

# Design, Synthesis, and Preliminary Evaluation of Doxazolidine Carbamates as Prodrugs Activated by Carboxylesterases

*David J. Burkhart,<sup>‡</sup> Benjamin L. Barthel,<sup>‡</sup> Glen C. Post,<sup>‡</sup> Brian T. Kalet,<sup>‡</sup> Jordan W.*

*Nafie,<sup>‡</sup> and Richard K. Shoemaker,<sup>‡</sup> and Tad H. Koch<sup>\*§‡</sup>*

Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO

80309-0215 and Colorado Cancer Center, Aurora, Colorado 80010

## Supporting Information

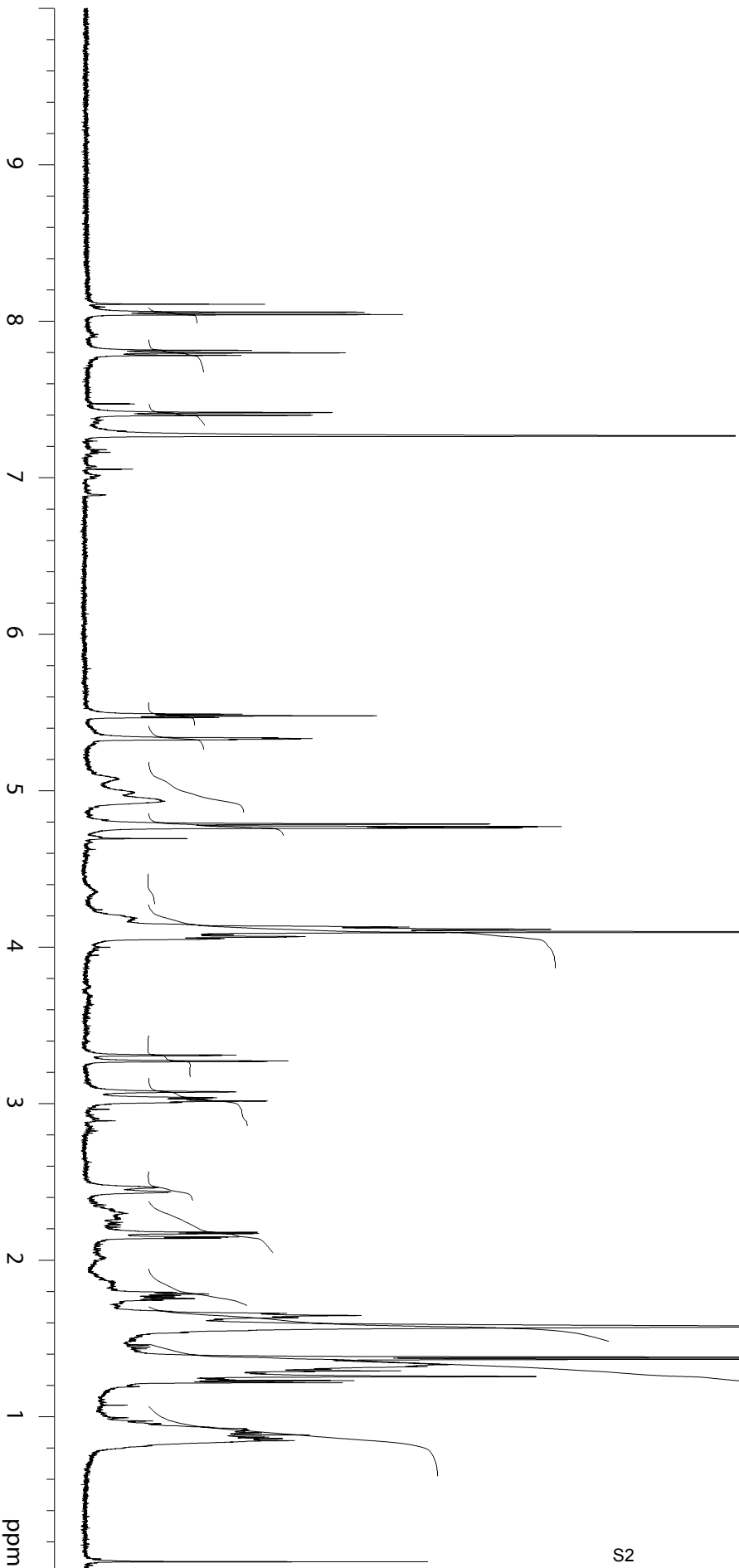
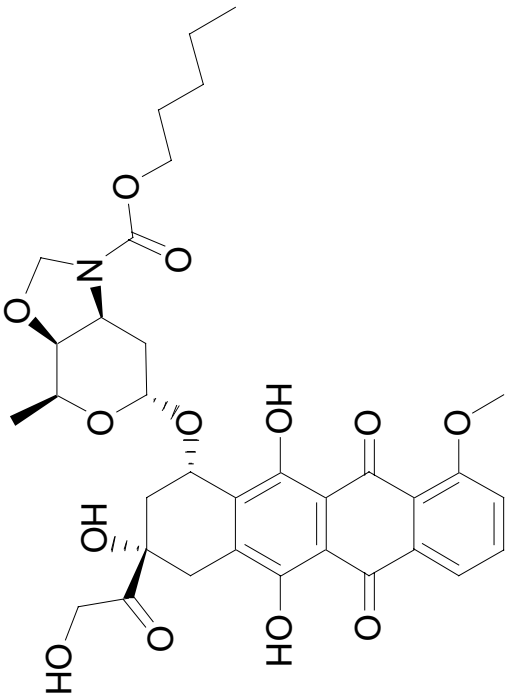
C18 reverse phase HPLC chromatograms of final products with two different eluting methods: method DXFRM5.M used 20 mM pH 7.4 triethylammonium acetate gradient with acetonitrile and method DOXAZ1.M used 0.1% trifluoroacetic acid gradient with acetonitrile. With both methods, the gradient started at 80% aqueous phase and proceeded to 70% aqueous phase at 5 min to 30% aqueous phase at 15 min and then isocratic to 25 min. The column was an Agilent Zorbax 5  $\mu$ m ODS column, 4.6 mm i.d. x 150 mm and was eluted at 1.0 mL/min and eluents were detected by absorption at 480 nm or 274 nm. Pentyl Doxaz-PABC was also detected by fluorescence at 550 nm.

<sup>1</sup>H NMR spectra of Doxaz ethyl carbamate, Doxaz butyl carbamate, Doxaz pentyl carbamate, butyl PABC-Doxaz, and pentyl PABC-Doxaz and homonuclear COSY spectra of pentyl PABC-Doxaz and Doxaz ethyl carbamate. HSQC and HMBC spectra for pentyl PABC-Doxaz are also provided

Arrhenius and Eyring plots of the rate constant for chair-twist boat interconversion of Doxaz ethyl carbamate as a function of temperature.

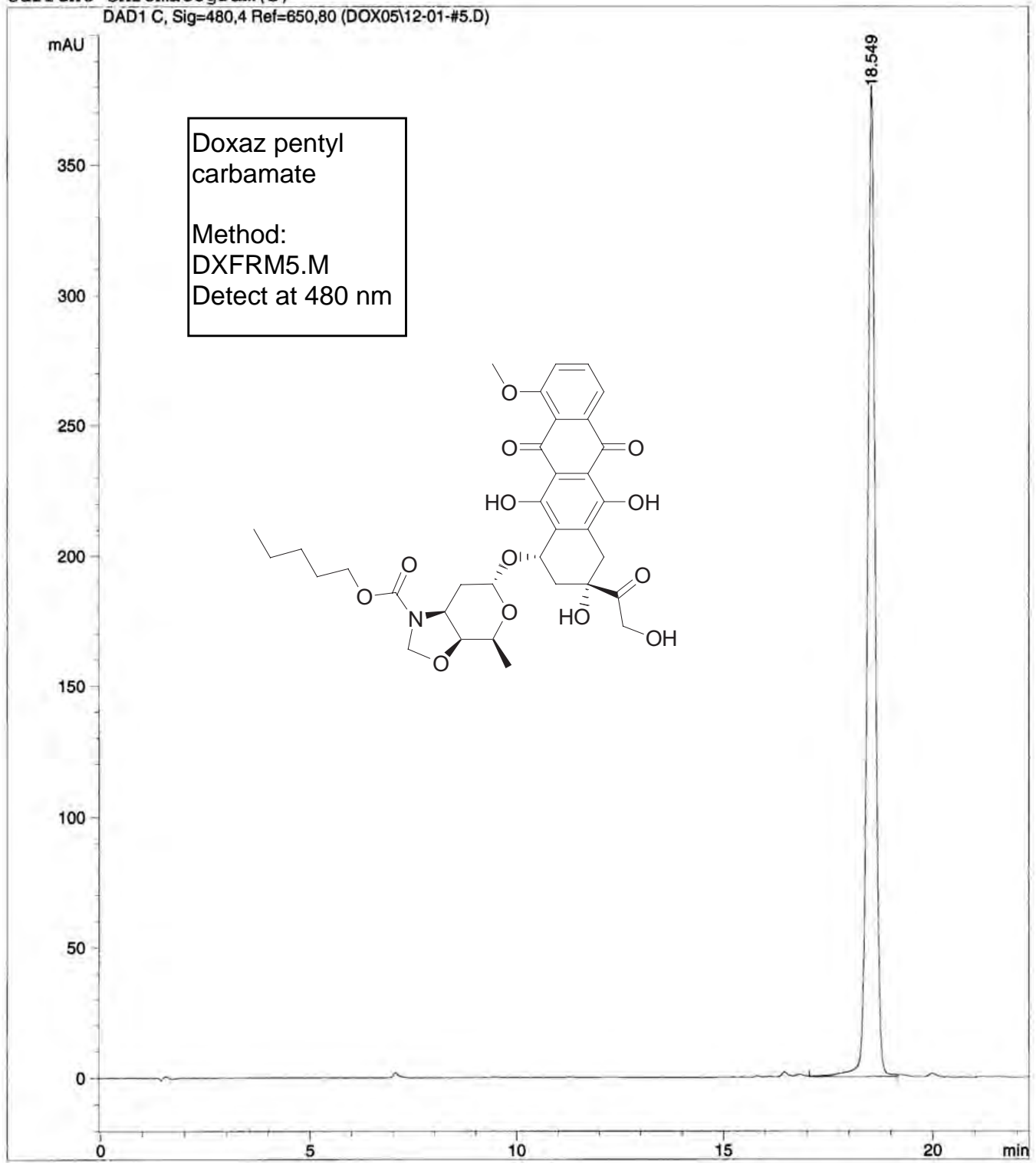
CES1 and CES2 primer sequences for RT-PCR experiment.

Doxaz pentyl/  
carbamate  
proton NMR



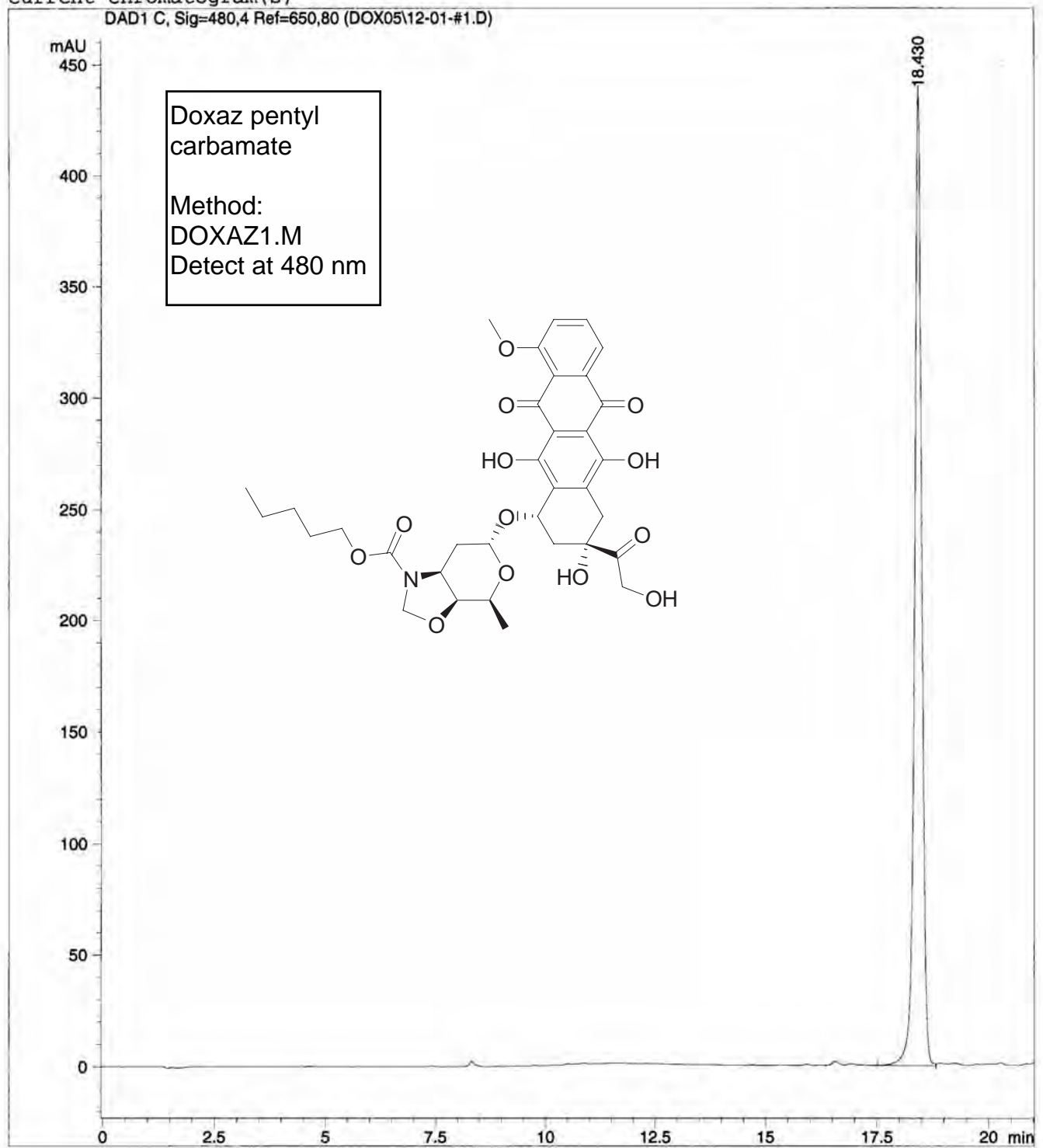
Current Chromatogram(s)

DAD1 C, Sig=480,4 Ref=650,80 (DOX05\12-01-#5.D)



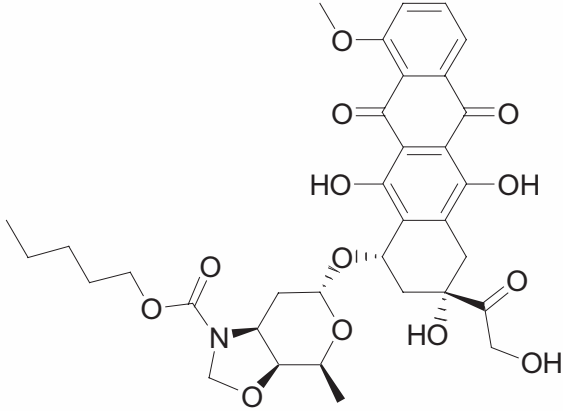
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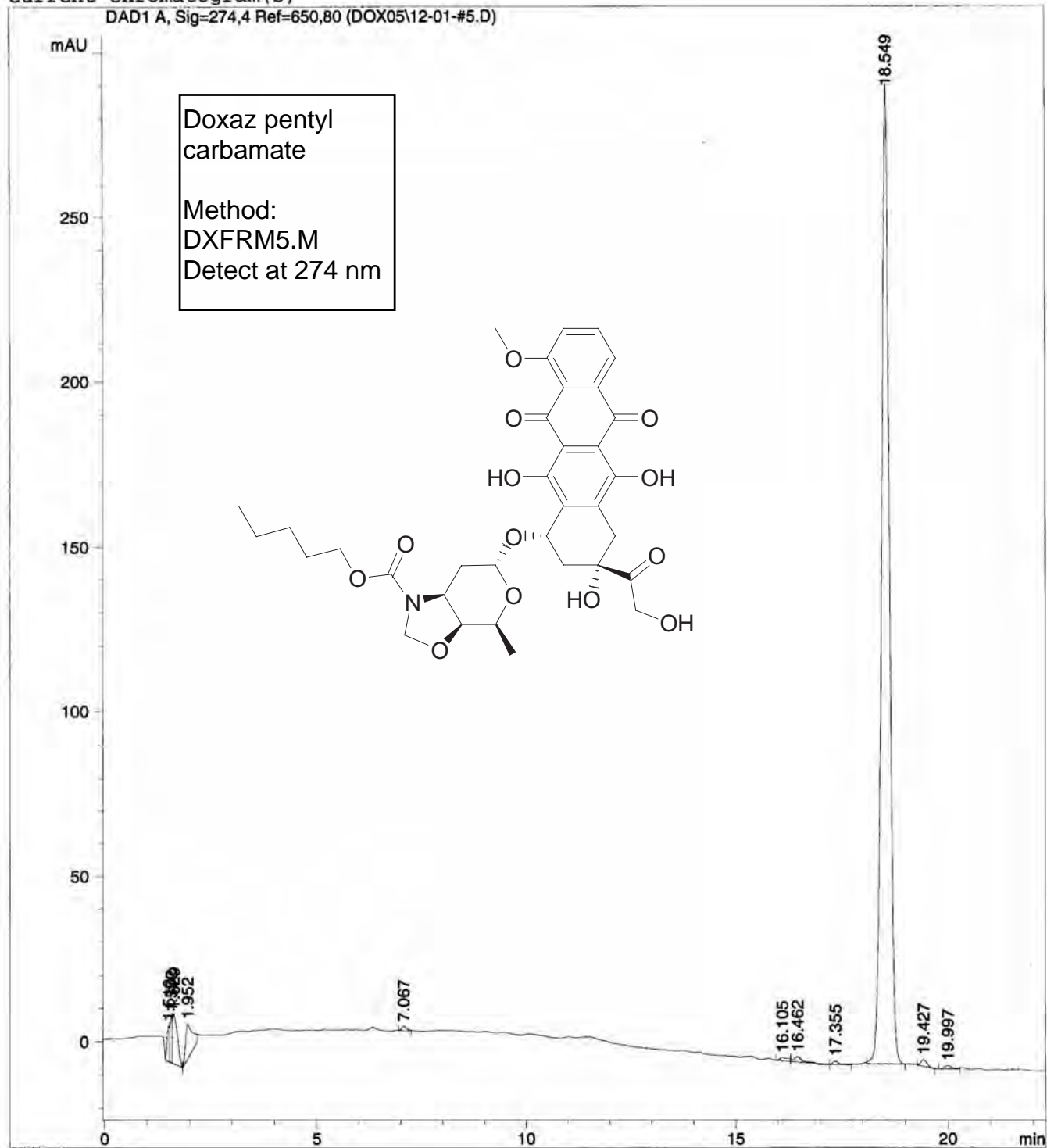
Doxaz pentyl  
carbamate

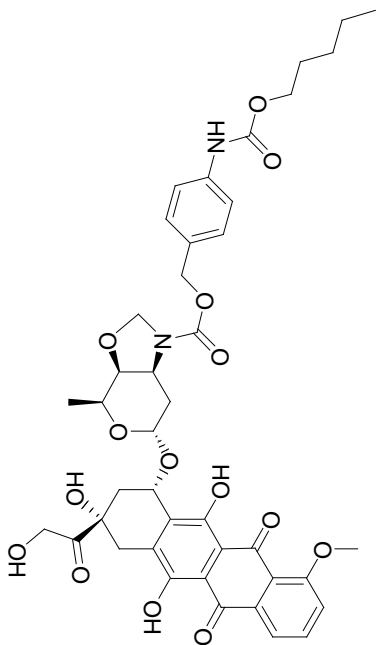
Method:  
DOXAZ1.M  
Detect at 480 nm



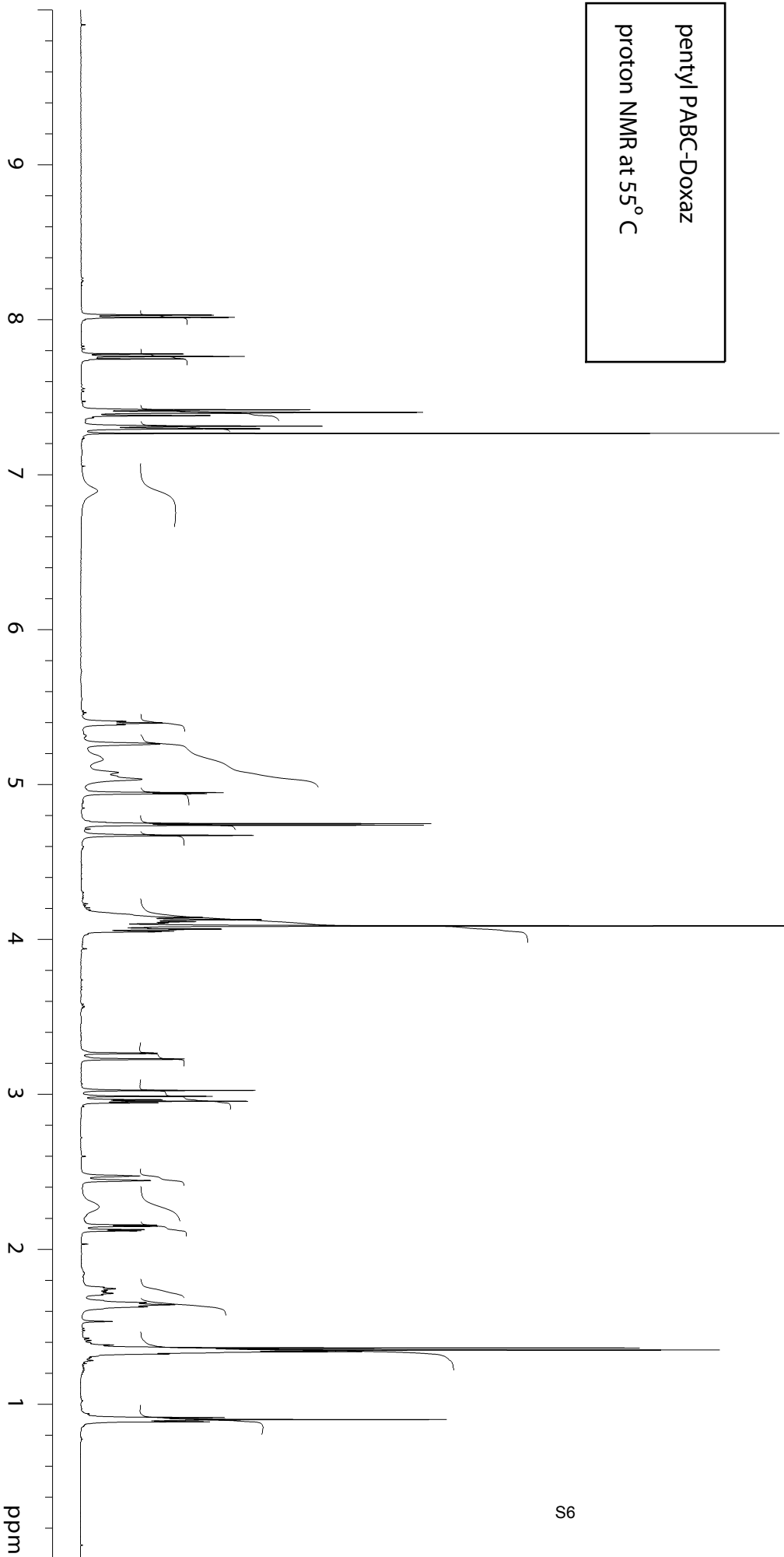
Current Chromatogram(s)

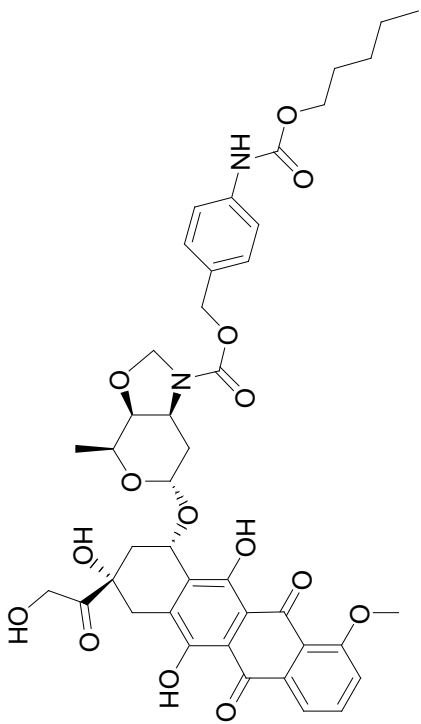
DAD1 A, Sig=274,4 Ref=650,80 (DOX05\12-01-#5.D)



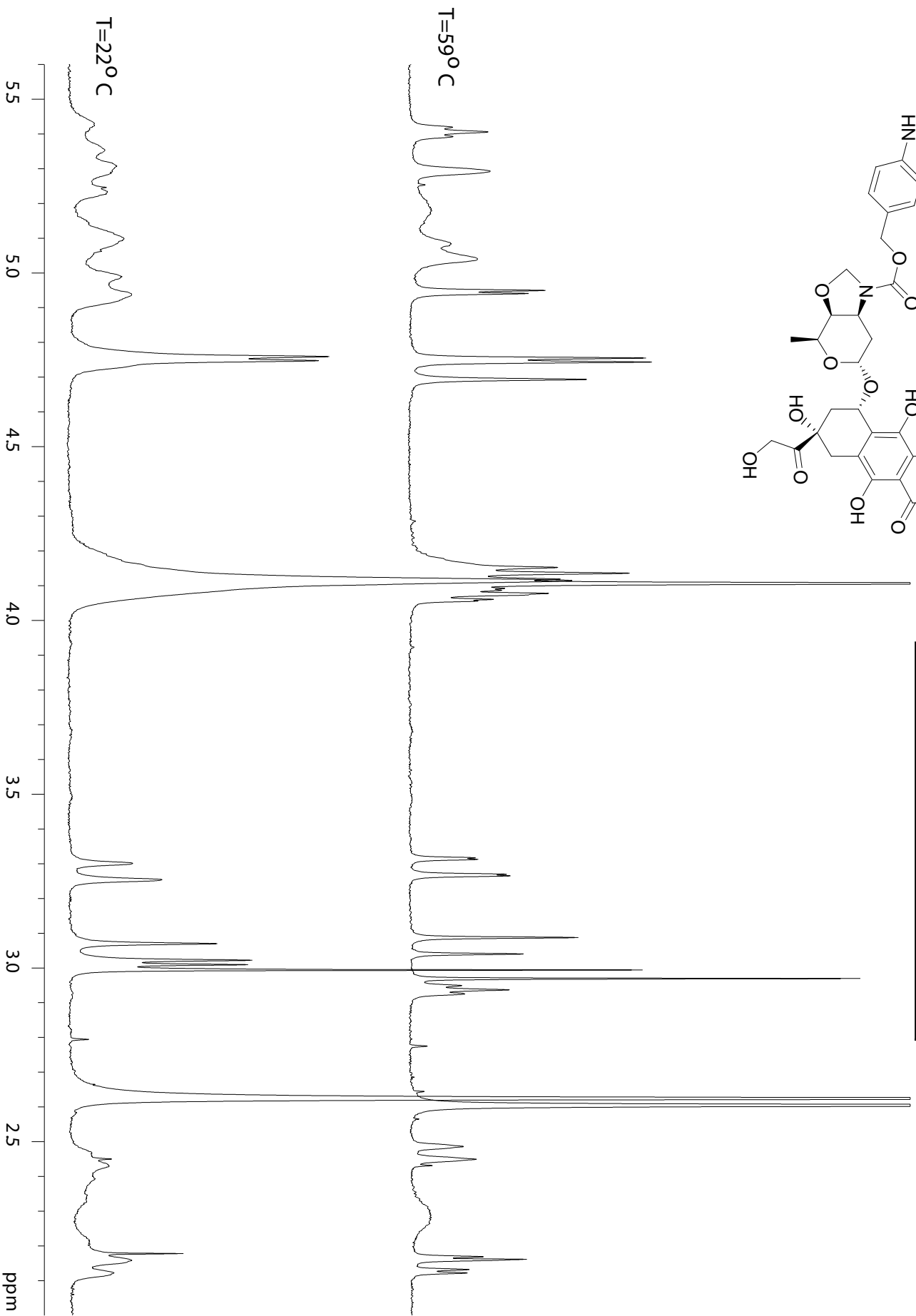


pentyl PABC-Doxaz  
proton NMR at 55° C

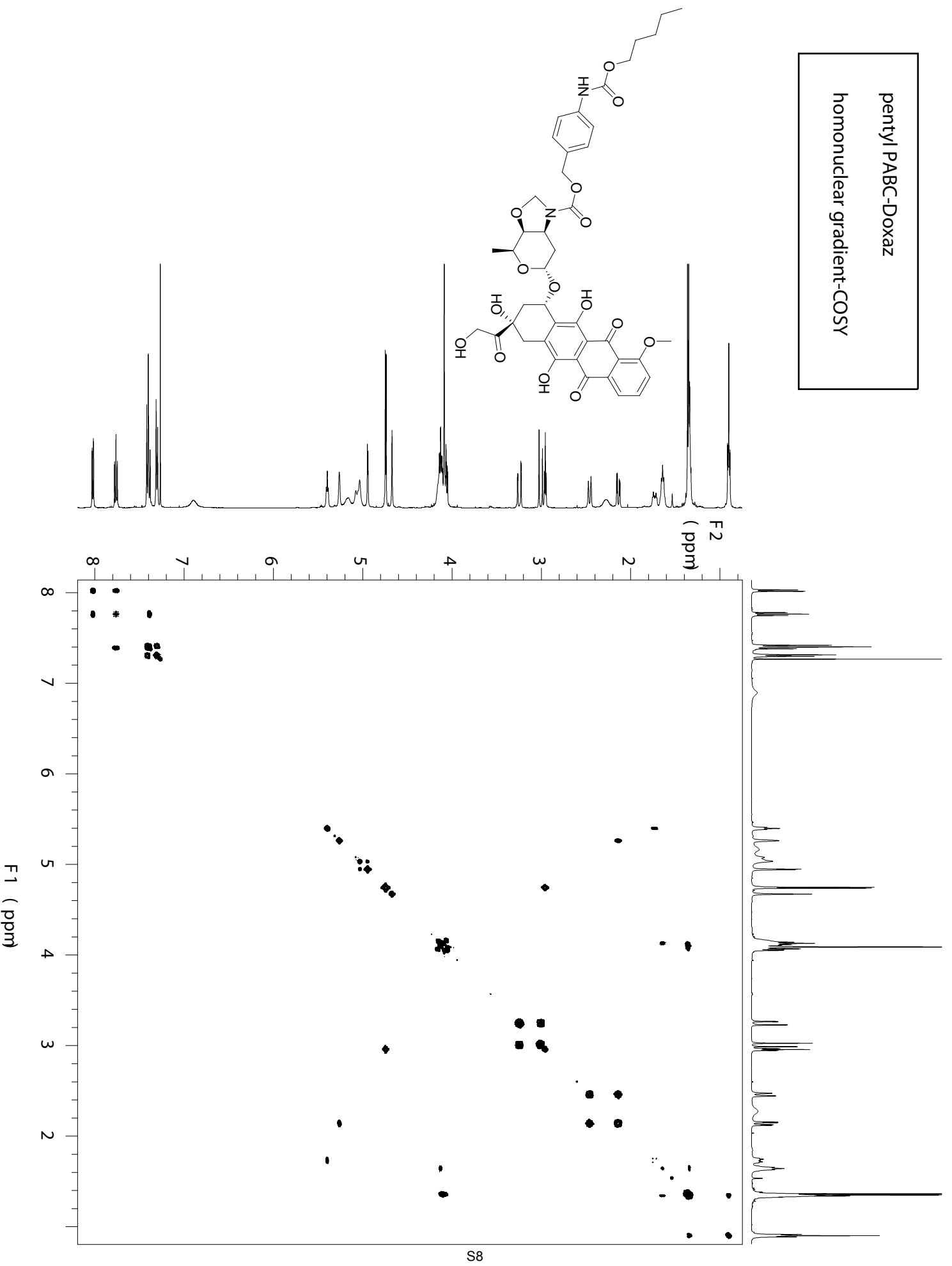




pentyl PABC-Doxaz  
proton NMR at 22° and 59° C



pentyl PABC-Doxaz  
homonuclear gradient-COSY

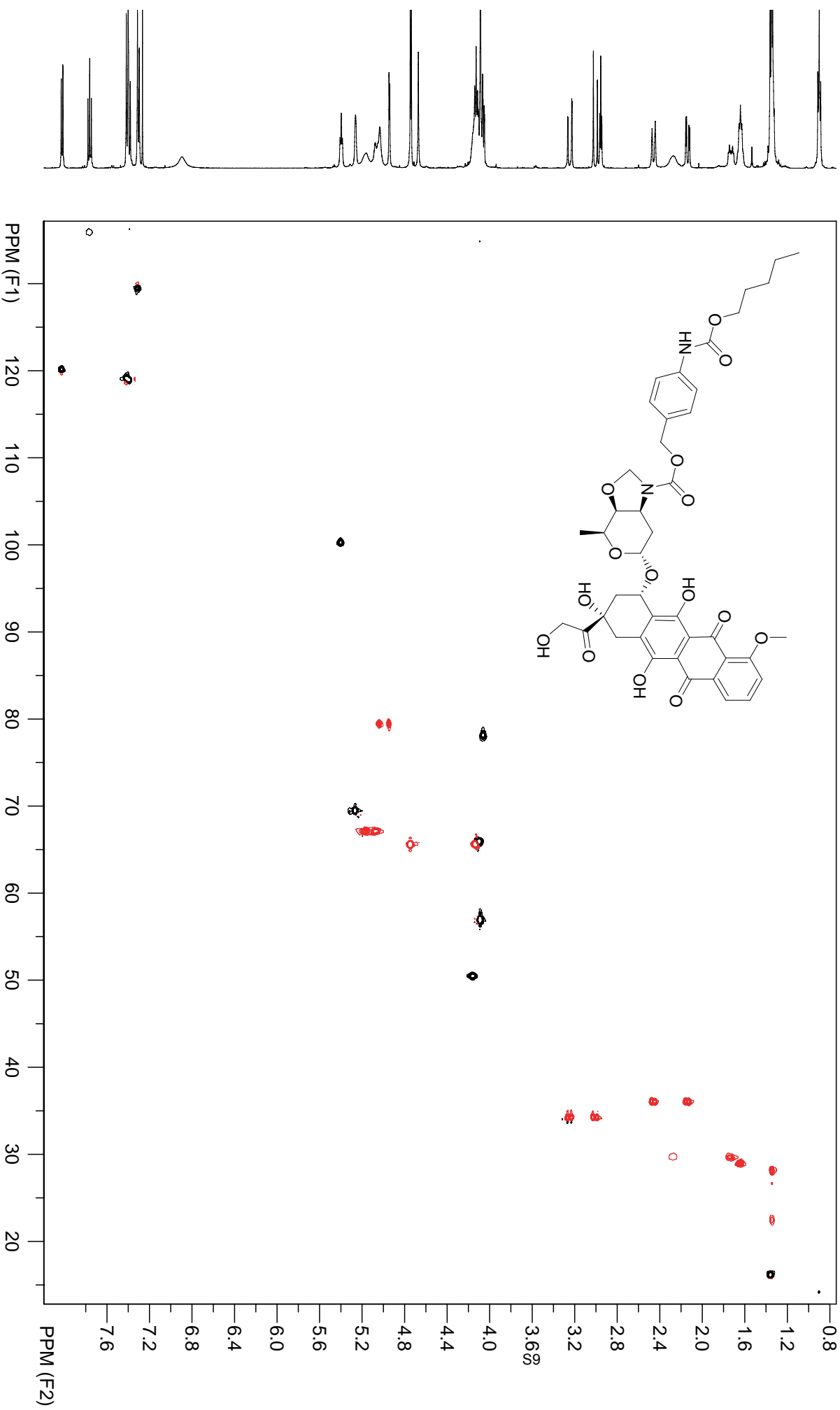
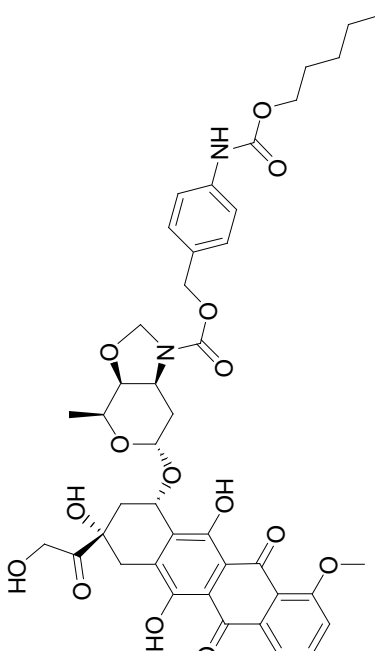


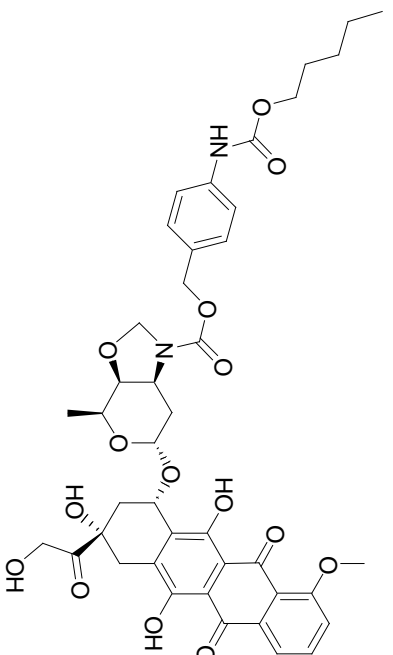


pentyl PABC-Doxaz

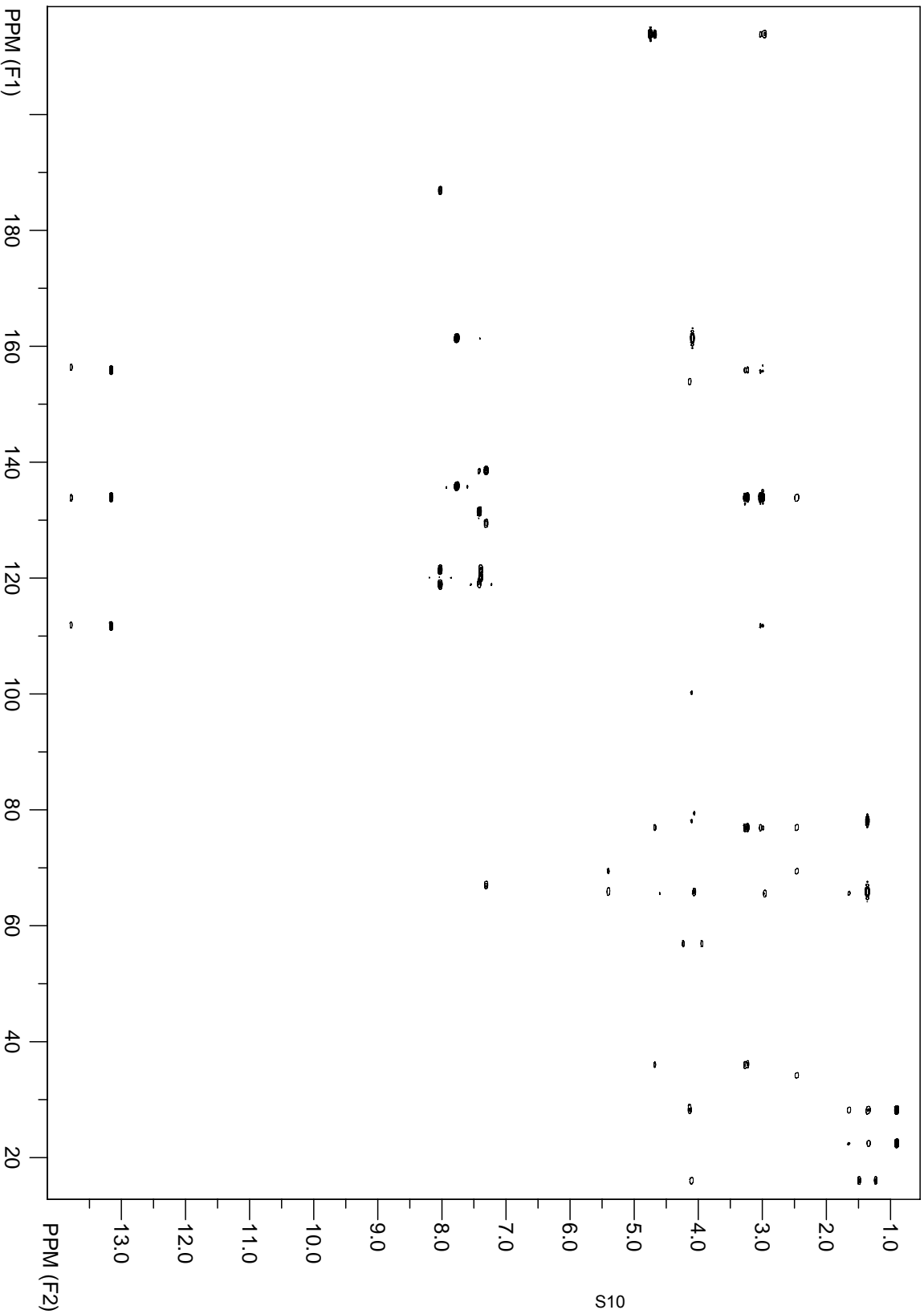
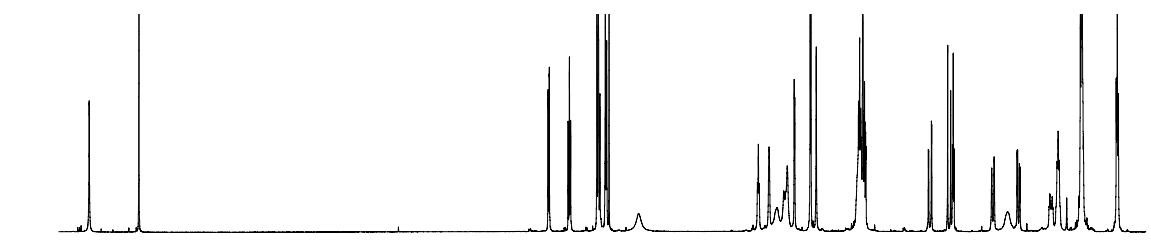
gradient-selected HSQC, multiplicity edited

Black: CH/CH<sub>3</sub> Red: CH<sub>2</sub>



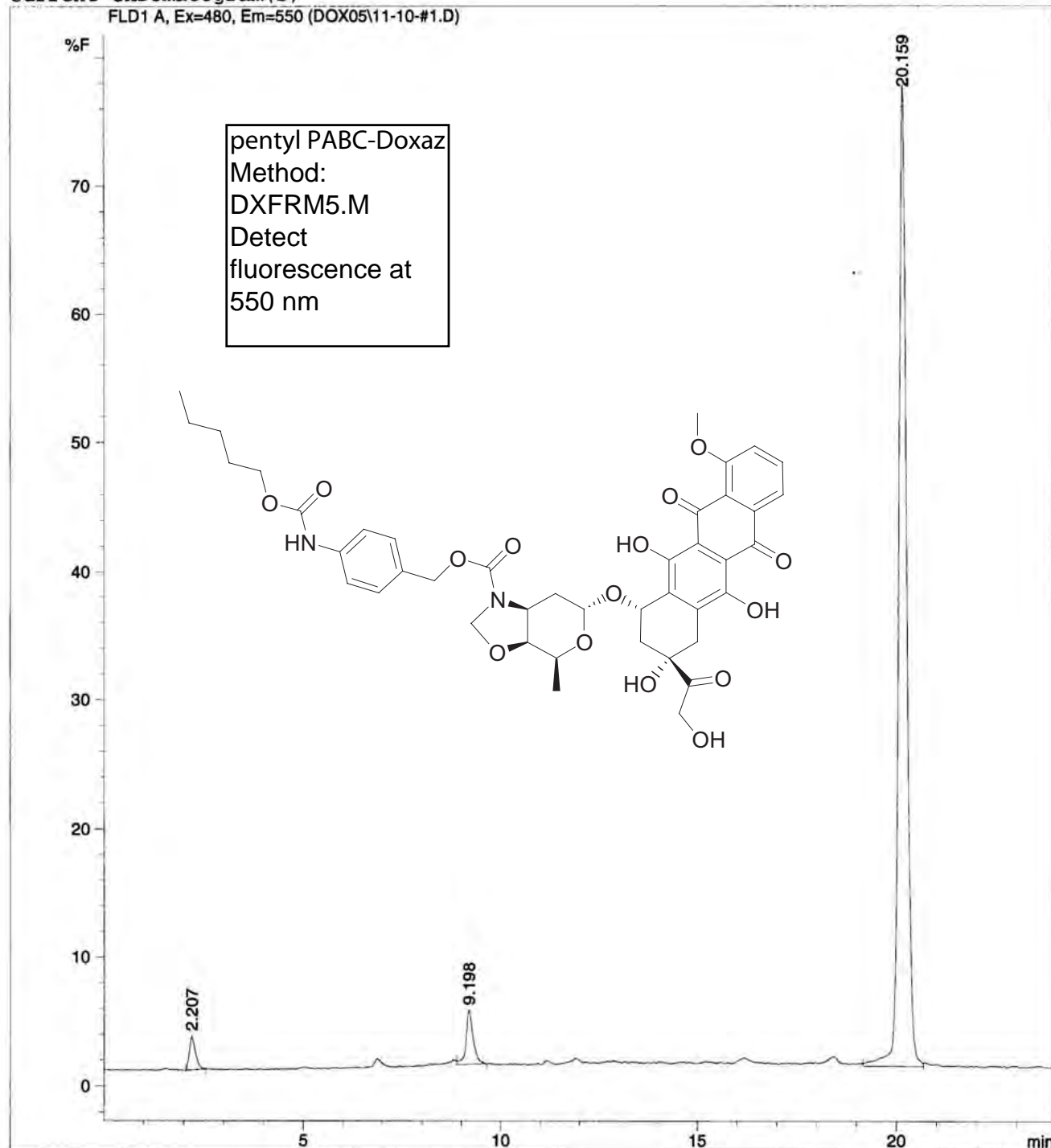


pentyloxy PABC-Doxaz  
 gradient-selected HMBC  
 optimized for  $j_{CH}=7.5$  Hz

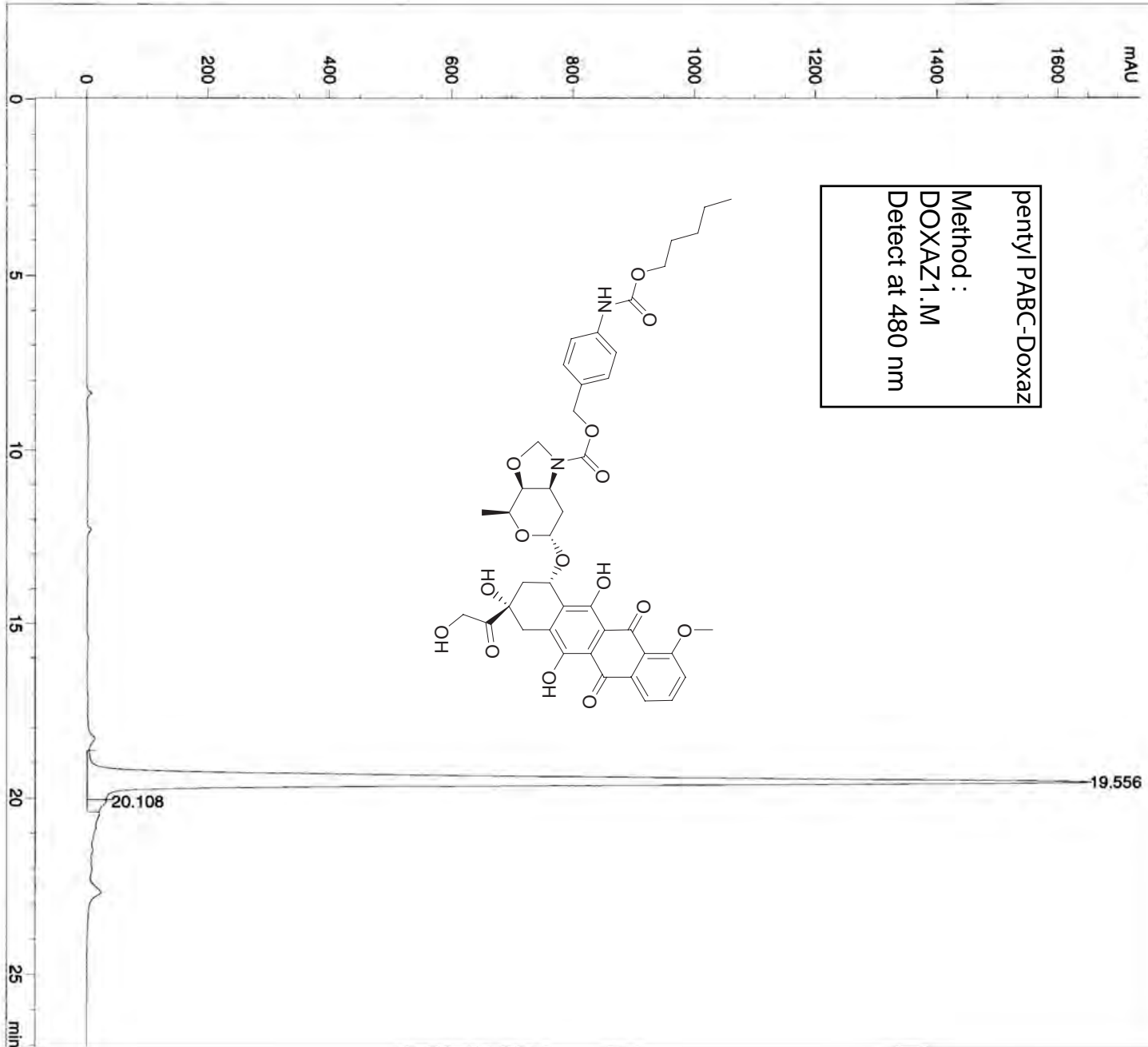
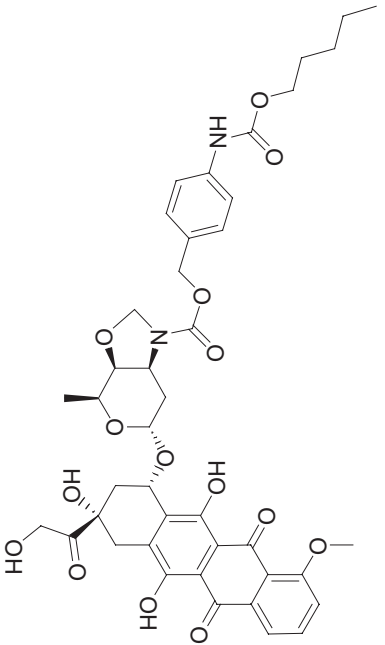


Current Chromatogram(s)

FLD1 A, Ex=480, Em=550 (DOX05\11-10-#1.D)

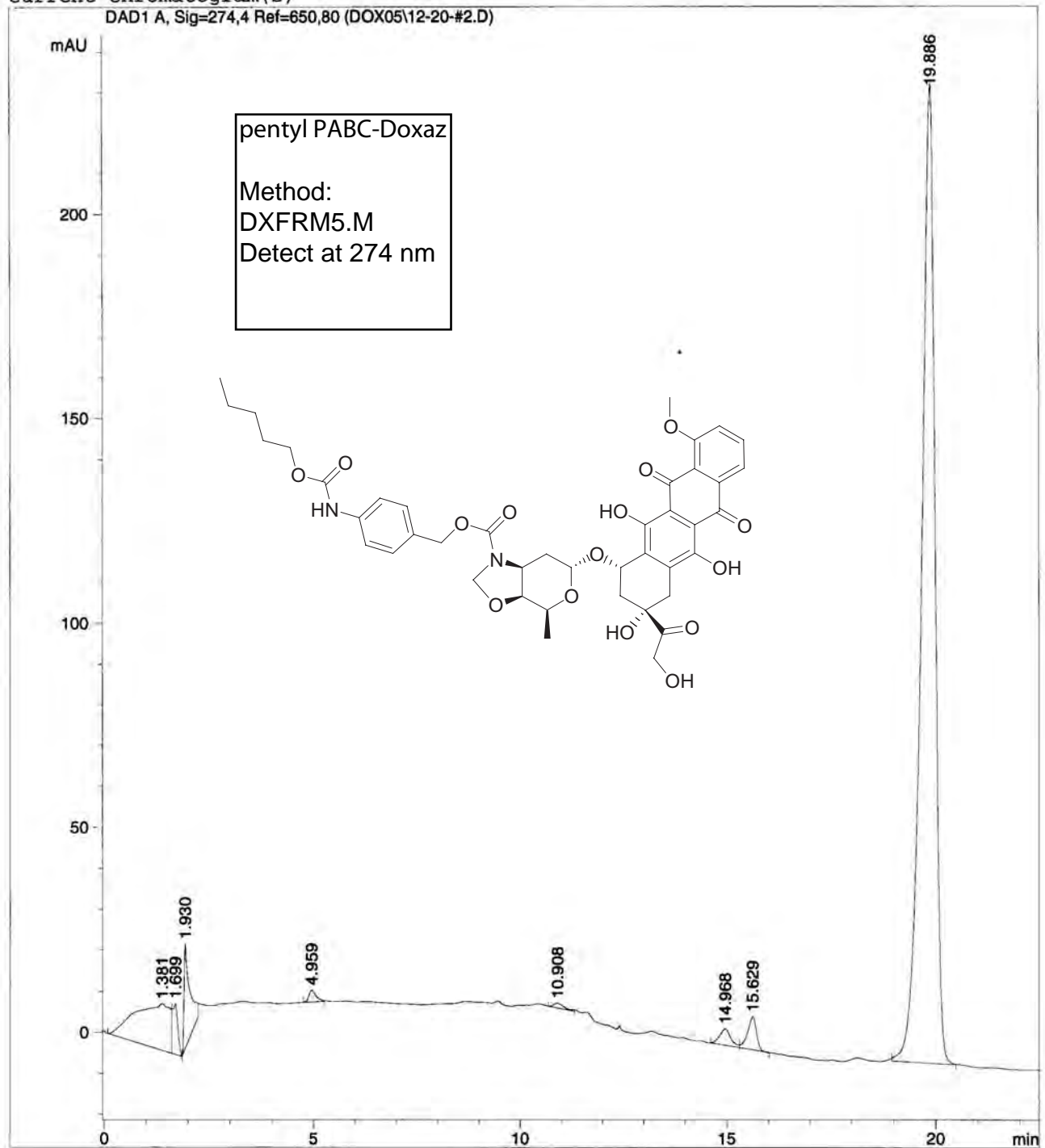


pentyl PABC-Doxaz  
Method :  
DOXAZ1.M  
Detect at 480 nm

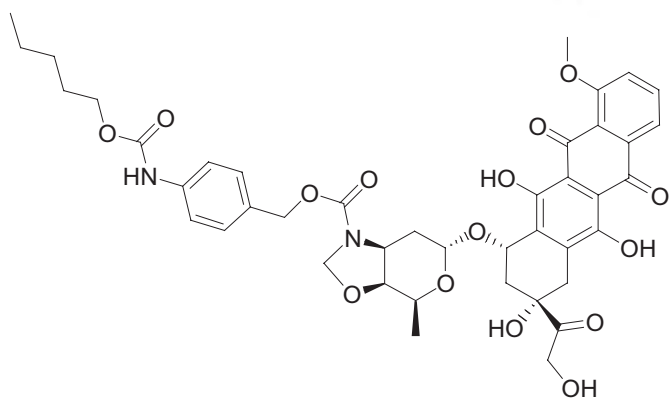


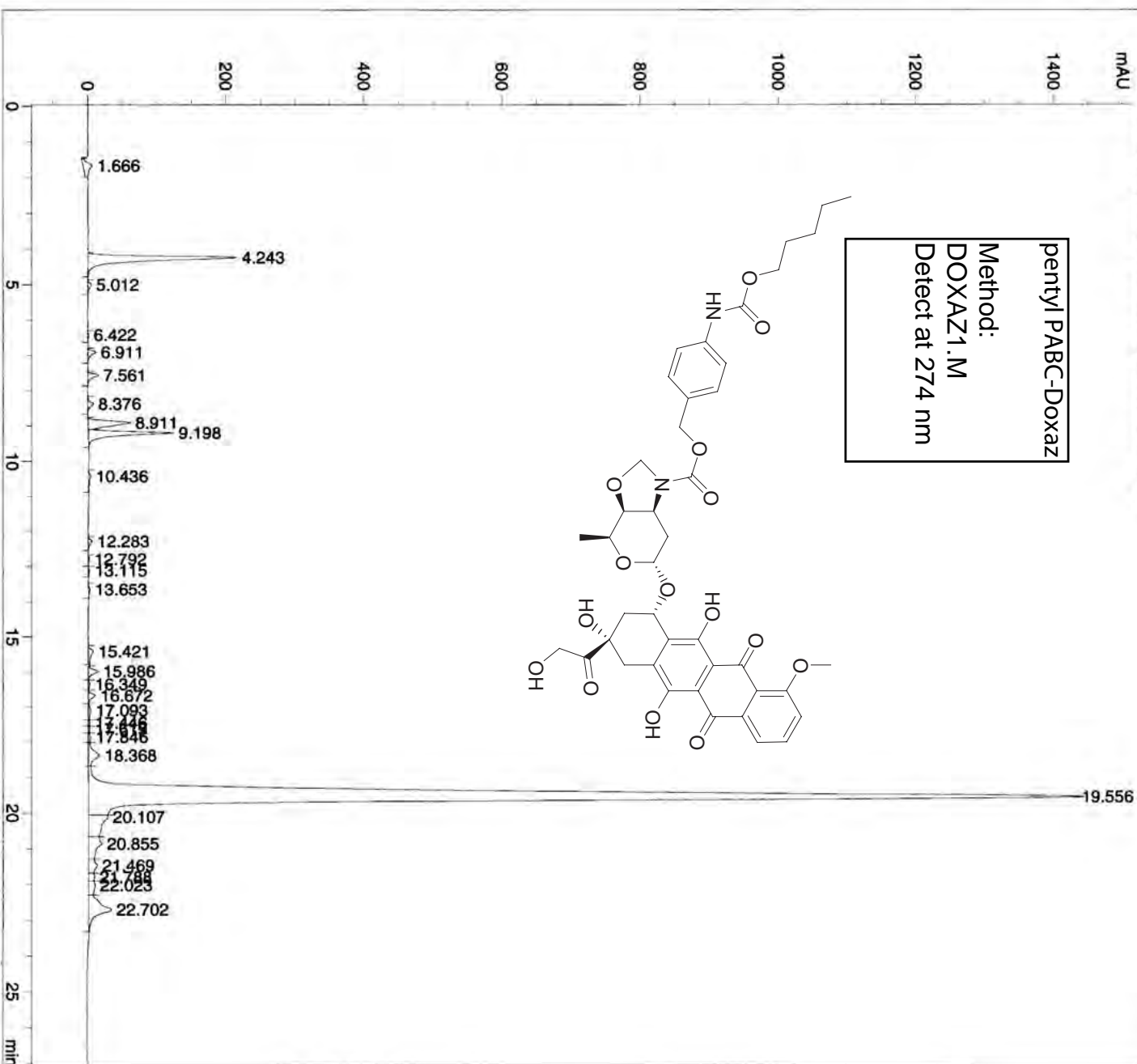
Current Chromatogram(s)

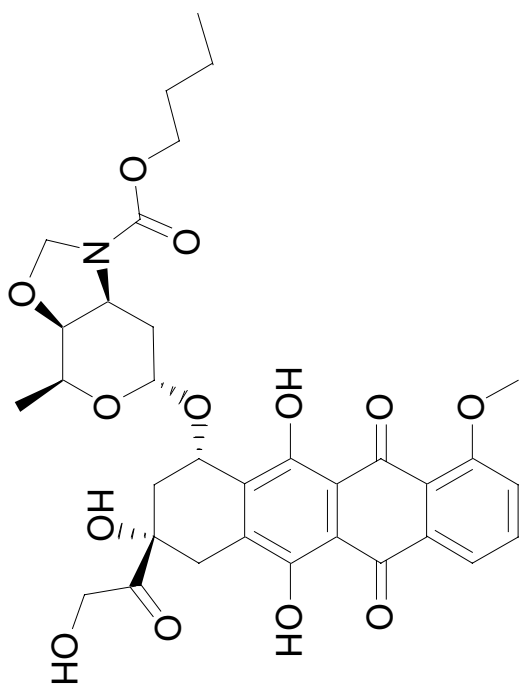
DAD1 A, Sig=274,4 Ref=650,80 (DOX05\12-20-#2.D)



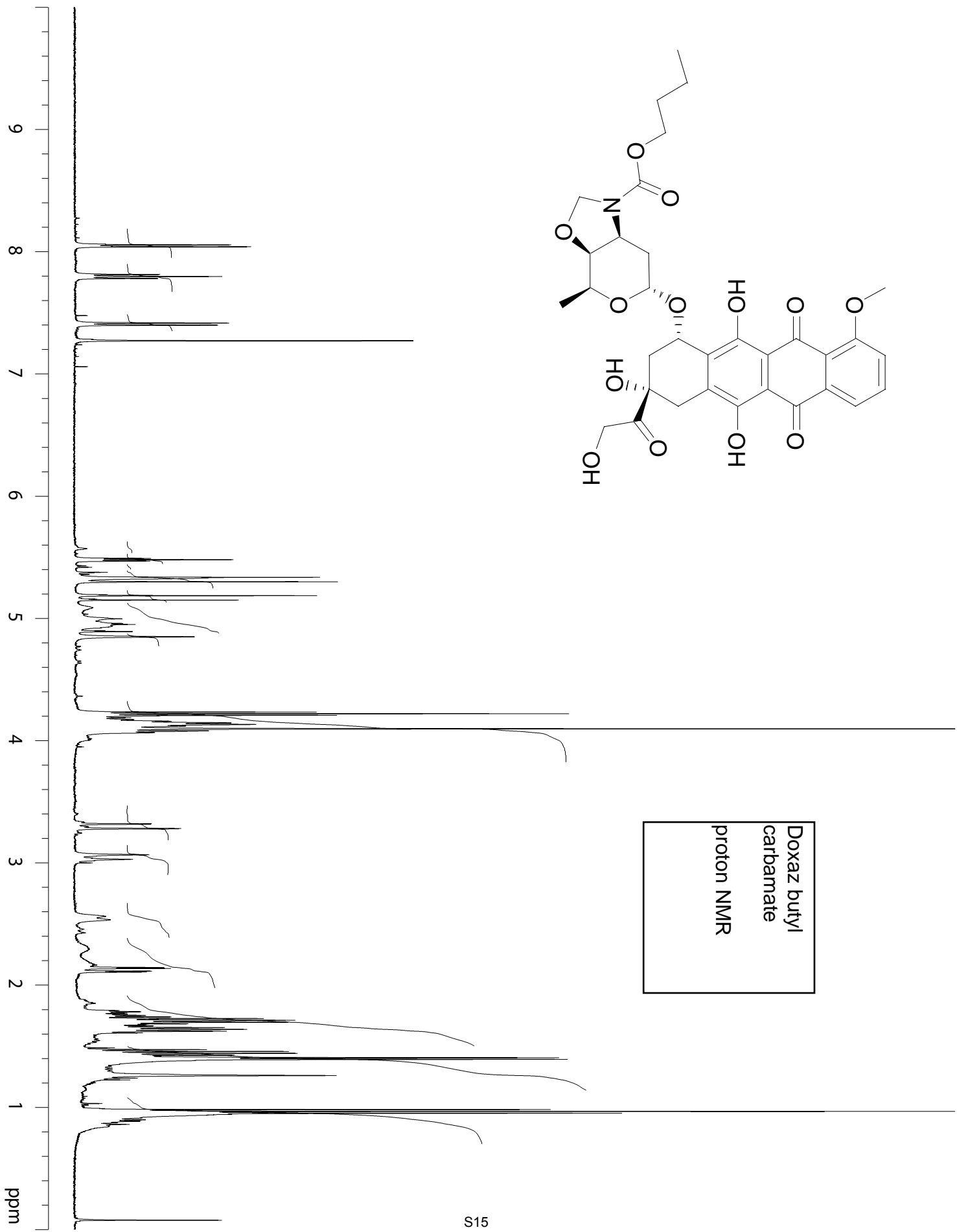
pentyl PABC-Doxaz  
Method:  
DXFRM5.M  
Detect at 274 nm



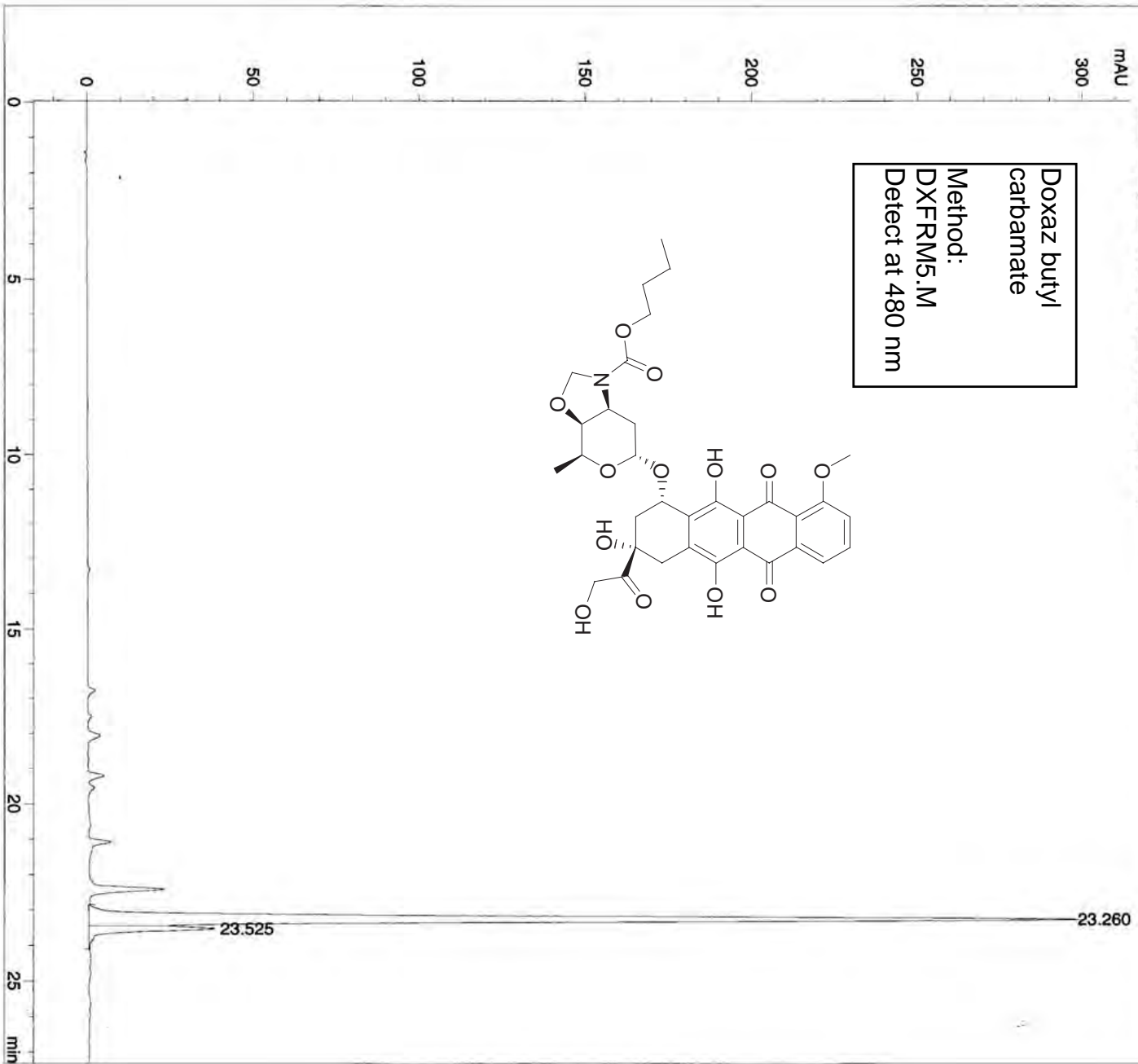
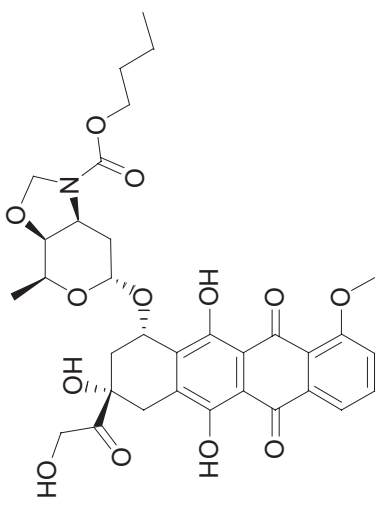




Doxaz butyl  
carbamate  
proton NMR

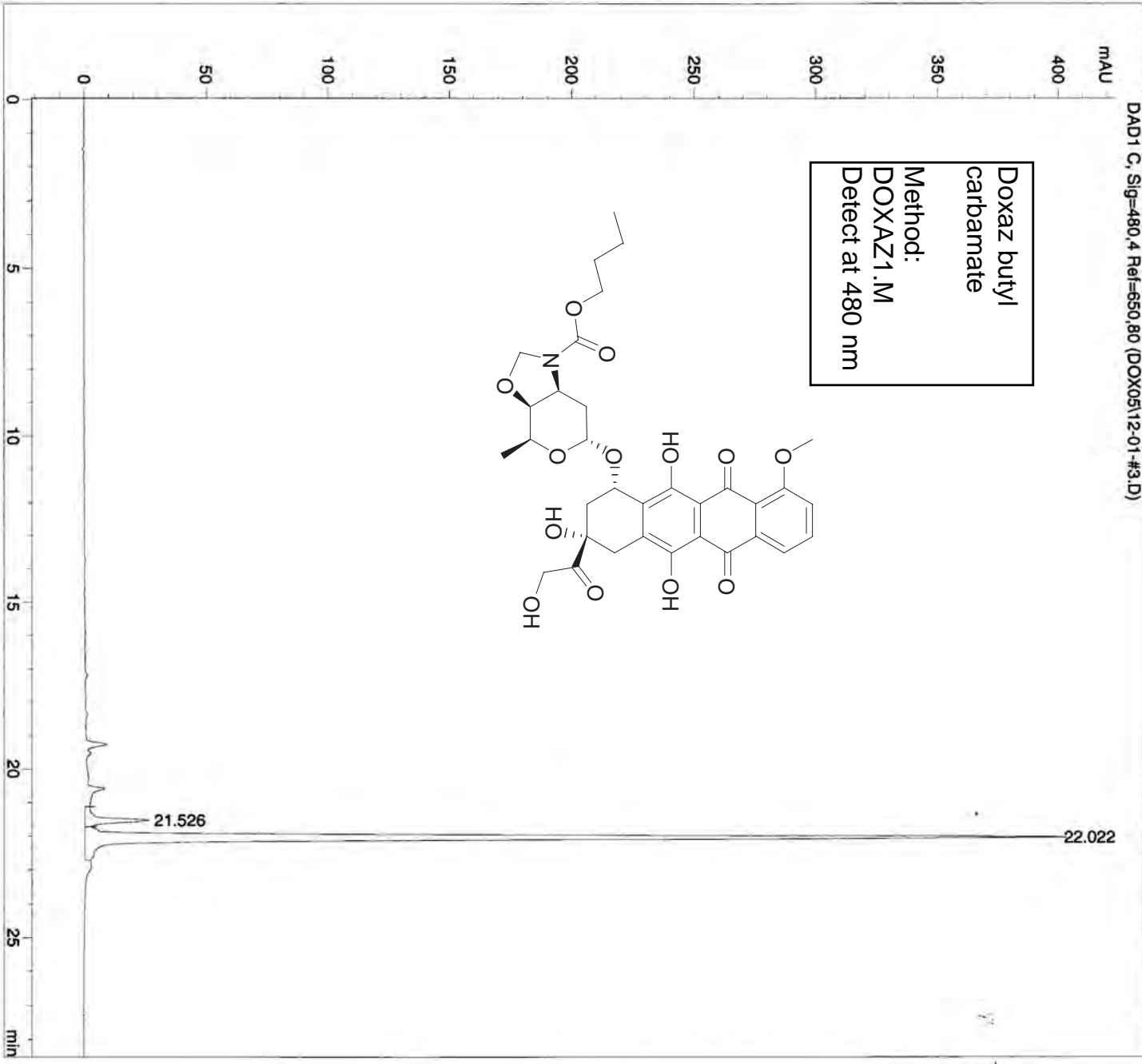
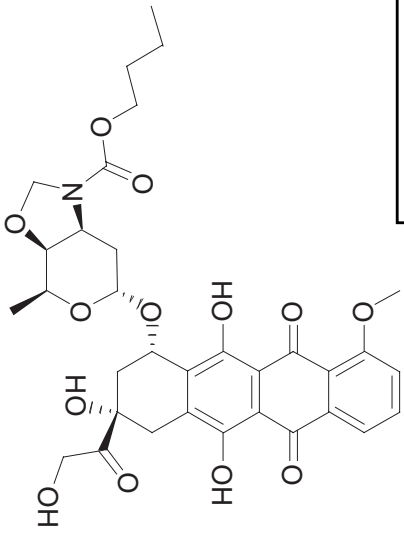


Doxaz butyl  
carbamate  
Method:  
DXFRM5.M  
Detect at 480 nm

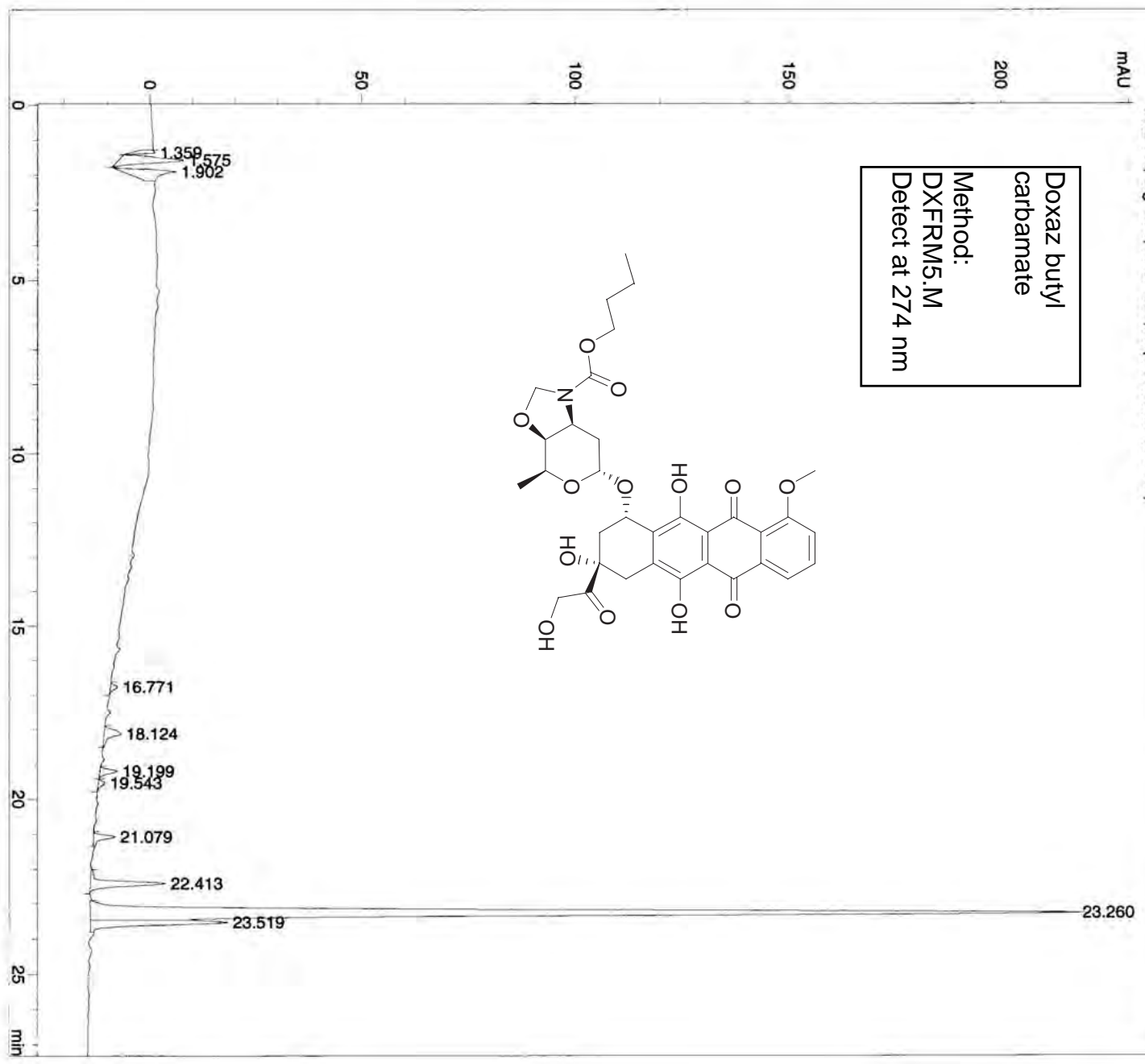
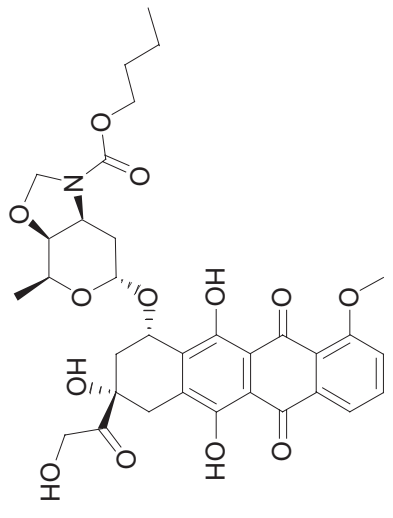




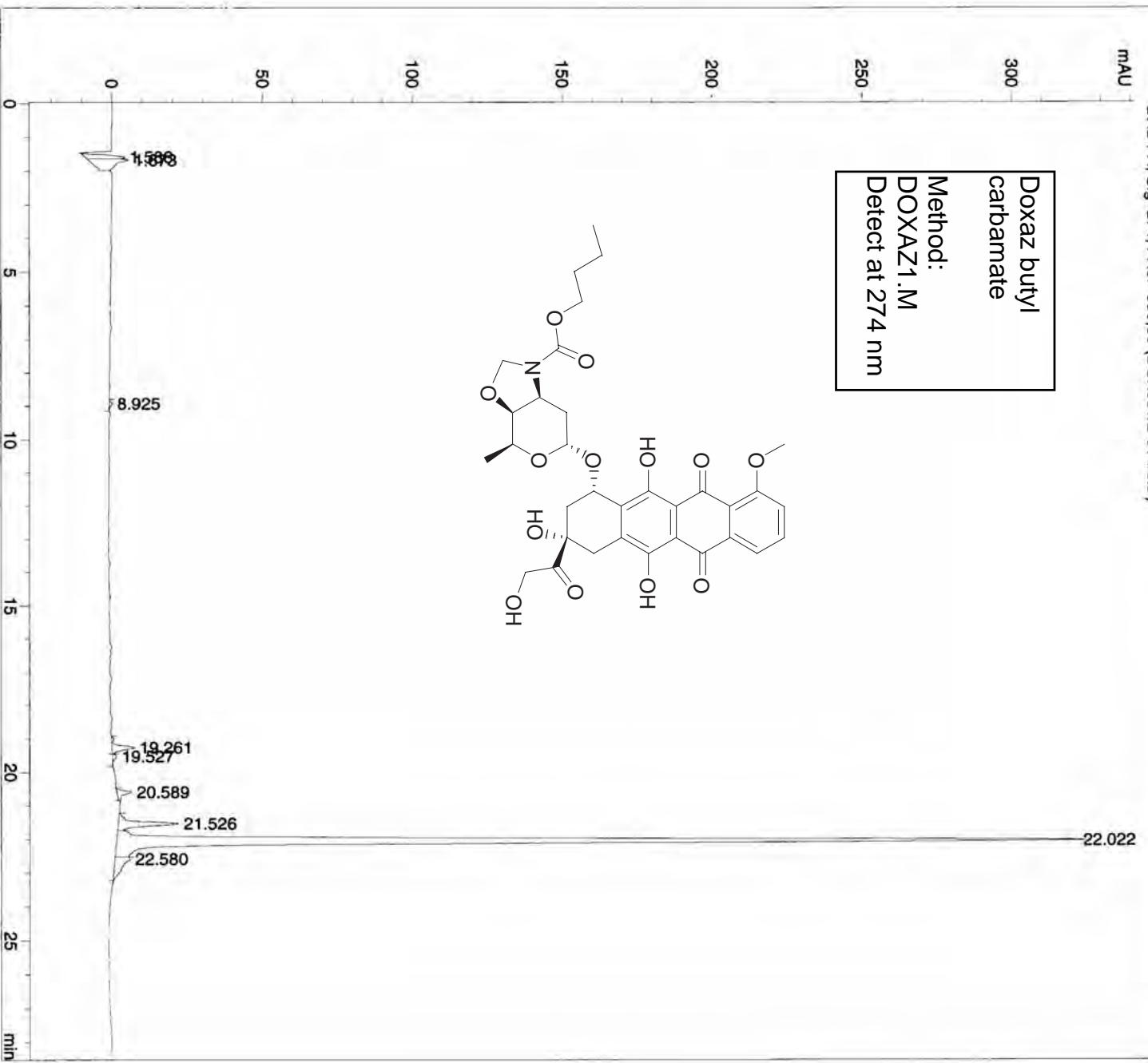
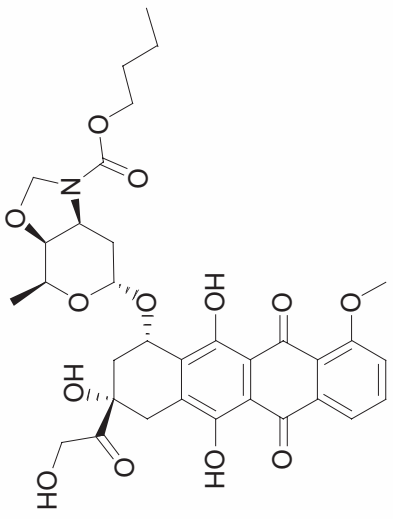
Doxaz butyl  
carbamate  
Method:  
DOXAZ1.M  
Detect at 480 nm

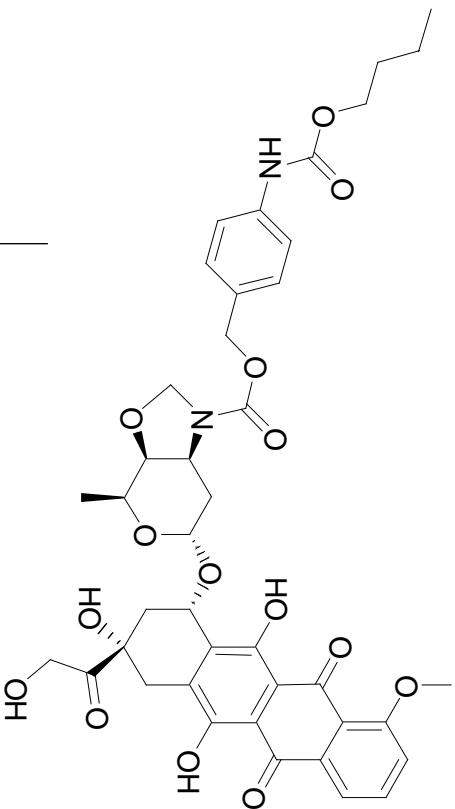


Doxaz butyl/  
carbamate  
Method:  
DXFRM5.M  
Detect at 274 nm

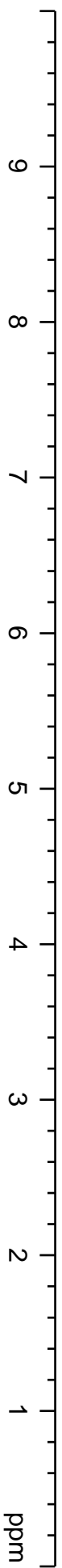


Doxaz butyl  
carbamate  
Method:  
DOXAZ1.M  
Detect at 274 nm





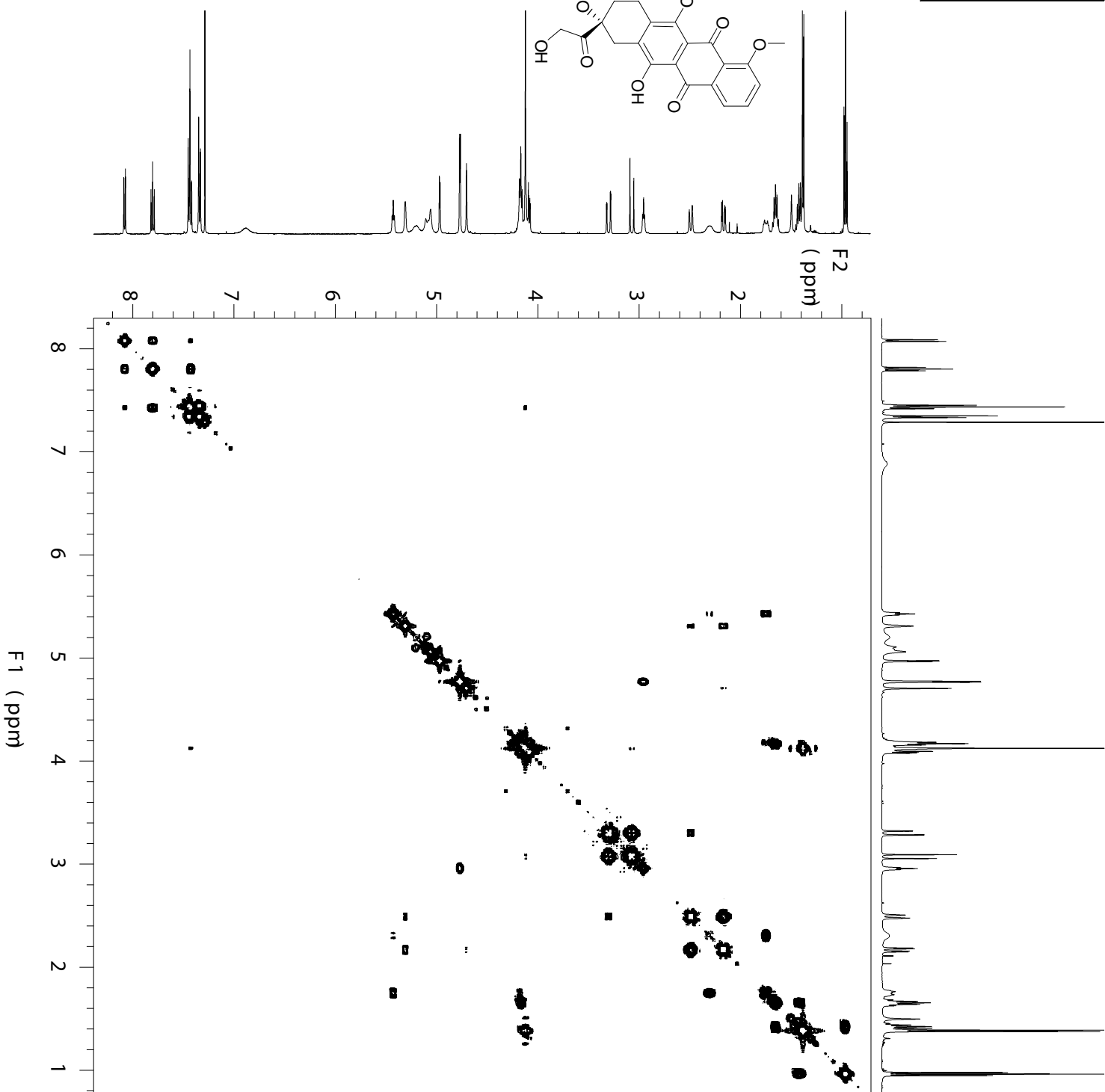
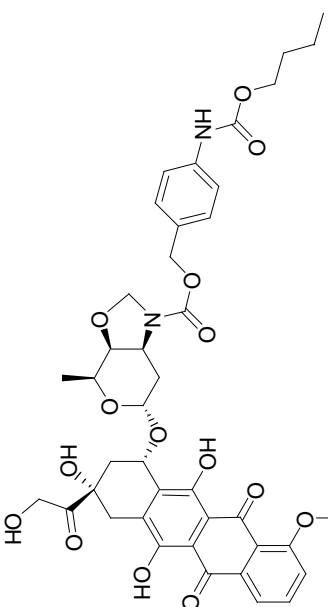
butyl PABC-Doxaz  
proton NMR, T=55°C.



butyl PABC-Doxaz

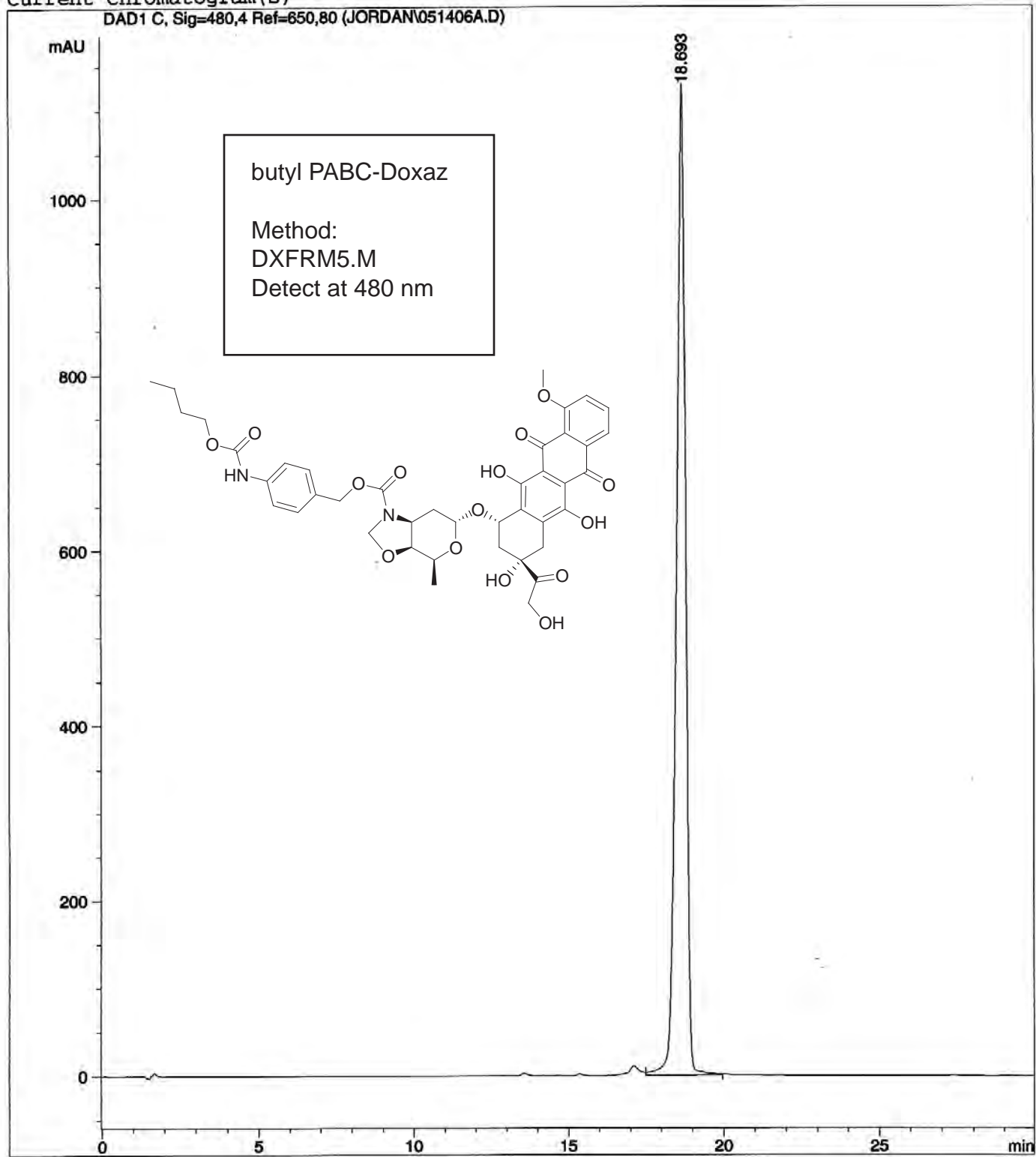
2D-gCOSY NMR

Temp. = 55°C.



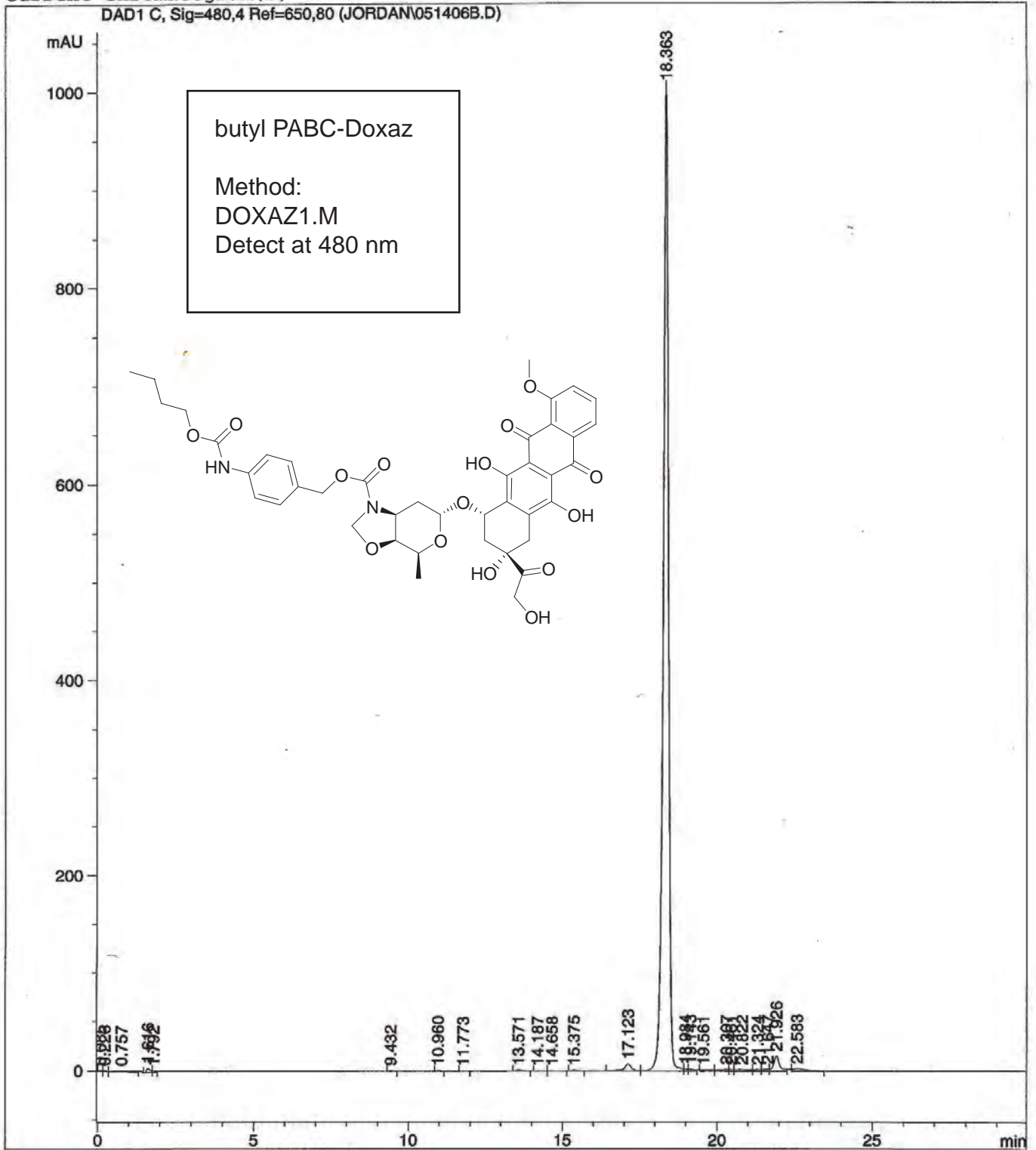
Current Chromatogram(s)

DAD1 C, Sig=480,4 Ref=650,80 (JORDAN\051406A.D)



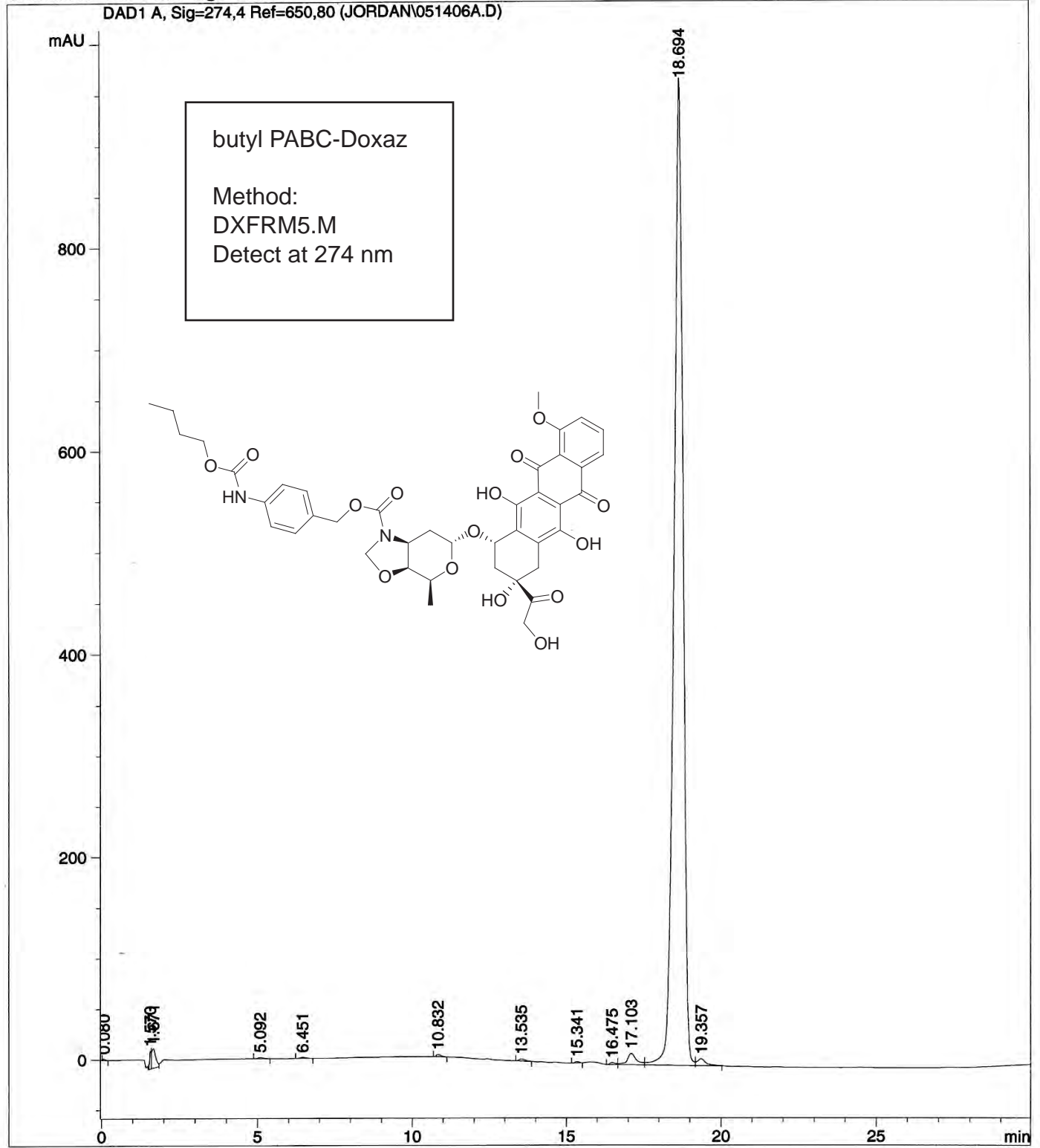
Current Chromatogram(s)

DAD1 C, Sig=480,4 Ref=650,80 (JORDAN\051406B.D)



Current Chromatogram(s)

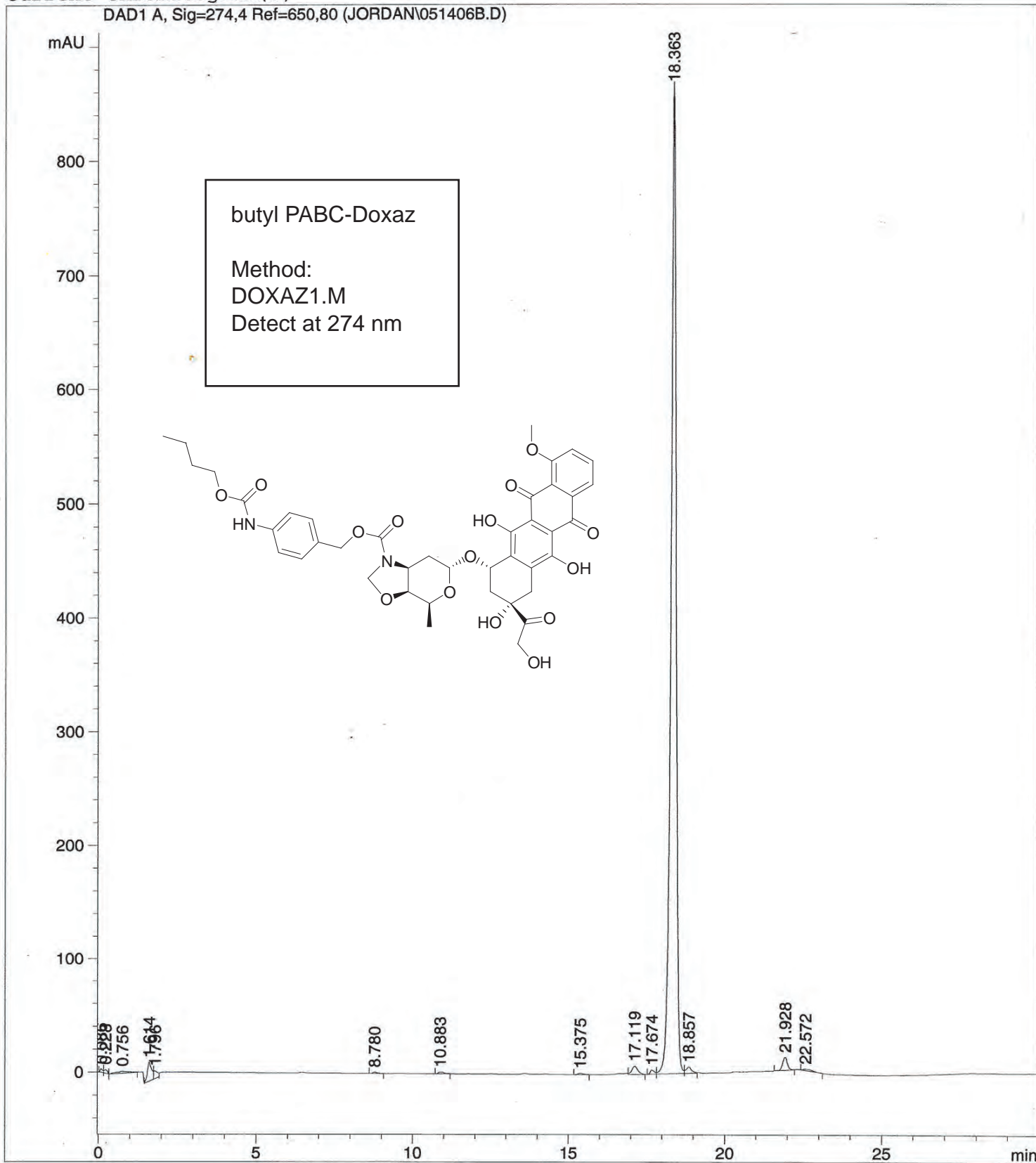
DAD1 A, Sig=274,4 Ref=650,80 (JORDAN\051406A.D)

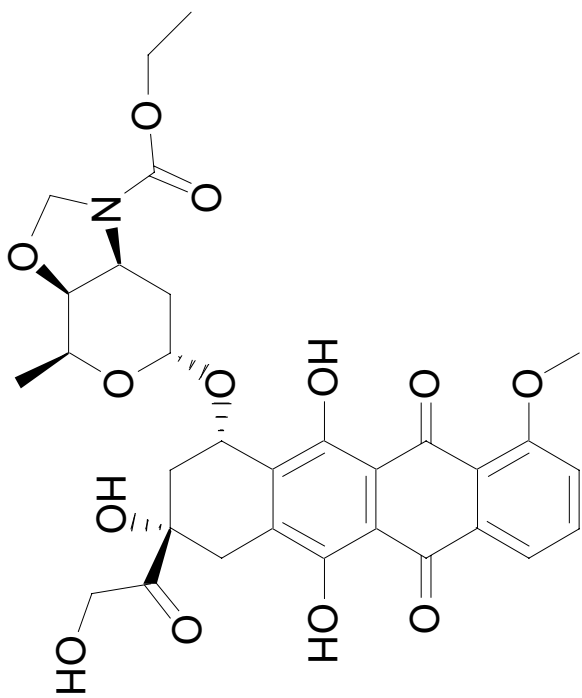




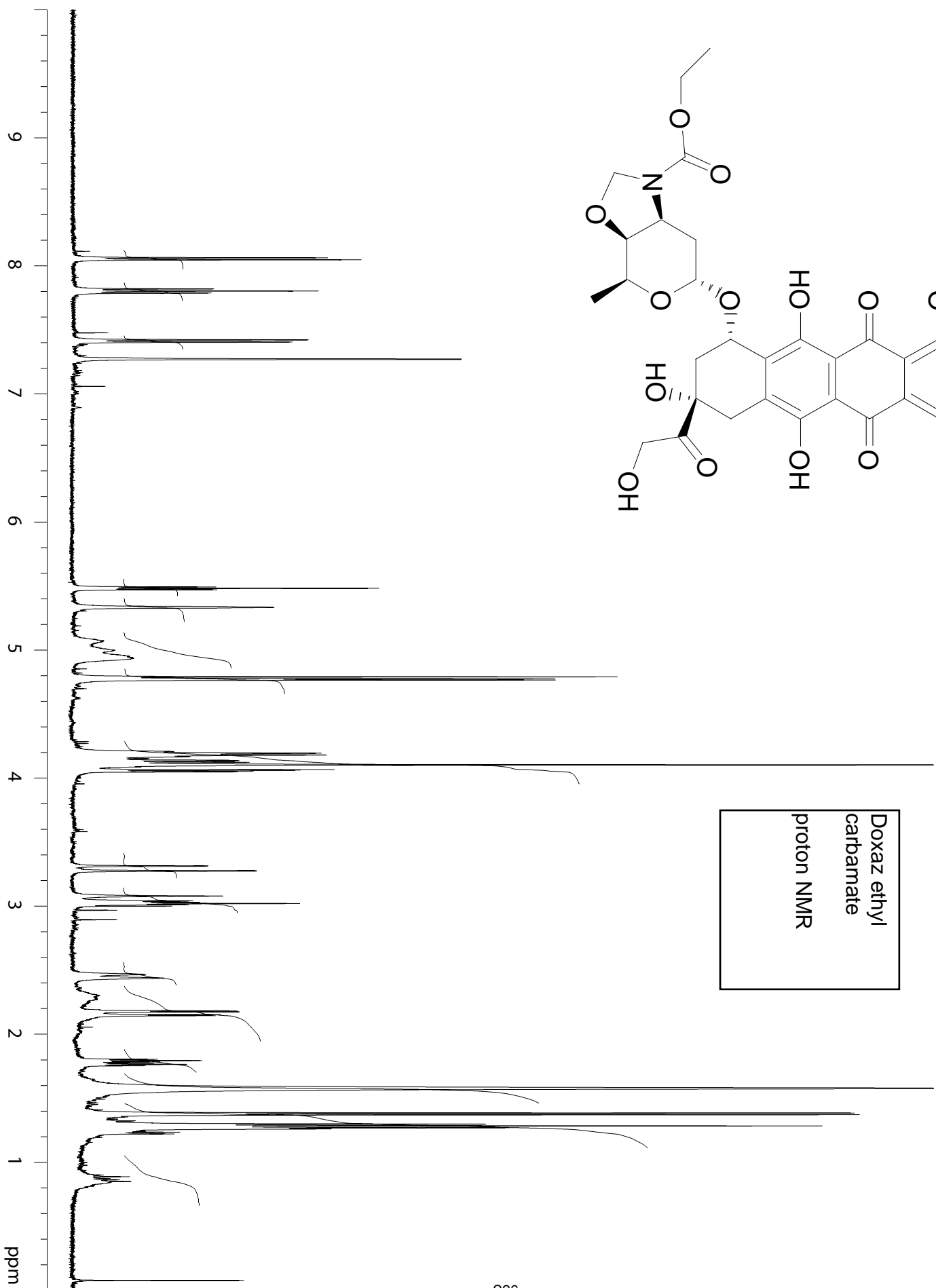
Current Chromatogram(s)

DAD1 A, Sig=274,4 Ref=650,80 (JORDAN\051406B.D)





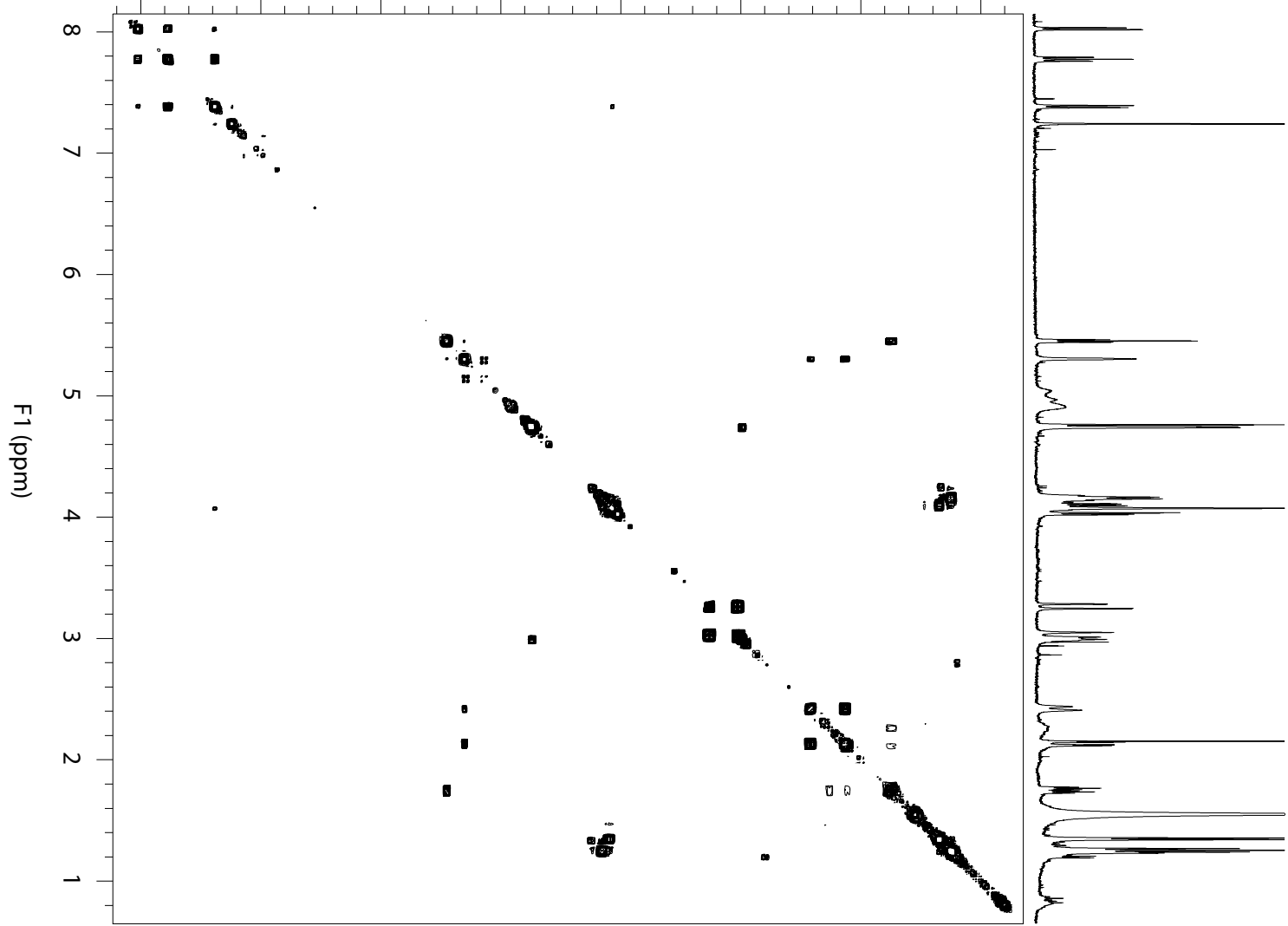
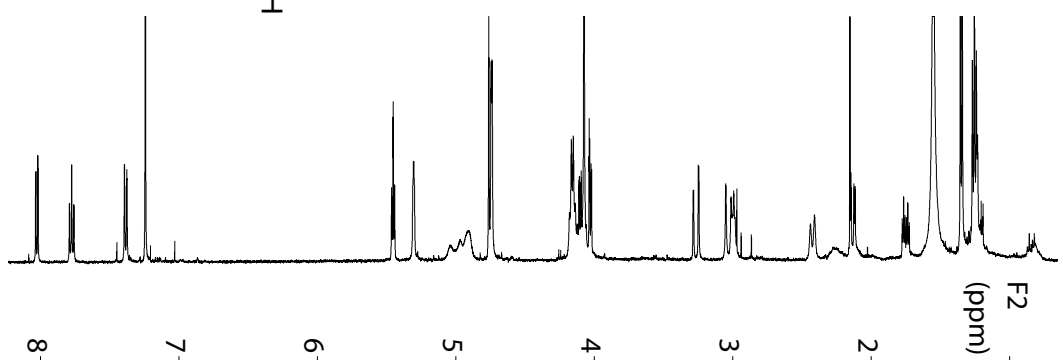
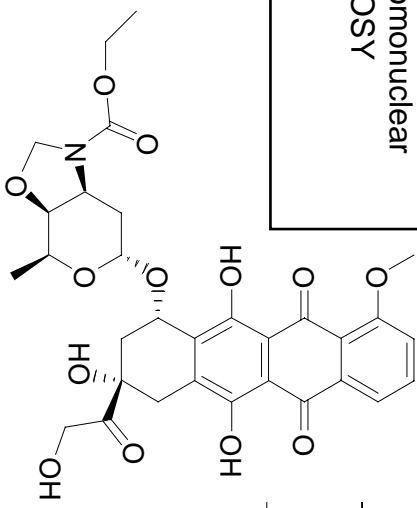
Doxaz ethyl  
carbamate  
proton NMR



UCB Varian Inova-500  
Standard Proton Parameters  
NALORAC 4N Probe  
Pulse Sequence: gCOSY

Solvent: CDCl3  
Ambient temperature  
Relax. delay 1.000 sec  
Acq. time 0.237 sec  
Width 4321.8 Hz  
2D Width 4321.8 Hz  
5 repetitions  
256 increments  
OBSERVE H1, 500.3674488 MHz  
DATA PROCESSING  
Sq. sine bell 0.118 sec  
F1 DATA PROCESSING  
Sq. sine bell 0.030 sec  
FT size 2048 x 2048  
Total time 27 min, 37 sec

Doxaz ethyl  
carbamate  
homonuclear  
COSY

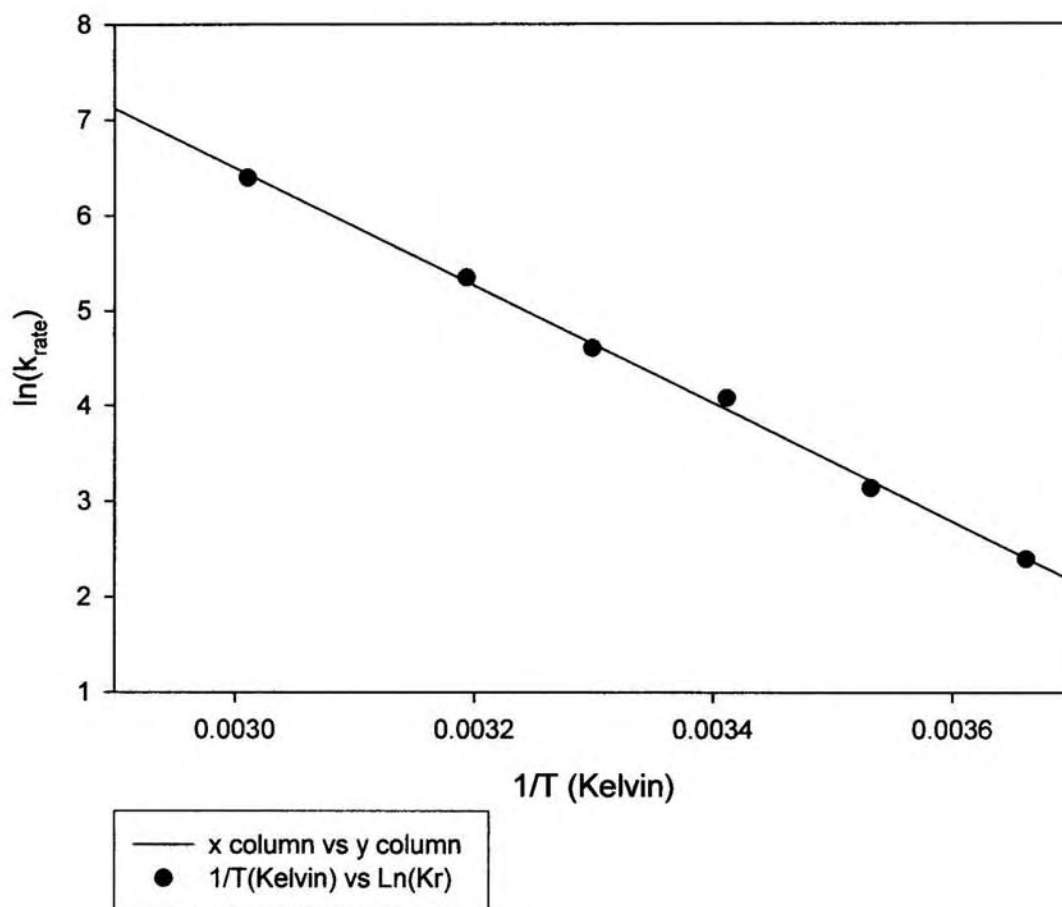


# Ethyl Carbamate, VT NMR Results

Arrhenius Plot {ln(k) vs. 1/T}

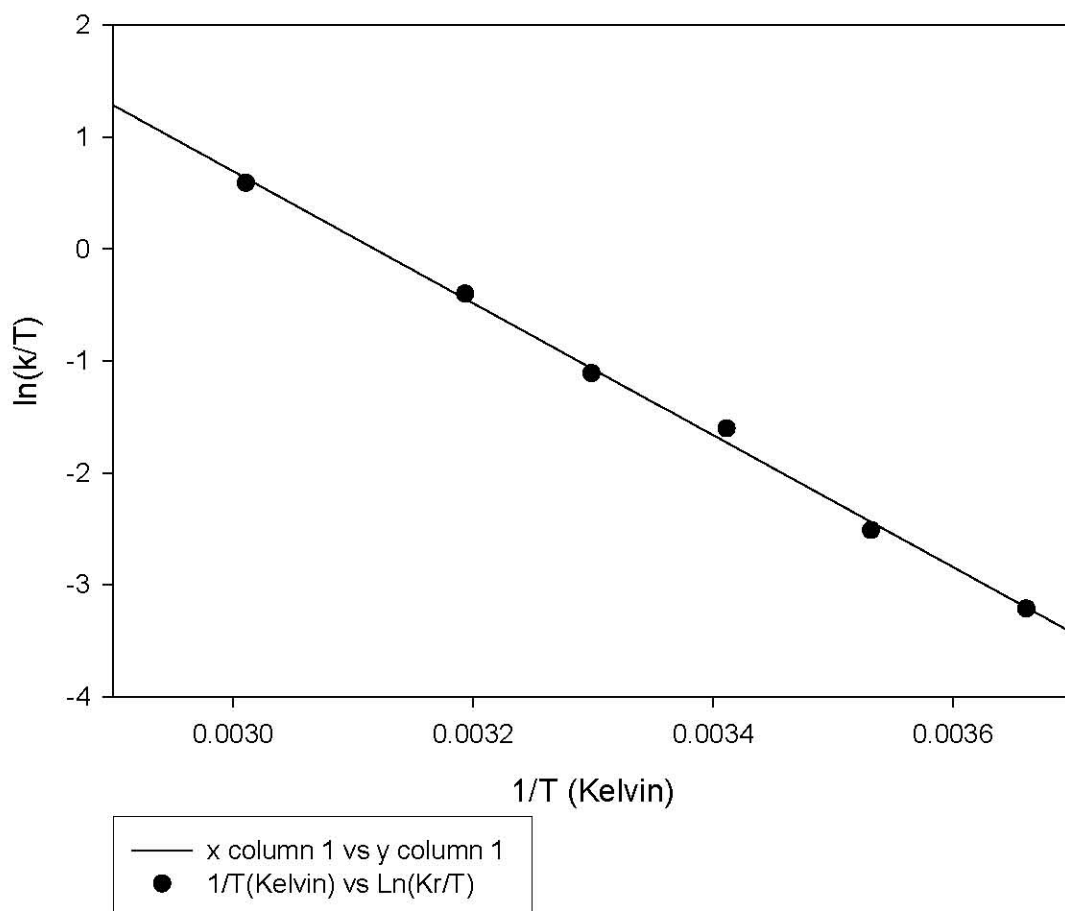
Slope = -6193.9348 (Ea = 12.3 Kcal/Mol)

Y-int = 25.08 (A = 7.80 x10<sup>10</sup>)



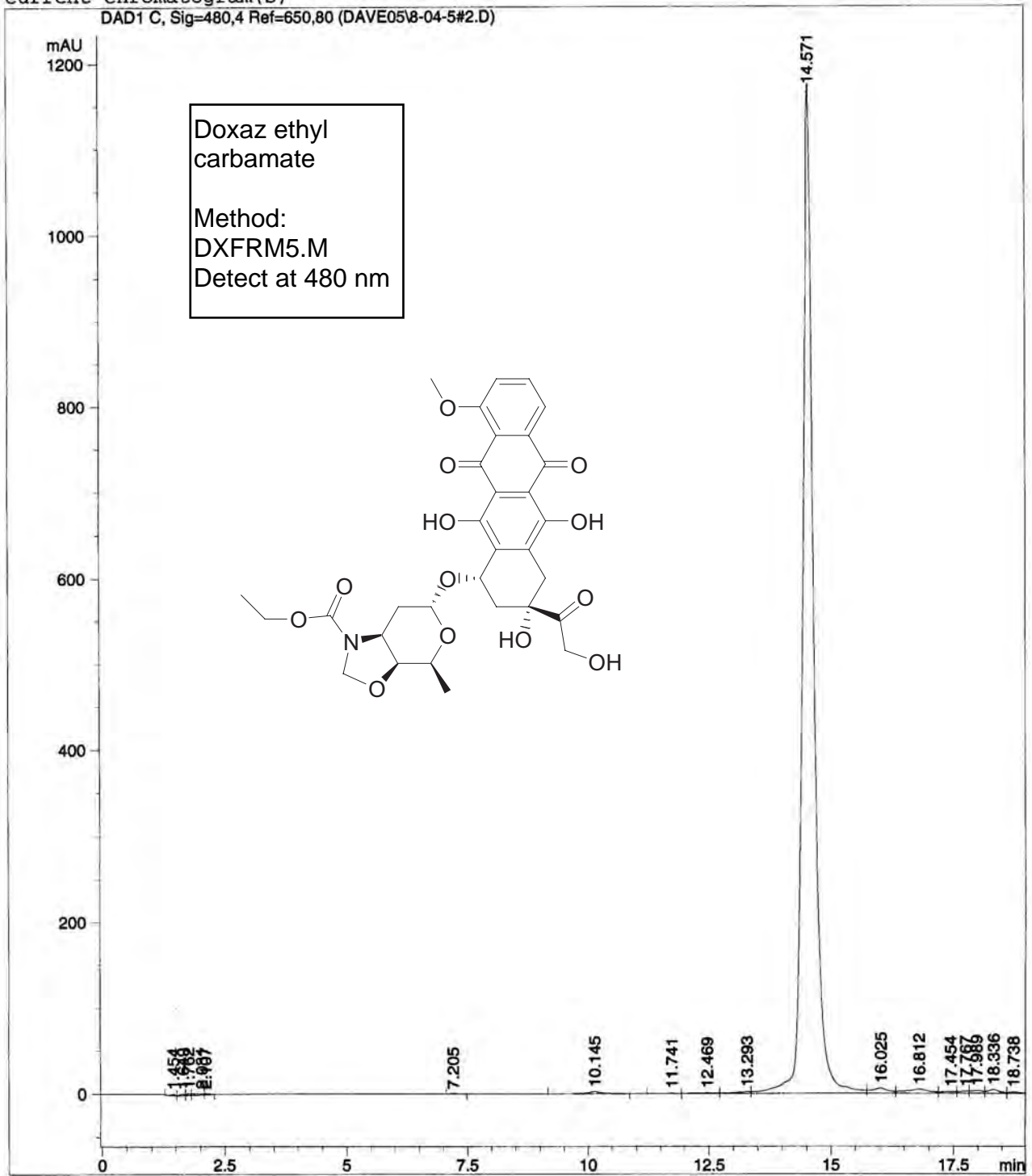
### Eyring Plot { $\ln(k/T)$ vs $1/T$ }

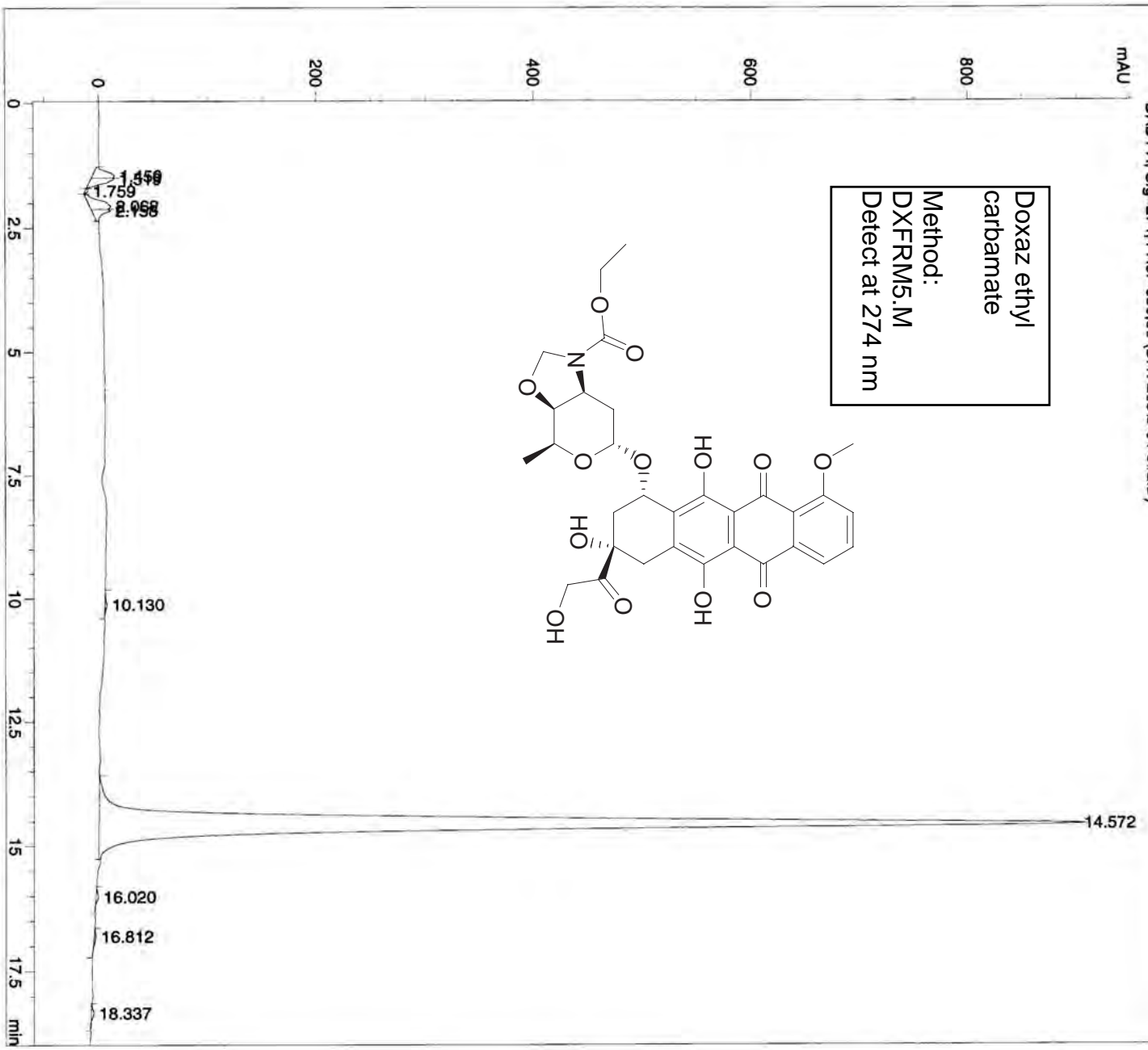
Slope =  $-5893.49$  { $\Delta H^\ddagger 11.71$  Kcal/Mol  $\pm$   $0.31$  Kcal/Mol}  
Y-int =  $18.4$  { $=\Delta S^\ddagger/R + 23.76$ },  $\Delta S^\ddagger = -10.69$  Cal/Mol-Kelvin  $\pm$   $0.99$   
(note: std.error in slope =  $155.3$ , std.error in Y-int= $0.52$ )



Current Chromatogram(s)

DAD1 C, Sig=480,4 Ref=650,80 (DAVE0518-04-5#2.D)





### Primer Sequences for the RT-PCR experiment

Gene-location	Forward Primer	Reverse Primer
hCES1-5'	5'-TGACCAAGAAAAACAGGCTGC-3'	5'-AGAATCCCCAGATGCCAG-3'
rCES1-5'	5'-TGACAAAACGTGATAGACTGC-3'	5'-AGAATCCCCAAATACCCAG-3'
h/rCES1-middle	5'-GGGATTCTTCAGCACAGG-3'	5'-GACTCTCCAAAGATGGTCACA-3'
hCES1-3'	5'-CAATGGAAACCCCAATGGG-3'	5'-TCCACTGCCTTCTTGGCAA-3'
rCES1-3'	5'-GAATGGGAATCCCAATGGA-3'	5'-CAGACCAGAAAGCCACTTCTTT-3'
hCES2-5'	5'-TCTAGGTCCGCTGCGATTTG-3'	5'-GAGTCGGAAGGGAAGGTCATG-3'
rCES2-5'	5'-AGGTCCGCTGCGCTTTG-3'	5'-GCGGGAATGGAAGACAGTATCAT-3'
h/rCES2-middle	5'-ATGTCTGAGGACTGCCTGTA-3'	5'-TCTCCAGTGCTGAAAAAGCCC-3'
hCES2-3'	5'-TGTGTCCCCCATATCCCAAG-3'	5'-CCACCAGGGCCTCAGAGTC-3'
rCES2-3'	5'-TATATCCCCCATGTCTCAAG-3'	5'-GCACCAGGGTCTCTGAGTC-3'

h before the gene name designates the human gene, while r designates it as the rat gene.