP~	dm	nnn
	EGN3.fid	dmm	c
ACQUISITION		dmf	200
sfrq	300.108	PROCESSING	
tn	H1	wtfile	
at	4.003	proc	ft
np	48052	fn	131072
sw	6002.4		
fb	not used	werr	
bs	4	wexp	
tpwr	54	wbs	
pw	8.0	wnt	
d1	0.050		
tof	867.7		
nt	32		
ct	24		
alock	n		
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
DISPLAY			
sp	-150.1		
wp	3001.0		
vs	93		
sc	0		
wc	250		
hzmm	12.00		
is	282.61		
rfl	2812.2		
rfp	2178.8		
th	20		
ins	3.000		
nm	ph		



41

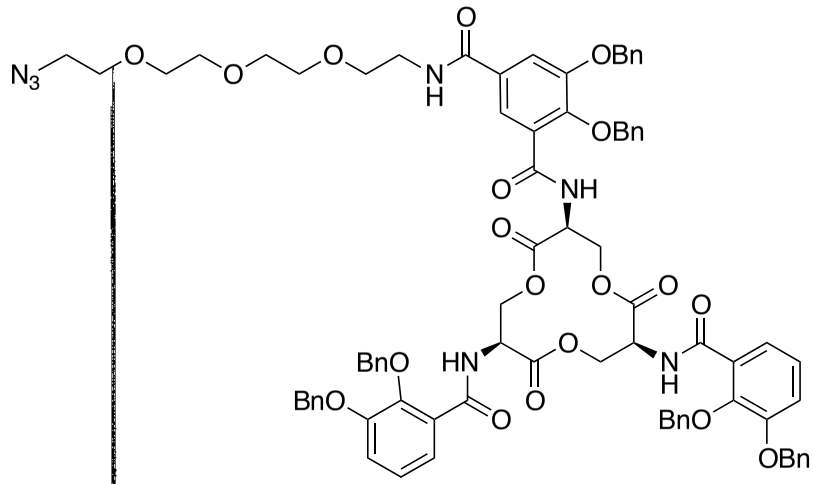


STANDARD CARBON PARAMETERS

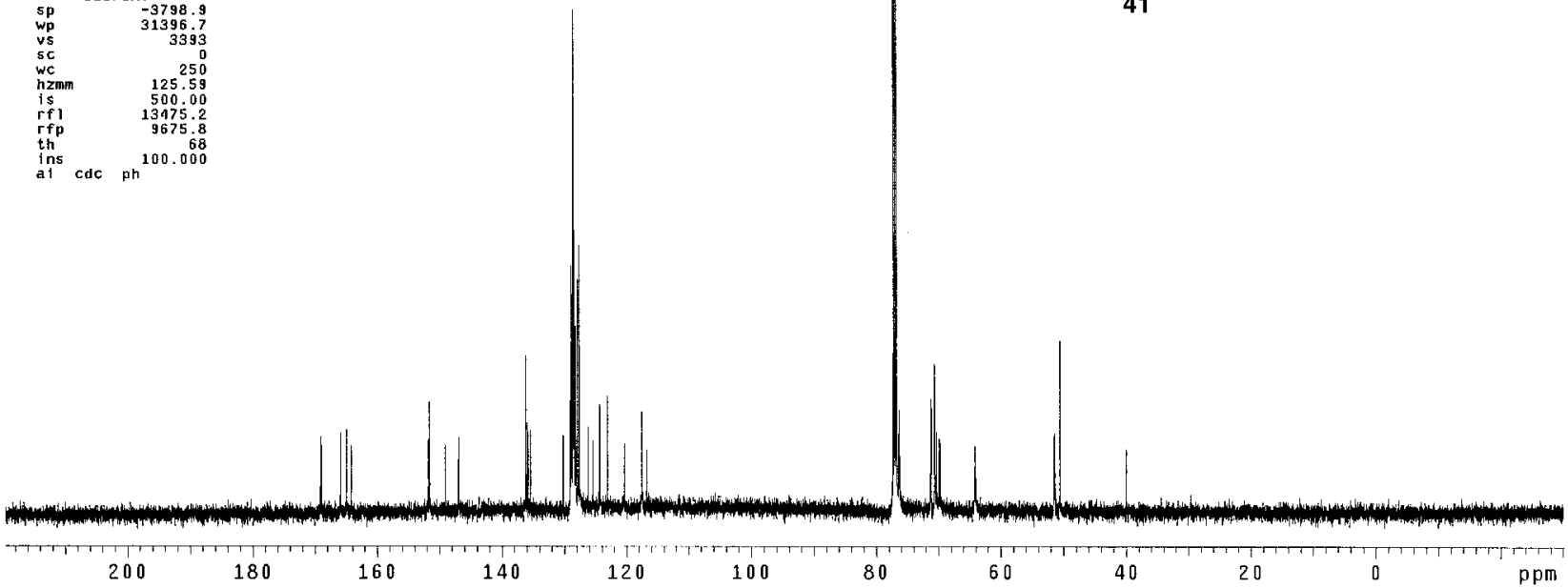
exp1 s2pul

SAMPLE		DEC. & VT	
date	Oct 21 2011	dfrq	499.744
solvent	CDCl3	dn	H1
file	/data/export/~	dpwr	34
home/nolan/NOtzhe/~		dof	0
bullwinkle/102111B~		dm	yyy
nEntPEGN3_C13.fid		dmm	w
ACQUISITION		dmf	10400
sfrq	125.672	dseq	
tn	C13	dres	1.0
at	2.000	homo	n
np	125588	PROCESSING	
sw	31397.2	lb	1.00
fb	not used	wtfile	
bs	16	proc	ft
tpwr	59	fn	131072
pw	6.7	math	f
d1	0		
tof	0	werr	
nt	99999	wexp	
ct	4640	wbs	
alock	n	wnt	
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
hs	nn		
DISPLAY			
sp	-3798.9		
wp	31396.7		
vs	3393		
sc	0		
wc	250		
hzmm	125.59		
is	500.00		
rfl	13475.2		
rfp	9675.8		
th	68		
ins	100.000		

a1 cdc ph



41



FT-IR (KBr pellet or NaCl disk, cm^{-1})

Boc-14

3443 (m), 3325 (s), 3074 (w), 2976 (s), 2930 (s), 2858 (s), 1698 (s), 1652 (s), 1535 (s), 1451 (s), 1391 (m), 1366 (s), 1351 (m), 1329 (w), 1273 (s), 1253 (s), 1217 (w), 1173 (s), 1126 (s), 1041 (w), 969 (w), 945 (w), 895 (w), 865 (w), 780 (w), 756 (m).

14

3307 (m), 3084 (m), 2932 (s), 2855 (m), 1680 (s), 1644 (s), 1542 (m), 1453 (w), 1433 (w), 1351 (w), 1321 (w), 1308 (w), 1273 (w), 1203 (s), 1178 (s), 1137 (s), 932 (w), 897 (w), 836 (w), 799 (w), 722 (w), 706 (w).

Boc-15

3438 (w), 3326 (m), 3050 (w), 2975 (m), 2933 (m), 2870 (m), 1708 (s), 1648 (s), 1592 (w), 1580 (w), 1530 (s), 1455 (w), 1391 (w), 1366 (m), 1351 (w), 1303 (m), 1254 (m), 1172 (s), 1124 (s), 1041 (w), 970 (w), 866 (w), 807 (w), 785 (m), 756 (w), 655 (w).

15

3270 (w), 3075 (m), 2918 (m), 2872 (m), 1680 (s), 1638 (s), 1592 (w), 1539 (m), 1480 (w), 1455 (w), 1429 (w), 1350 (w), 1307 (w), 1259 (w), 1203 (s), 1174 (s), 1135 (s), 945 (w), 880 (w), 834 (w), 798 (m), 785 (m), 721 (w), 706 (w).

Boc-16

3346 (s), 3092 (w), 3071 (w), 3027 (m), 2977 (s), 2929 (s), 2863 (s), 2708 (w), 2498 (w), 1956 (w), 1693 (s), 1649 (s),1543 (s),1505 (s),1454 (s),1392 (m), 1366 (s), 1301 (s),1251 (s), 1251 (s), 1201 (s), 1165 (s), 1131 (s),1030 (m), 943 (w), 863 (m), 831 (m), 801 (m), 753 (s), 720 (m), 700 (s).

16

3426 (m), 3027 (m), 2918 (m), 1948 (w), 1683 (m), 1632 (m), 1548 (m), 1505 (m), 1454 (m), 1420 (m), 1351 (m), 1309 (m), 1203 (m), 1134 (m), 1022 (w), 940 (w), 862 (w), 836 (w), 801 (m), 743 (m), 722 (m), 700 (m).

17

3412 (s), 2976 (m), 2919 (m), 2742 (m), 2673 (m), 2535 (m), 2497 (m), 1694 (s), 1616 (s), 1583 (s), 1521 (s), 1485 (s), 1445 (s), 1367 (s), 1398 (m), 1367 (s), 1310 (s), 1243 (m), 1212 (s), 1175 (s), 1098 (s), 1037 (m), 962 (w), 932 (w), 895 (w), 851 (w), 832 (w), 793 (m), 751 (m), 664 (w).

Fmoc-18

3309 (m), 3049 (w), 3010 (w), 2924 (m), 2868 (m), 1719 (s), 1627 (s), 1508 (m), 1465 (s),

1390 (w), 1337 (w), 1295 (w), 1260 (m), 1148 (w), 1112 (w), 1024 (w), 949 (w), 884 (w), 833 (w), 807 (w), 745 (m).

18

2918 (m), 2876 (m), 1718 (m), 1692 (s), 1628 (s), 1506 (m), 1470 (m), 1385 (w), 1340 (w), 1295 (w), 1251 (w), 1201 (m), 1178 (w), 1125 (m), 1025 (w), 947 (w), 886 (w), 831 (w), 799 (w), 776 (w), 748 (w), 720 (w).

20

3467 (s), 3177 (m), 3079 (m), 3005 (m), 2978 (m), 2955 (m), 2905 (m), 2854 (w), 1678 (s), 1640 (w), 1622 (m), 1485 (s), 1442 (s), 1411 (w), 1370 (m), 1319 (s), 1282 (s), 1235 (m), 1195 (s), 1168 (s), 1130 (m), 916 (m), 867 (w), 791 (s), 772 (w), 681 (m), 630 (w).

21

3062 (m), 3032 (m), 2976 (m), 2937 (m), 2912 (m), 2889 (m), 1692 (s), 1639 (w), 1603 (w), 1578 (w), 1482 (m), 1453 (w), 1414 (w), 1378 (w), 1331 (m), 1268 (m), 1219 (w), 1146 (w), 1049 (m), 1028 (w), 968 (w), 914 (w), 858 (w), 791 (w), 758 (w), 697 (m).

22

3084 (w), 3063 (m), 3031 (m), 2955 (m), 2941 (m), 2876 (m), 2846 (m), 1692 (s), 1599 (m), 1574 (m), 1498 (m), 1483 (m), 1454 (m), 1414 (m), 1377 (m), 1336 (m), 1272 (m), 1256 (m), 1218 (m), 1153 (m), 1081 (w), 1051 (s), 1028 (w), 958 (s), 940 (m), 914 (w), 887 (w), 859 (m), 843 (w), 782 (m), 757 (s), 744 (m), 727 (m), 695 (s), 650 (w).

23

3356 (m), 3065 (w), 3031 (w), 2954 (w), 2927 (w), 2876 (w), 1752 (s), 1660 (s), 1597 (w), 1576 (m), 1514 (s), 1455 (m), 1424 (w), 1376 (w), 1346 (m), 1312 (m), 1266 (s), 1205 (s), 1133 (w), 1082 (w), 1054 (w), 1029 (w), 962 (w), 914 (w), 852 (w), 806 (w), 754 (s), 698 (s), 668 (w).

24

3359 (m), 3062 (w), 3031 (w), 2957 (w), 2881 (w), 1752 (s), 1697 (m), 1660 (s), 1601 (w), 1577 (m), 1516 (s), 1454 (m), 1376 (m), 1345 (m), 1312 (m), 1266 (s), 1205 (s), 1135 (w), 1081 (w), 1053 (w), 1027 (w), 956 (w), 915 (w), 851 (w), 754 (s), 698 (s), 668 (w).

25

3654 (w), 3628 (w), 3359 (m), 3088 (w), 3064 (w), 3032 (w), 2946 (w), 2933 (w), 2874 (w), 1761 (s), 1660 (m), 1599 (w), 1576 (m), 1519 (m), 1490 (m), 1454 (m), 1427 (w), 1374 (m), 1345 (m), 1267 (m), 1203 (m), 1134 (w), 1081 (w), 1048 (w), 1028 (w), 954 (w), 912 (w), 849 (w), 807 (w), 754 (s), 734 (s), 697 (s).

26

3356 (m), 3058 (w), 3032 (w), 2929 (w), 2881 (w), 1750 (s), 1660 (s), 1597 (w), 1576 (m), 1514 (s), 1455 (m), 1375 (w), 1346 (w), 1312 (w), 1266 (s), 1204 (s), 1133 (w), 1082 (w), 1053 (w), 1027 (w), 962 (w), 914 (w), 851 (w), 802 (w), 754 (s), 698 (m).

27

3358 (m), 3062 (w), 3031 (w), 2957 (w), 2928 (w), 2876 (w), 1751 (s), 1697 (m), 1660 (s), 1577 (m), 1517 (s), 1455 (m), 1375 (w), 1346 (w), 1303 (w), 1266 (s), 1206 (m), 1134 (w), 1082 (w), 1054 (w), 1022 (w), 957 (w), 914 (w), 851 (w), 806 (w), 754 (s), 698 (m).

28

3356 (m), 3088 (w), 3064 (w), 3032 (w), 2957 (w), 2924 (w), 2872 (w), 1753 (s), 1718 (m), 1662 (s), 1599 (w), 1577 (s), 1517 (s), 1454 (s), 1427 (w), 1375 (m), 1346 (m), 1267 (s), 1205 (s), 1133 (w), 1112 (w), 1082 (m), 1052 (m), 1029 (m), 957 (m), 914 (m), 851 (w), 808 (w), 755 (s), 736 (s), 699 (s).

Bn_6-29

3356 (m), 3064 (w), 3033 (w), 2931 (m), 2872 (m), 1752 (s), 1710 (m), 1661 (s), 1577 (m), 1516 (s), 1455 (s), 1367 (m), 1346 (m), 1266 (s), 1205 (s), 1130 (m), 1083 (m), 1047 (m), 958 (w), 915 (w), 851 (w), 808 (w), 755 (m), 699 (m).

Bn_6-30

3353 (m), 3065 (w), 3032 (w), 3010 (w), 2930 (m), 2855 (m), 1752 (s), 1660 (s), 1593 (w), 1577 (m), 1518 (s), 1454 (m), 1424 (w), 1374 (w), 1346 (m), 1301 (m), 1265 (s), 1206 (s), 1130 (m), 1083 (m), 1050 (m), 1022 (w), 957 (w), 915 (w), 850 (w), 809 (w), 755 (s), 699 (m), 659 (w).

Bn_6-31

3354 (m), 3062 (w), 3032 (w), 3006 (w), 2929 (m), 2855 (m), 1752 (s), 1660 (s), 1577 (m), 1518 (s), 1454 (m), 1374 (w), 1346 (w), 1303 (w), 1265 (m), 1206 (m), 1130 (w), 1083 (w), 1061 (w), 1031 (w), 957 (w), 915 (w), 850 (w), 811 (w), 755 (s), 698 (m).

Bn_6-32

3353 (m), 3067 (w), 3028 (w), 2997 (w), 2924 (m), 2859 (m), 1751 (s), 1659 (s), 1577 (m), 1519 (s), 1455 (m), 1372 (w), 1346 (w), 1295 (m), 1205 (m), 1122 (w), 1083 (w), 1053 (w), 1027 (w), 958 (w), 914 (w), 845 (w), 806 (w), 785 (w), 754 (m), 698 (m).

Bn_6-33

3356 (m), 3071 (w), 3031 (w), 2924 (m), 2868 (m), 1751 (s), 1660 (s), 1576 (m), 1519 (s), 1454 (m), 1374 (w), 1346 (m), 1301 (m), 1265 (s), 1205 (m), 1130 (w), 1083 (w), 1057 (w), 1022 (w), 957 (w), 915 (w), 851 (w), 806 (w), 754 (s), 698 (m).

Bn_6-34

3350 (m), 3088 (w), 3065 (w), 3033 (w), 2931 (m), 2868 (m), 1752 (s), 1660 (s), 1616 (m), 1577 (s), 1518 (s), 1455 (s), 1369 (m), 1348 (m), 1309 (s), 1266 (s), 1211 (s), 1152 (w), 1129 (m), 1083 (m), 1053 (w), 1027 (w), 958 (w), 912 (m), 844 (s), 794 (w), 734 (s), 699 (m).

Bn_6-35

3369 (m), 3075 (w), 3036 (m), 3015 (m), 2950 (m), 2907 (m), 2855 (m), 1750 (s), 1662 (s), 1627 (s), 1577 (m), 1512 (s), 1464 (s), 1375 (m), 1339 (m), 1302 (m), 1263 (s), 1208 (s), 1121 (m), 1082 (m), 1053 (m), 1027 (m), 962 (m), 910 (w), 884 (w), 867 (w), 849 (w), 806 (w), 754 (s), 699 (m), 664 (w).

36

2954 (m), 2922 (m), 2859 (m), 1718 (m), 1682 (s), 1616 (s), 1469 (s), 1385 (w), 1333 (w), 1255 (w), 1199 (m), 1165 (m), 1139 (m), 1022 (w), 828 (w), 799 (w), 720 (w).

38

3345 (m), 3019 (m), 2967 (m), 2931 (m), 2863 (m), 1707 (s), 1628 (s), 1509 (s), 1467 (s), 1389 (m), 1365 (w), 1338 (m), 1301 (w), 1260 (s), 1170 (m), 1109 (w), 1026 (m), 988 (w), 940 (w), 888 (w), 834 (w), 807 (w), 751 (m), 711 (w), 665 (w).

39

3354 (m), 3065 (w), 3028 (m), 3009 (m), 2933 (m), 2859 (w), 1751 (s), 1658 (s), 1619 (s), 1577 (m), 1499 (s), 1455 (s), 1374 (m), 1346 (s), 1308 (s), 1262 (s), 1208 (s), 1128 (w), 1083 (m), 1028 (m), 979 (w), 957 (w), 914 (w), 851 (w), 806 (w), 754 (s), 699 (s), 665 (m).

41

3409 (s), 3002 (w), 2963 (m), 2918 (m), 2855 (m), 1748 (m), 1660 (s), 1576 (w), 1541 (w), 1437 (m), 1403 (m), 1342 (w), 1313 (m), 1260 (m), 1204 (w), 1025 (s), 953 (m), 845 (w), 798 (w), 703 (w).