THESIS

DESIGN AND SYNTHESIS OF ANALOGS OF THE PEPTIDYLNUCLEOSIDE ANTIBIOTICS THE MUREIDOMYCINS

Submitted by

David Michael Bender

Department of Chemistry

In partial fulfillment of the requirements

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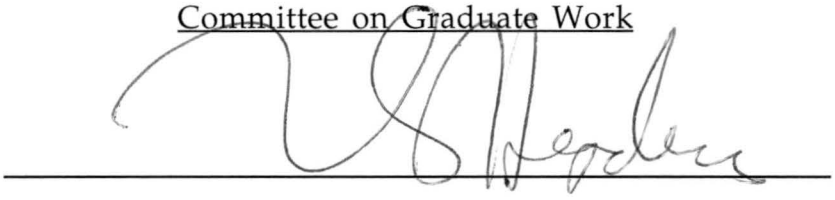
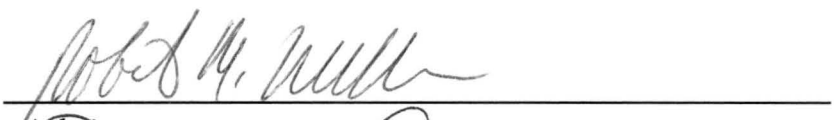
WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY DAVID MICHAEL BENDER ENTITLED THE DESIGN AND SYNTHESIS OF ANALOGS OF THE PEPTIDYLNUCLEOSIDE ANTIBIOTICS THE MUREIDOMYCINS BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

Committee on Graduate Work





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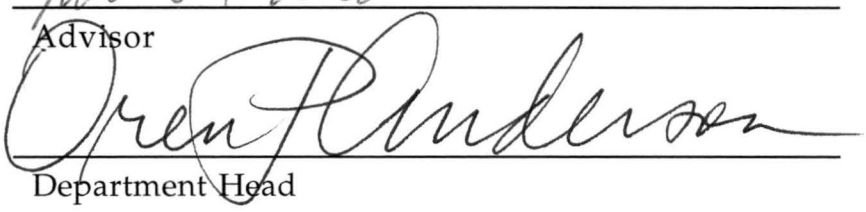

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ABSTRACT OF THESIS

DESIGN AND SYNTHESIS OF ANALOGS OF THE PEPTIDYLNUCLEOSIDE ANTIBIOTICS THE MUREIDOMYCINS

The recent emergence of strains of bacteria which are resistant to many commercially available antibiotics requires a continual search for new drugs to combat infection. A new family of antibiotics, the mureidomycins, has recently been reported. These compounds have been shown exhibit activity against *Pseudomonas aeruginosa* through inhibition of phospho-N-acetylmuramyl translocase, an enzyme involved in the lipid cycle of peptidolycan biosynthesis. Progress toward the total synthesis of this series of natural products is described. Specifically, methodology for the production of 4′,5′-unsaturated enamine nucleosides has been investigated. In addition, two unnatural amino acids, (S)-*m*-tyrosine and 2-amino-3-N-methylaminobuyric acid, have been synthesized in optically pure form.

Molecular modeling was used to predict structural analogs of the mureidomycin antibiotics. These compounds are simple peptidylnucleosides and are derived from coupling of small amino acid subunits to an aminonucleoside core. The synthesis of these analogs is described, including a novel route to 3′,5′-dideoxy-5′aminouridine.

David Michael Bender
Chemistry Department
Colorado State University
Fort Collins, CO 80523
Spring 1998

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I would like to thank Prof. Robert M. Williams for his intellectual and financial support during my career at Colorado State University. The

diversity of his research interests has provided me a unique opportunity to gain exposure to science both within and outside the field of chemistry. In addition, he has fostered a learning environment in which I have been permitted to conduct research in a creative and independent manner.

Lastly, I would like to express my heartfelt thanks to Molly and to my family for their unwavering patience and support.

This work is dedicated to my family.

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Abbreviations

AIBN	1,1-azobisisobutyrlnitrile
AMBA	2-amino-3-N-methylaminobutyric acid
Boc	*tert*-butoxycarbonyl
CBz	benzyloxycarbonyl
DEAD	diethylazodicarboxylate
DIEA	diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	N,N-dimethylformamide
ES	electrospray
FAB	fast atom bombardment
Fmoc	fluorenylmethoxycarbonyl
HMPA	hexamethylphosphoramide
HOAc	acetic acid
HRMS	high resolution mass spectrum
LRMS	low resolution mass spectrum
NaHMDS	sodium hexamethyldisilamide
Phth	phthalimide
TMS	trimethylsilyl
TBS	*tert*-butyldimethylsilyl
TBDPS	*tert*-butyldiphenylsilyl
TFA	triflouroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography

Chapter 1

A. Introduction

Disruption of the peptidoglycan layer of bacterial cell walls has been the most prominent target in the develpment of new drugs used in the treatment of bacterial infection. Gram-(+), Gram-(-), and acid-fast (*Mycobacteria* sp.) bacteria are extremely susceptible to agents that inhibit these biosynthetic pathways, as they are both unique and essential to these organisms. Peptidoglycan biosynthesis is catalyzed by a series of cytoplasmic and membrane-associated enzymes, and has generally been organized into three distinct stages.[1] The first stage involves the formation of nucleotide-linked cell wall precursors which serve as the building blocks of the peptidoglycan. In the second stage, these precursors are transferred across the cytoplasmic membrane onto a lipid carrier and transported to the site of growing peptidoglycan. Incorporation of these monomeric units into the cell wall, followed by cross-linking constitutes the third and final stage of this cycle. A great deal of research has focused on the development of drugs that interfere with these processes.

1. The Role of Translocase in Peptidoglycan Biosynthesis

The enzyme responsible for the transfer of the cell wall precursor, phospho-N-acetylmuramyl-pentapeptide, from the nucleotide carrier uridine diphosphate to the lipid carrier undecaprenyl phosphate is phospho-N-acetylmuramyl-pentapeptide translocase (*translocase I*).[2] (**Figure 1**) Very little is known about this reaction, and currently there are no commercial drugs which exhibit activity as a result of inhibition of this enzyme.

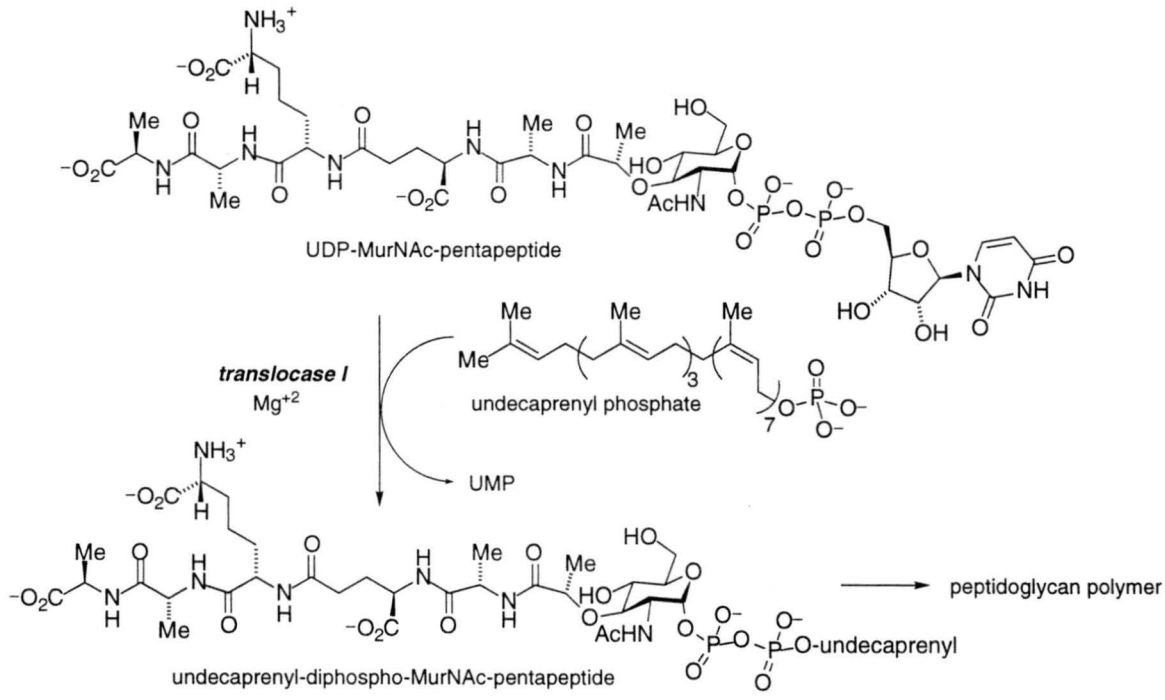
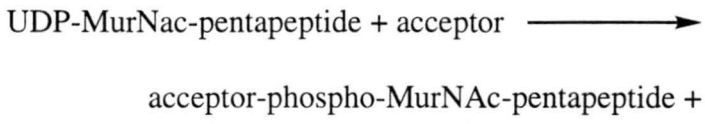


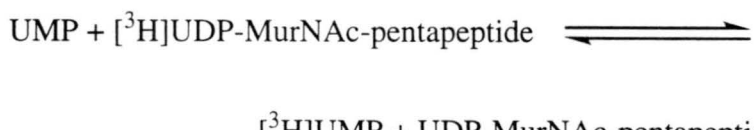
Figure 1 The Role of Translocase

The existence of translocase was first postulated by Neuhaus and co-workers.[2] Membrane preparations from *Staphylococcus aureus* were incubated with UDP-MurNAc-[^{14}C]-pentapeptide in the presence of Mg^{2+}, afterwhich was observed a transfer of radioactivity to some acceptor associated with the membrane. Additional labeling studies showed that one of the

phosphate groups from UDP was incorporated into the membrane, while the second phosphate remained with the nucleotide to form uridine monophosphate (UMP). This transfer reaction is summarized by the following:



The structure of the acceptor was later determined by Higashi, Strominger, and Sweeley to be the C_{55} isoprenoid alcohol undecaprenyl monophosphate.[3] In further studies by Neuhaus[4], a decrease in incorporation of labeled material into the membrane over time was observed as a result of the build up of UMP, which was found to react with the lipid bound substrate in the reverse reaction. This finding was supported by incubating labeled [^{3}H]UDP-MurNAc-pentapeptide with unlabeled UMP, which resulted in the production of [^{3}H]UMP.



Following this work, kinetic studies were performed[4] on the natural system as denoted by the following reversible reaction:

UDP-MurNAc-pentapeptide + undecaprenyl-P $\rightleftharpoons$

undecaprenyl-PP-MurNAc-pentapeptide + UMP

An equilibrium constant (K_{eq}) was calculated to be 0.25 $\pm$ 0.04, corresponding to a $\Delta G^{\circ\prime}$ of +0.98 kcal, indicating that the transphosphorylation proceeds without significant loss in free energy.

The first structural data to be obtained for translocase was presented in 1991 by Matsuhashi et al[5]. In *Escherichia coli*, several gene clusters, referred to as murein synthetic gene clusters *mra*, *mrb*, *mrc*, *mrd*, and *mre* were studied in order to determine which genes encode for enzymes involved in peptidoglycan biosynthesis. Within the largest of these clusters, *mra*, the gene *mraY* was identified as encoding for a 39.5 kDa protein shown to catalyze the transfer reaction reported by Neuhaus. Accordingly, the complete amino acid sequence of translocase was determined from the base sequence of *mraY*.

2. Inhibition of Translocase

There are currently no commercial drugs which target translocase. However, several compounds have been discovered which inhibit the activity of this enzyme.[6]. In 1953, amphomycin (**1**) was reported to inhibit peptidoglycan biosynthesis. The structure of this antibiotic was elucidated decades later[7], and was found to consist of a fatty acid moiety linked to an eleven amino acid peptide. Omura and co-workers[8,9] later showed that the compound's activity was accompanied by the accumulation of UDP-MurNAc-

pentapeptide, and that the mechanism of action involved inhibition of the transfer of phospho-N-acetylmuramyl pentapeptide to undecaprenyl phosphate. Amphomycin also interferes with mammalian glycoprotein biosynthesis by inhibiting the production of dolichyl phosphoryl mannose (Man-P-Dol), dolichyl phosphoryl glucose (Glc-P-Dol), and dolichyl pyrophosphoryl N-acetylglucosamine (GlcNAc-P-P-Dol).[10,11] This toxicity prevents its use as an antibiotic.

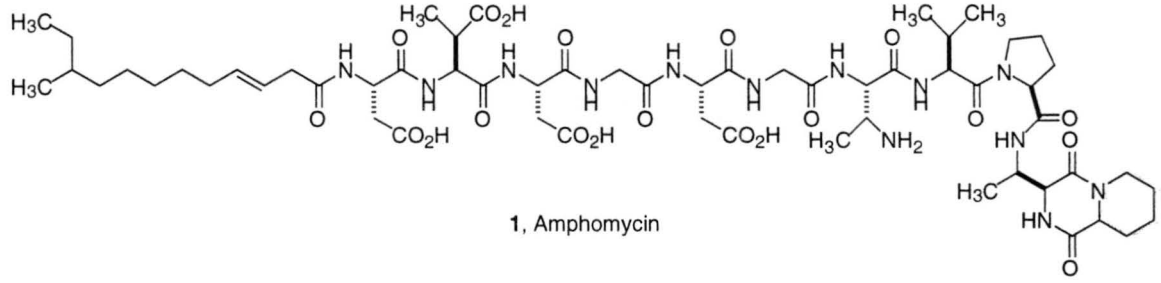
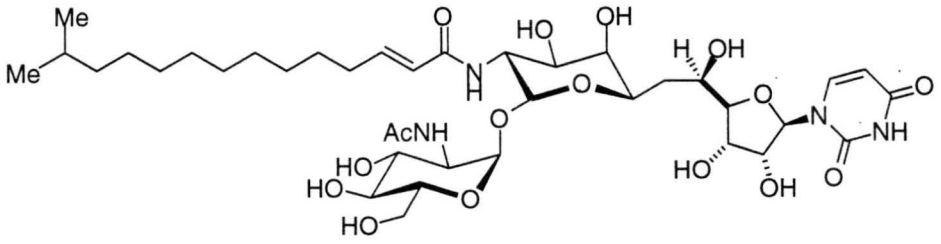


Figure 2 Amphomycin

Tunicamycin (**2**) was isolated from *Streptomyces lysosuperficus* in 1971 by Takatuski *et al.*[12] This compound exhibits a wide range of activity against animal and plant viruses, Gram-(+) bacteria, yeast and fungi. It was later determined that the site of antibacterial activity was phospho-MurNAc-pentapeptide translocase and that was exhibited with an ID_{50} of 12 μg/mL.[13] The structure of this natural product contains the nucleotide uracil which is attached to the unusual aminodialdose tunicamine. N-acetylglucosamine is attached to the anomeric position of tunicamine, while a fatty acid is linked to the amine. The total synthesis of tunicamycin was recently completed by

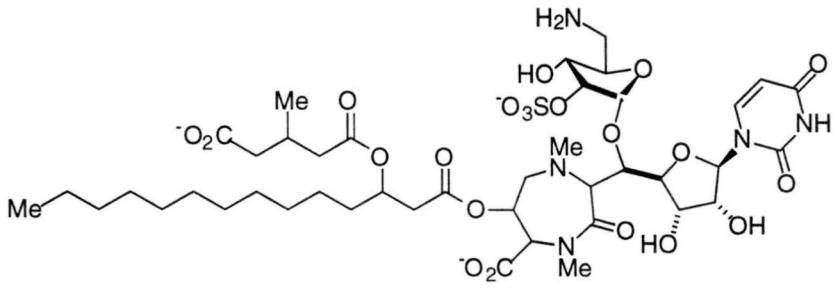
Myers.[14] As in the case of amphomycin, tunicamycin also inhibits the synthesis of animal cell membranes[15] and is therefore toxic.



2, Tunicamycin-V

Figure 3 Tunicamycin

A structurally similar group of antibiotics, the liposidomycins (**3**) were isolated from *Streptomyces griseosporeus* and were found to inhibit translocase, having activity approximately three orders of magnitude greater than tunicamycin (ID_{50}=0.038 μg/mL).[16] These compounds also contain the uridine nucleotide core, as well as a sulfated aminosugar and a fatty acid. As their similarity to tunicamycin predicts, the liposidomycins are also potent inhibitors of glycoprotein synthesis.



3, Liposidomycin C

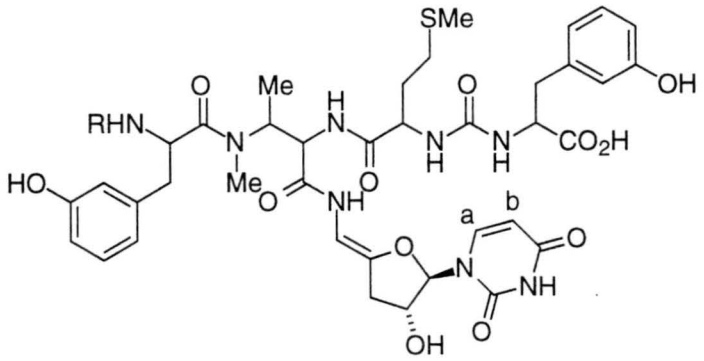
Figure 4 Liposidomycin

In summary, although all three of these compounds exhibit *in vitro* antibacterial activity through inhibition of translocase, their interference with mammalian biosynthesis renders them too toxic for use as commercial antibiotics.

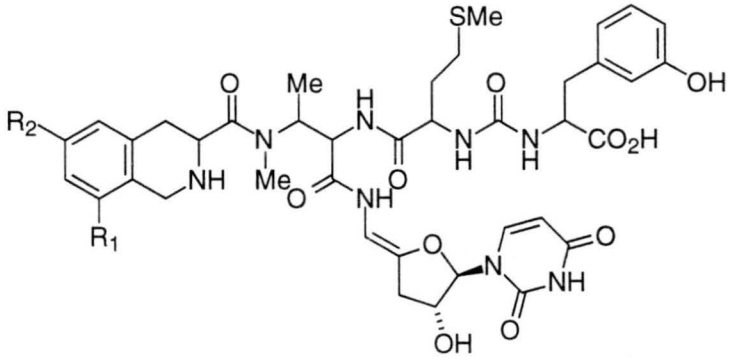
3. Inhibition of Tranalocase by Peptidylnucleoside Antibiotics

In 1989, researchers at the Sankyo Co., Ltd. reported the isolation of a series of new antibiotics known as the mureidomycins[17] (**4**) (**Figure 5**). These compounds are produced by *Streptomyces flavidovirens* SANK 60486 and were isolated from soil samples. Initially, four compounds, mureidomycins A~D, were identified. In 1993, two additional members of this family of antibiotics, mureidomycins E and F, were also discovered.[18]

The mureidomycin antibiotics are referred to as peptidylnucleosides based on their structural properties. All six compounds contain the modified nucleoside 3′-deoxyuridine attached to a tetrapeptide *via* an unusual enamine sugar linkage. Analysis of the amino acid sequence of MRDs A~D revealed the presence of two unnatural amino acids, 2-amino-3-N-methylaminobutyric acid (AMBA) and *m*-tyrosine, as well as the naturally occurring methionine. In addition, one of the amide bond linkages is replaced by a urea. Mureidomycins E and F are structurally identical with the exception of the N-terminal tyrosine residue which has undergone condensation with formaldehyde yeilding a tetrahydroisoquinoline. Mureidomycins A~F were screened against a number of Gram-(+) and



4a, Mureidomycin A, R =H
4b, Mureidomycin B, R = H, $\Delta^{a,b}$ reduced
4c, Mureidomycin C, R = glycyl
4d, Mureidomycin D, R = glycyl, $\Delta^{a,b}$ reduced



4e, Mureidomycin E, R_1 = OH, R_2 = H
4f, Mureidomycin F, R_1 = H, R_2 = OH

Figure 5 The Mureidomycin Antibiotics

Gram-(-) organisms and were found to be specifically active against *Pseudomonas aeruginosa*.[17c, 18]

The most active compound, MRD C with MICs of 0.1 to 3.13 μg/mL, was assayed along with with several β-lactam antibiotics, including fosfomycin (FOS), cefsoludin (CFS), ceftazidime (CAZ), cefoperazon (CPZ), carbenicillin (CBPC), sulbenicillin (SBPC), piperacillin (PIPC), and cefotaxime

Table 1 Antimicrobial Activity of Mureidomycins

Test Organism	A	B	C	D
Staphylococcus aureus FDA 209PJC1	200	>200	>200	>200
Escherichia coli NIHJ JC-2	>200	>200	>200	>200
Proteus mirabilis B-30-1	>200	>200	>200	>200
Serratia marcescens	>200	>200	>200	>200
Klebsiella pneumoniae PCI 602	25	25	12.5	25
Pseudomonas aeruginosa SANK	6.25	6.25	1.56	6.25
P. aeruginosa SANK 75775	6.25	12.5	1.56	6.25
P. aeruginosa SANK 1080	25	50	3.13	12.5
P. aeruginosa SC 8753	12.5	25	3.13	12.5
P. aeruginosa SANK 73279	12.5	25	1.56	6.25
P. aeruginosa NRRL B1000	25	50	3.13	6.25
P. aeruginosa ATCC 13388	25	50	3.13	6.25
P. aeruginosa SANK 70479	6.25	12.5	1.56	6.25
P. aeruginosa SANK 70579	<0.1	0.2	<0.1	6.25
P. aeruginosa NCTC 10490	12.5	25	0.4	6.25

Mueller-Hinton agar. Innoculum size 10^6 cells/mL.
Activity reported in μg/mL.

(CTX). MRD C had equal or greater potentcy against *Pseudomonas* relative to all drugs tested (**Table 2**). MRD-resistant strains were produced from cultures grown at four times the MIC. Although the aquisition of resistance to MRD was found to be somewhat higher than that for β-lactam antibiotics, no cross resistance between MRDs and β-lactam drugs was observed.

In vivo assays for the mureidomycins were performed on mice infected with *P. aeruginosa* SANK 75775.[17c] Drugs were administered subcutaneously immediately and four hours after infection and ED_{50}'s were calculated for MRDs A~D as 69, 75, 50 and >100 mg/kg respectively. Mice tolerated intravenous doses of 400 mg/kg of mureidomycins A and C for a

Table 2 Activities of MRD C, CBPC, SBPC, CTX, PIPC, and CPZ against *P. aeruginosa*

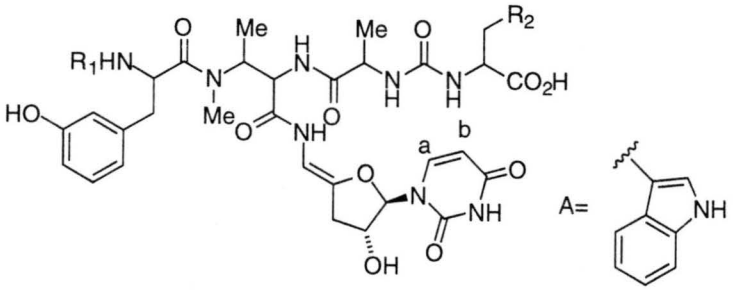
Test Organism	MRD C	CBPC	SBPC	CTX	PIPC	CPZ
P. aeruginosa 433	3.13	12.5	NT	NT	0.78	1.56
P. aeruginosa 638	6.25	50	NT	NT	3.13	6.25
P. aeruginosa 1008	3.13	50	NT	NT	3.13	3.13
P. aeruginosa 1872	6.25	100	NT	NT	1.56	3.13
P. aeruginosa SANK	3.13	50	NT	NT	NT	3.13
P. aeruginosa SC 8753	1.56	>200	>200	>200	NT	NT
P. aeruginosa B2-5	3.13	50	50	6.25	NT	NT
P. aeruginosa SANK	3.13	50	50	12.5	NT	NT
P. aeruginosa ATCC	3.13	100	50	6.25	NT	NT
P. aeruginosa NRRL	3.13	100	50	12.5	NT	NT
P. aeruginosa SANK	1.56	25	12.5	3.13	NT	NT
P. aeruginosa SANK	<0.1	<0.2	<0.2	<0.2	NT	NT
P. aeruginosa NCTC	0.4	<0.2	0.4	<0.2	NT	NT

Mueller-Hinton agar. Innoculum size 10^6 cells/mL.
Activity reported in μg/mL.
(CBPC) carbenicillin, (SBPC) sulbenicillin, (CTX) cefotaxime, (PIPC) piperacillin, (CPZ) cefoperazon.

period of 14 days without the observance of any toxic symptoms. Assays of the tissues of mice treated with 100mg/kg of MRD C showed that after one hour, the drug was only detected in small amounts in the liver and kidneys, and that no mureidomycin was present in the system after twenty-four hours. Two additional families of peptidylnucleoside antibiotics have been reported (**Figure 6**). The pacidamycins[20] (**5**) are produced by *Streptomyces coeruleorubidus* and were isolated by the anti-infective division of Abbott Laboratories. The biological activity of these compouns has been studied in great detail. *In vitro* activity was similar to that of the mureidomycins[20c], in

that specific activity against *P. aeruginosa* was observed. MICs ranged from 8 to 64 μg/mL.

When the assays were performed in the presence of human serum, the MIC's increased by approximately a factor of two. In addition, the activity of pacidamycin A was reduced by a factor of two when the pH was raised from 6.5 to 7.2, and by a factor of four at pH 8.0. Interestingly, the pacidamycins showed no *in vivo* activity (ED_{50} > 100 mg/kg) in studies with mice.

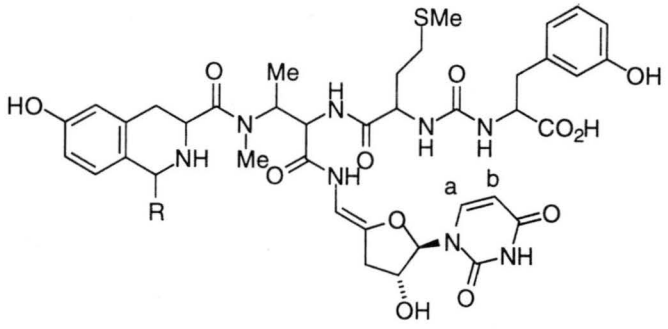


5a, Pacidamycin 1, R_1 = Alanyl, R_2= **A**
5b, Pacidamycin 2, R_1 = Alanyl, R_2= **B**
5c, Pacidamycin 3, R_1 = Alanyl, R_2= **C**
5d, Pacidamycin 4, R_1 = H, R_2= **A**
5e, Pacidamycin 5, R_1 = H, R_2= **B**
5f, Pacidamycin 6, R_1 = glycyl, R_2= **A**
5g, Pacidamycin 7, R_1 = glycyl, R_2= A

B=

C=

OH



6a, Napsamycin A, R = H
6b, Napsamycin B, R = Me
6c, Napsamycin C, R = H, $\Delta^{a,b}$ reduced
6d, Napsamycin D, R = Me, $\Delta^{a,b}$ reduced

Figure 6 Pacidamycin and Napsamycin

The napsamycins[21] (**6**) are produced by *Streptomyces* sp. HIL Y-82,1372 as reported recently by workers at Hoechst, India. Inspection of the structures of these compounds revealed that napsamycin A was identical to the previously reported mureidomycin F. Limited biological studies have shown that the napsamycins are active *in vitro* against *P. aeruginosa.*

The mechanism of action of the mureidomycins was determined[22] to be inhibition of phospho-N-acetylmuramyl translocase (translocase I) by Inukai using labeling studies with [C^{14}]-UDP-MurNAc-pentapeptide. Unlike previous translocase inhibitors, mureidomycin had no affect on glycoprotein biosynthesis[23], including the production of dolichyl phosphoryl mannose (Man-P-Dol), dolichyl phosphoryl glucose (Glc-P-Dol), and dolichyl pyrophosphoryl N-acetylglucosamine (GlcNAc-P-P-Dol). In 1996, Bugg *et al.* conducted fluorescence-based assays to study the kinetics of inhibition by mureidomycin.[24] Translocase was overexpressed in *E. coli* and solubilized using Triton X-100. Solubilized enzyme was incubated with dodecaprenyl phosphate and the modified substrate dansyl-UDP-MurNAc-pentapeptide which also binds translocase with high affinity (K_M = 19 $\pm$ 3 μM). Mureidomycin A inhibited translocase in a time-dependent fashion, consistent with being a slow-binding inhibitor. The kinetics exhibit an initial decrease in rate followed by transition to a steady-state after several minutes. Therefore, inhibition results from conversion of the initial E-I complex to a more tightly bound E-I* complex. K_i and K_i* (simple competitive and slow-binding) equilibrium constants were calculated as 36 $\pm$ 6 nM and 2.0 $\pm$ 0.6 nM,

respectively. This corresponds to a 500 fold difference between K_m and K_i and a 10,000 fold difference between K_m and K_i*, indicating an incedible affinity for mureidomycin.

The mureidomycin antibiotics are selective inhibitors of phospho-N-acetylmuramyl-pentapeptide tanslocase (translocase I). These are the first compounds to be discovered which target this enzyme while having no adverse affects on mammalian glycoprotein biosynthyesis. Antimicrobial assays have shown these peptidylnucleoside antibiotics to possess activity (IC_{50} = 0.05 μg/mL) against *Pseudomonas aeruginosa* exclusively. This is significant in that there are very few commercial antibiotics with good antipseudomonal activity due to the ability of these organisms to acquire resistance. Furthermore, *in vitro* assays using solubilized enzymes from membrane fractions have demonstrated that these compounds are active against translocase from *P. aeruginosa*, *E. coli*, and *S. aureus* as well. This data clearly makes the mureidomycins attractive targets for further research.

B. Results and Discussion

The total synthesis of Mureidomycin A was proposed to proceed according to the retrosynthetic analysis in **Figure 7**. The most important and challenging bond connection is that between uridine and the peptide portion of the molecule, which was envisioned to be installed in the final step of the synthesis. The nucleotide core would evolve from 3'-deoxyuridine which

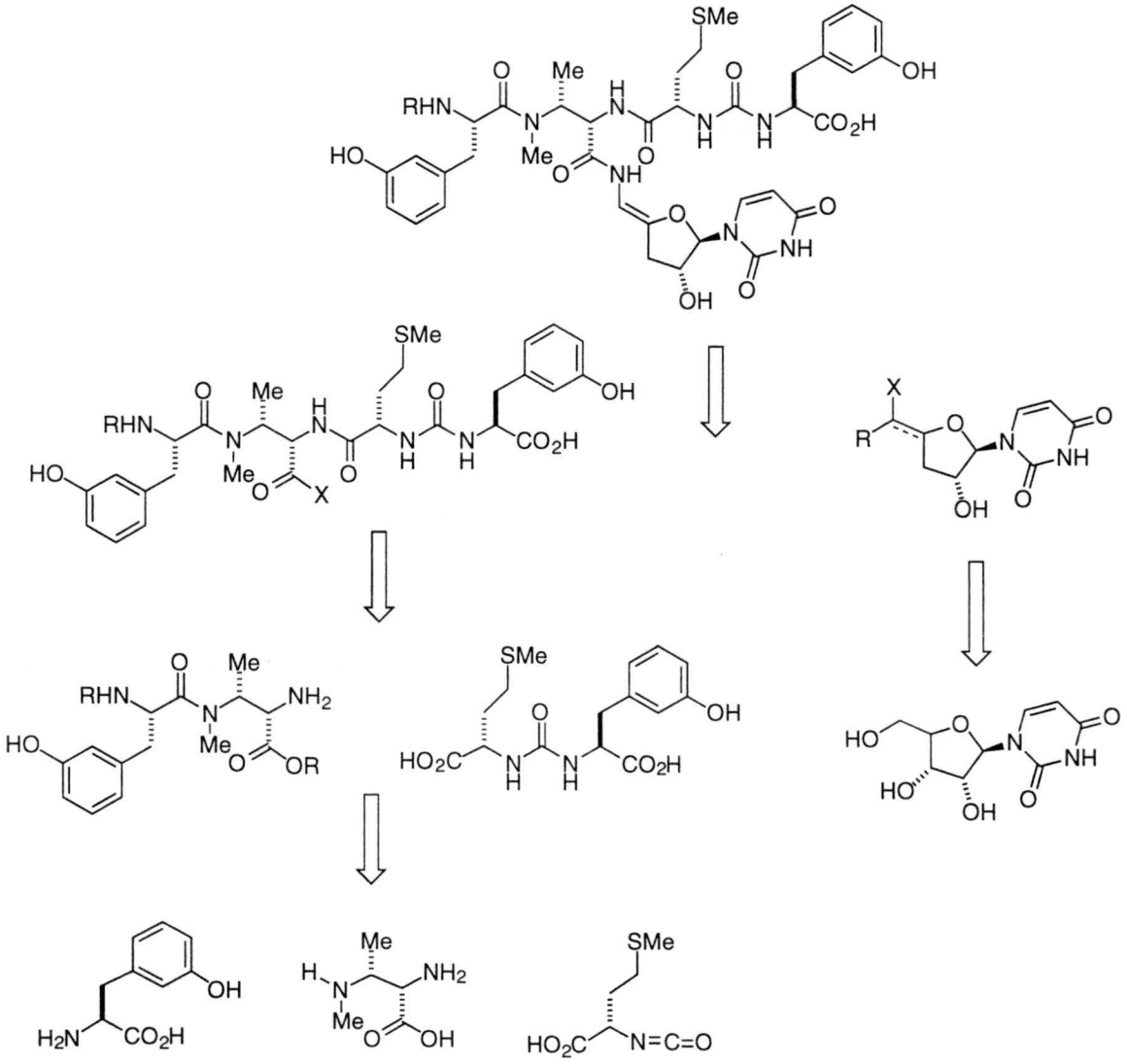


Figure 8 Retrosynthetic Analysis

could be synthesized from commercially available uridine. In addition, the two unnatural amino acids, *m*-tyrosine and 2-methyl-3-N-methylaminobutyric acid (AMBA), were required for the synthesis of the peptide half of the molecule. For the *m*-tyrosine residue, methodology developed in the Williams group several years ago was used. The synthesis of AMBA was reported by researchers at Sankyo during the course of their structural elucidation of the mureidomycins.

1. Synthesis of 3′-deoxyuridine

3′-deoxyuridine (**10**) has previously been synthesized. Walton *et al.* utilized classical Hilbert-Johnson methodology to produce the compound (**Figure 9**).[25] Under these conditions, 2,4-dimethoxypyrimidine **8** was coupled with a protected 3′-deoxyribose derivative (**7**) , which was synthesized from 2-deoxyribose. Further elaboration yielded the desired 3′-deoxy nucleoside. This route lead to the production of both α and β anomers (**9**) which were separated at the conclusion of the synthesis.

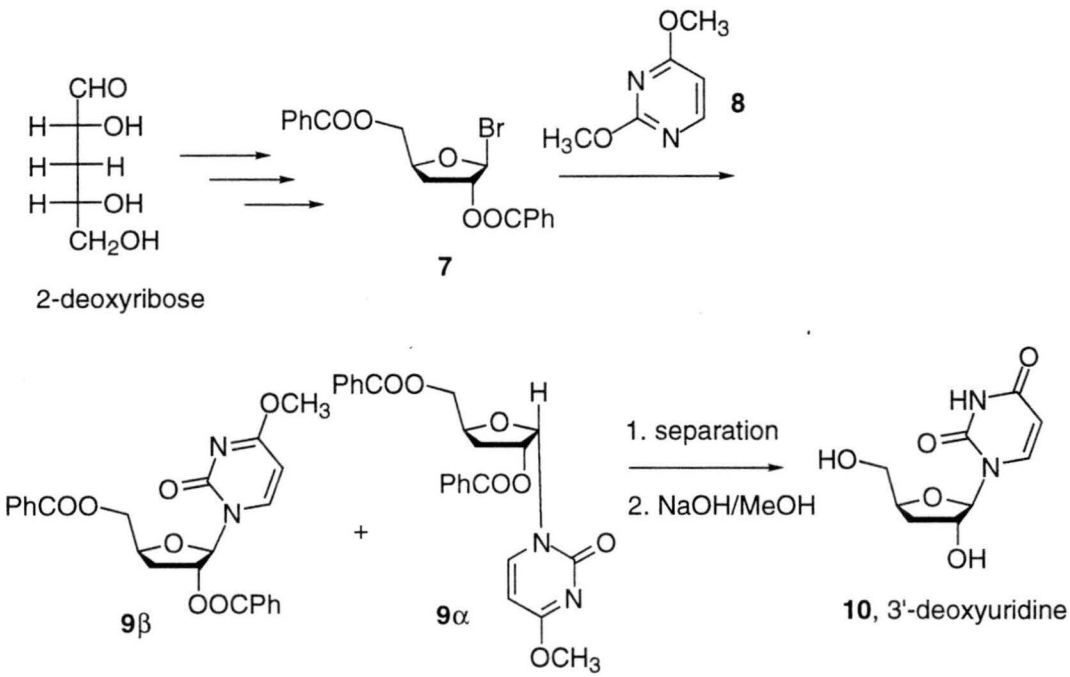


Figure 9 Hilbert-Johnson Synthesis of 3′-Deoxyuridine

More recently, Lin and co-workers reported[26] a synthesis of 3′-deoxyuridine starting from commercially available uridine. The nucleoside was first protected as the 5′-trityl ether followed by selective protection of the 2′-OH as the silyl ether. Conversion of the remaining secondary alcohol tothe thiocarbonate derivative followed by radical deoxygenation with tri-*n*-butyltin hydride gave the corresponding deoxy compound. Cleavage of the silyl ether with TBAF and subsequent removal of the trityl group by heating in 80% acetic acid at reflux gave **10**.

Multiple gram quanities of 3′-deoxyuridine were required for studies toward the synthesis of the mureidomycins, and although both published syntheses provide access to this compound, neither was applicable to large-scale production of the nucleoside. Lin's synthesis was completed to generate

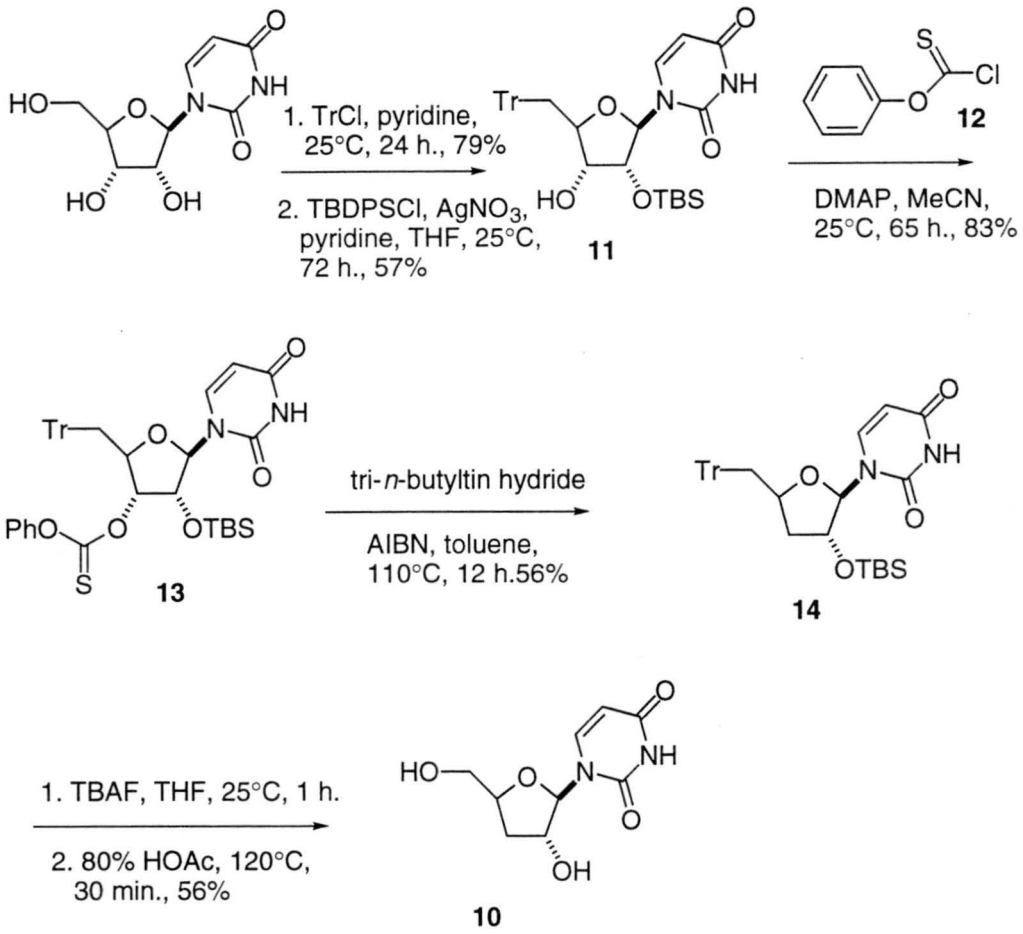
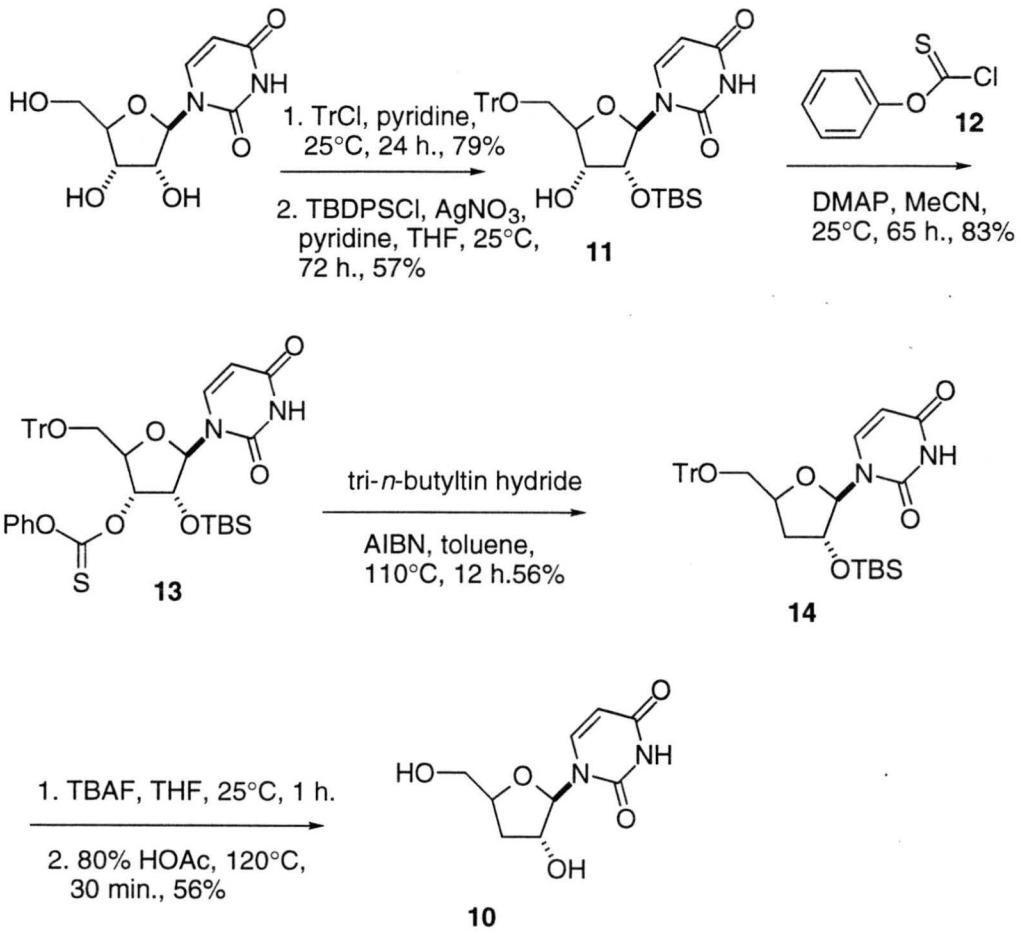


Figure 10 Lin's Synthesis of 3′-Deoxyuridine

an authentic sample of 3′-deoxyuridine. The low yields encountered were a result of difficulties in purifying the synthetic intermediates which could only be isolated using preparative thin layer chromatography. In addition, the synthesis of the mureidomycins requires that the 5′-trityl protecting group be removed in the presence of the 2′-silyl ether, which proved difficult. The synthesis outlined in **Figure 11**, which is a modification of that published by Lin, produced adequate quanities of the desired 3′-deoxynucleoside.



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presence of imidazole. Phenylchlorothionocarbonate (**12**) was prepared on large scale by the reaction of phenol and thiophosgene. The silyl ether was combined with this reagent in dry acetonitrile in the presence of DMAP to give thiocarbonate **16** in excellent yield. It is interesting to note that this reaction required 72 hours when 0.1 - 1 equivalents of DMAP were used. However, this reaction time was greatly reduced by increasing the amount of DMAP, achieving completion in only 6 hours in the presence of 15 equivalents of this reagent. The thiocarbonate was subjected to Barton radical deoxygenation conditions[28-31] using triphenyltin hydride in toluene at reflux in the presence of the radical initiator AIBN to yield **17**. Selective removal of the primary silyl ether[32] was acomplished by stirring at room temperature in a solvent system consisting of HOAc/H_2O/THF (13:7:3) to give 2′-O-(*tert*-butyldimethylsilyl)-3′-deoxyuridine (**18**).

2. Synthesis of 4′,5′-unsaturated nucleosides

The most interesting structural feature of the mureidomycin antibiotics is the unusual enamine ribose moiety, which to date, has not been identified in any other natural product. Two different approaches were studied in order to introduce this functionality into the molecule.

(i) Formation of the amide bond linkage between the nucleoside and the peptide by nucleophilic displacement of a 5′ halide followed by oxidative elimination to the enamine.

(ii) Acylation of an alkyl imine accompanied by tautomerization to the enamine.

In 1991, McCarthy *et al.* synthesized S-adenosyl-L-homocysteine hydrolase (SAH hydrolase) inhibitors *via* the oxidative elimination of 5′-phenylsulfoxides of adenosine. As detailed in **Figure 12**, fluorine[34] or chlorine[35] was introduced α to the sulfoxide, followed by elimination to the vinyl halide.

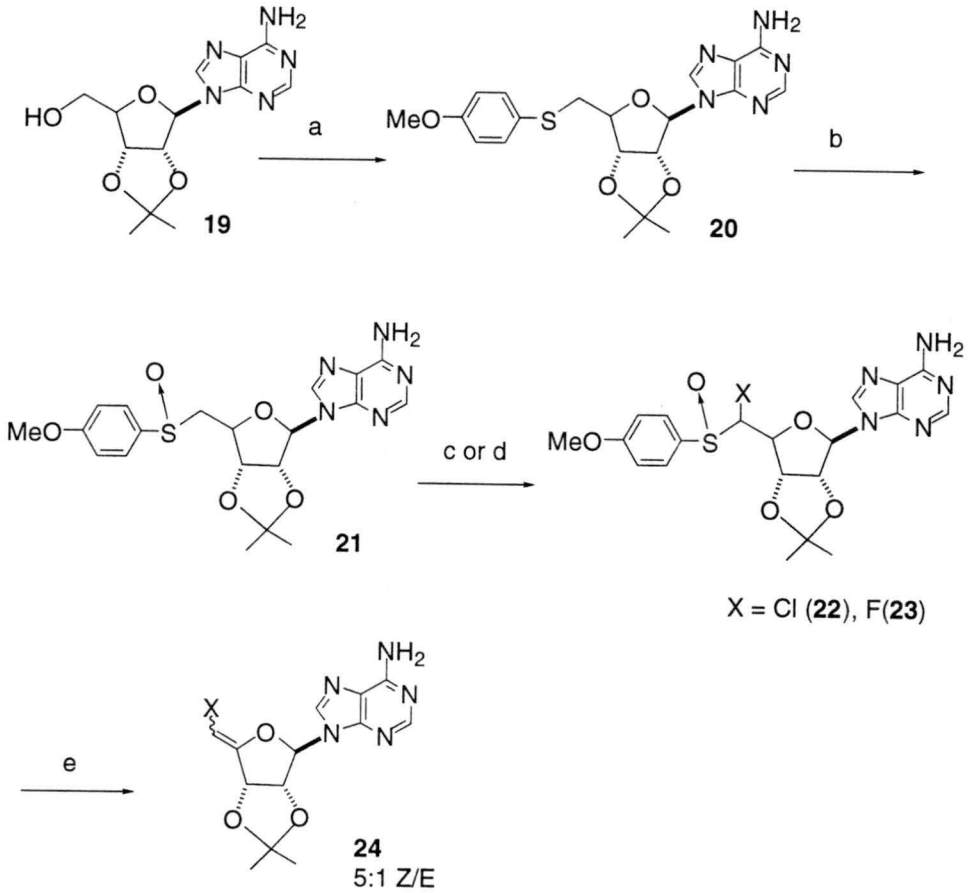


Figure 12 **McCarthy's Synthesis of SAH Hydrolase Inhibitors**

The corresponding uridine thioester[36] was synthesized from uridine as in **Figure 13**. However, attempts to synthesize the α-chloro species failed, as the SO_2Cl_2 underwent preferential reaction with the uracil base. In addition, the vigorous conditions necessary for the elimination of the sulfoxide may not be applicable to the fully-assembled mureidomycin skeleton.

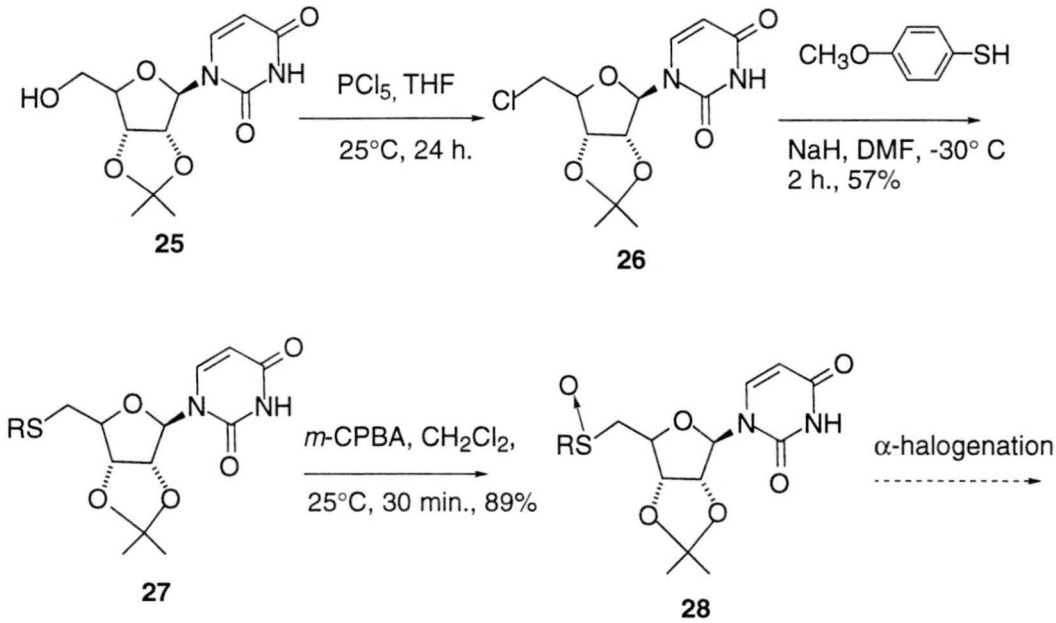


Figure 13 Synthesis of Uridine Thioesters

Selenium offered an excellent alternative to the use of these sulfoxides. Haraguchi and co-workers[37] reported the synthesis of vinyl nucleosides from the oxidative elimination of 5′-phenylselenides, which were derived from the reaction of cyclonucleosides with the anion generated from diphenyl diselenide. 5′-*o*-nitro-phenylselenides are easily prepared by the reaction of deoxyuridine **18** and commercially available *o*-nitrophenyl selenocyanate. Under these conditions, several grams of the selenonucleoside **29** were synthesized. Oxidative elimination of this compound gave vinyl uridine **31**.

The mild reaction conditions employed were believed to be much more suitable for the elimination of the peptidylnucleoside. Chlorination was accomplished by reaction with N-chlorosuccinimide in carbon tetrachloride to give **32** as a diastereomeric mixture. This compound was inert to nucleophilic attack by acetamide as well as nucleophiles such as azide. Further studies are necessary to determine whether substrates such as **32** will be useful intermediates in the total synthesis of the mureidomycins.

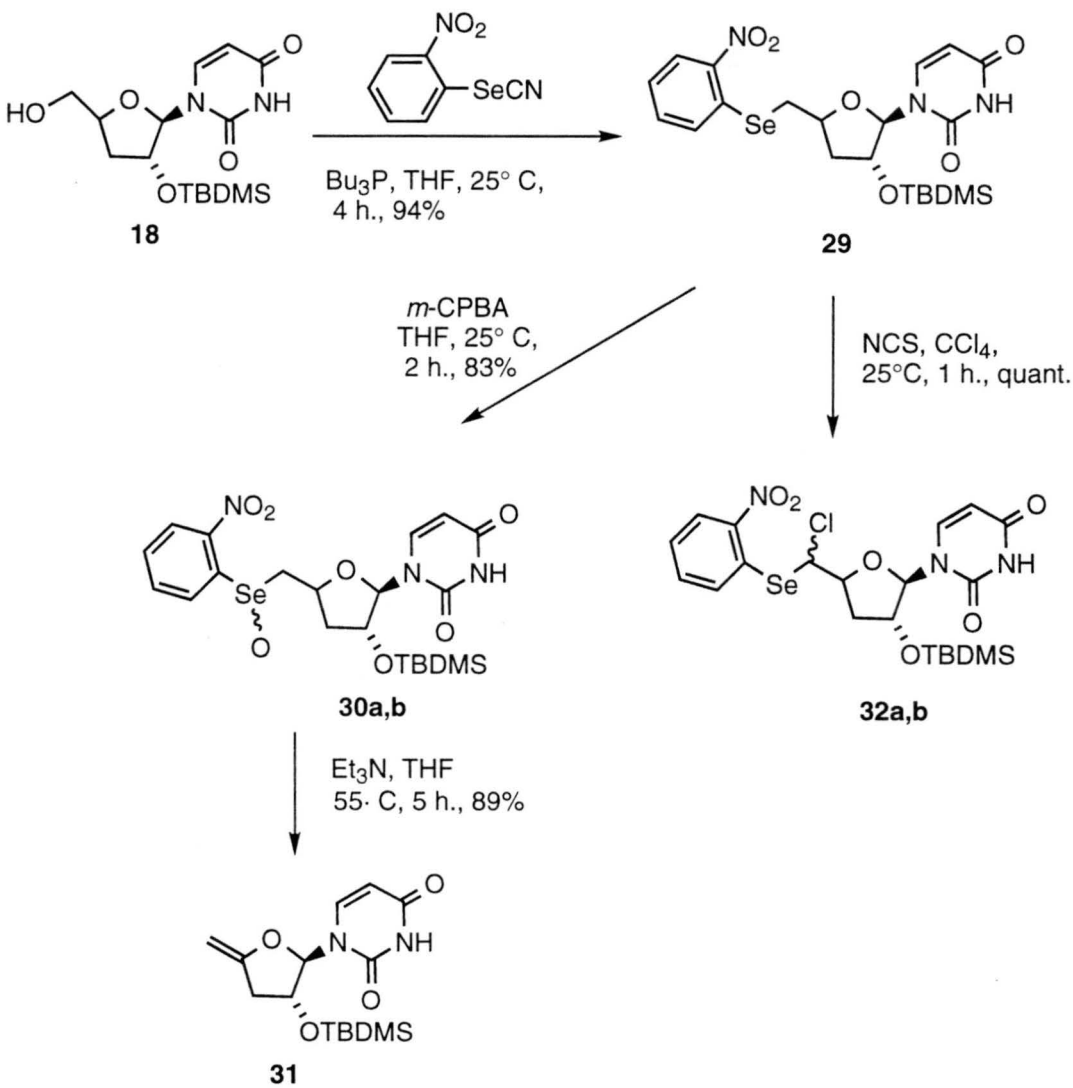


Figure 14 Synthesis of 5′-*o*-nitro-phenylselenides

A second method for the introduction of the enamine sugar was explored. N-alkyl imines react with silanes[38-40] or with acid chlorides[41] to yield the corresponding enamines. The latter was utilized in the total synthesis of the natural product pederine[42] as detailed in **Figure 15**. Imine **33** was acylated with acid chloride **34** to give acylimidate **35**. This compound was immediately reduced to give the desired natural product.

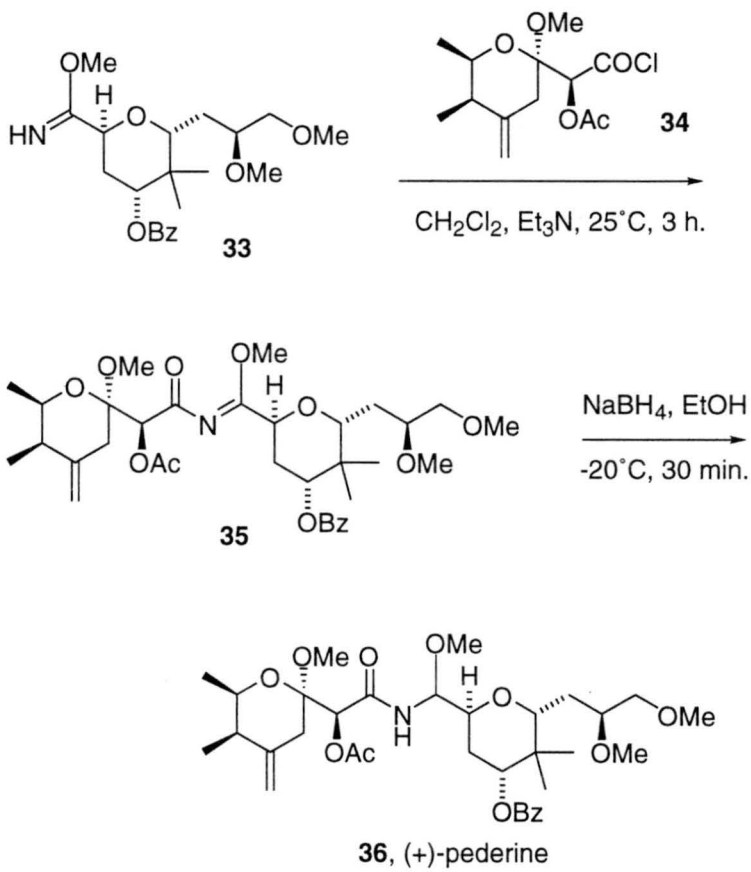


Figure 15 Synthesis of (+)-pederine

Following this methodology, deoxyuridine **18** was oxidized to the aldehyde (**37**) using the the Swern[43] or Dess-Martin oxidation[44]. The aldehyde was used immediately and condensed with benzylamine under dehydrating conditions to give imine **38**. Attempts to acylate the imine using acetyl

chloride produced complex reaction mixtures and **39** was isolated in very low yield.

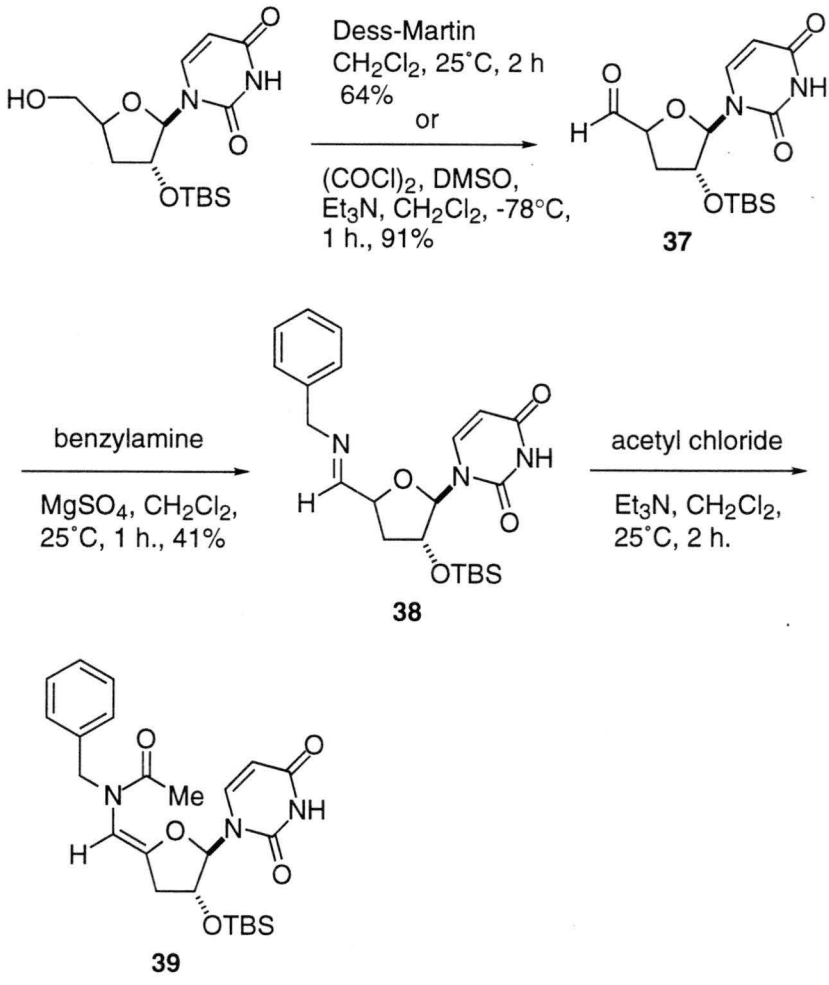


Figure 16 N-Acylation of Imines

3. Synthesis of (S)-*m*-tyrosine

Sankyo's report[17b] on the structural elucidation of the mureidomycins did not contain information regarding the absolute configuration of the amino acids which make up the peptide portion of the molecule. There is evidence[45], however, that the amino acids have the natural, or L

configuration. For the synthesis of *m*-tyrosine, methodology was required that would unambiguously produce the (R) or (S) enantiomer.

In 1988, the Williams research group described the synthesis of optically active α-amino acids utilizing chiral glycine templates.[46] The synthesis of these compounds[47] is outlined in **Figure 17**. Inexpensive benzoin was converted to the oxime and stereospecifically hydrogenated to the racemic *erythro*-amino alcohols **40a,b**. Separation of these enantiomers was accomplished by resolution of the corresponding L-glutamate salts. Alkylation with ethyl bromoacetate was followed by protection as the carbamate of choice. Lactonization in benzene at reflux with catalytic *p*-TSOH affords crystalline lactones **41** and **42**.

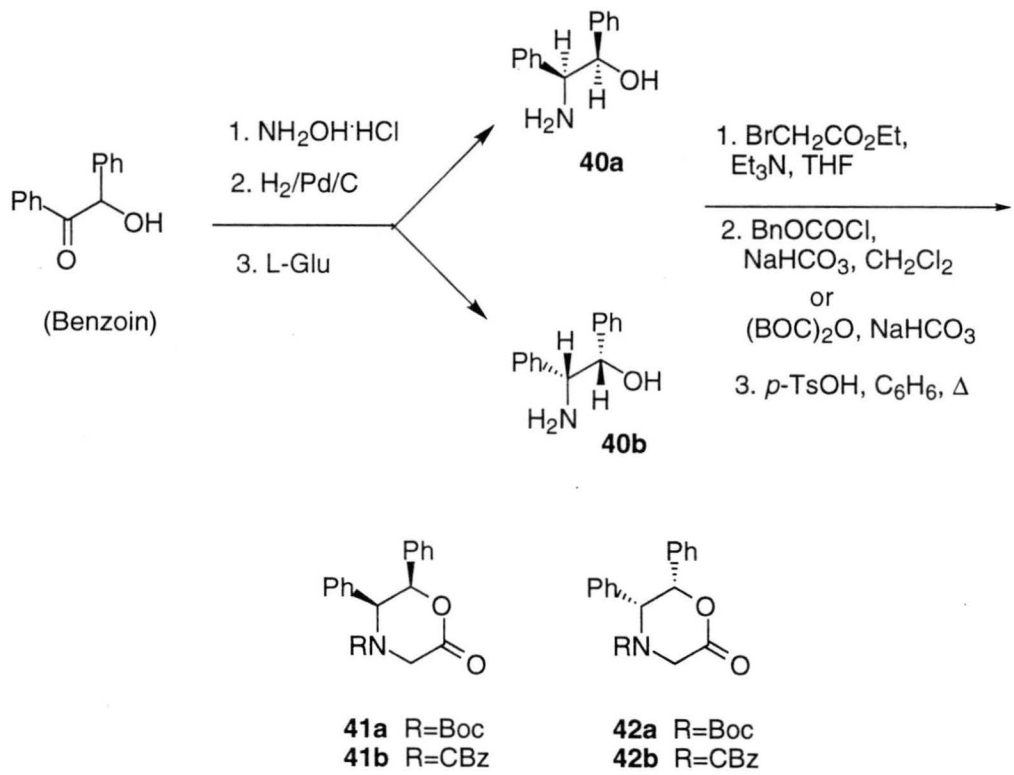


Figure 17 Synthesis of the Williams lactone

Oxazinone **41b** was condensed with *m*-benzloxy benzyl bromide **43** *via* formation of the sodium enolate. The benzyl halide was conveniently prepared from the commercially available benzyl alcohol by halogenation with Ph_3P/CBr_4.[48] Alkylated lactone **44** was produced in high yield in >99:1 dr. Catalytic hydrogenation with $PdCl_2$ gave *m*-tyrosine[49] in >98:2 er as determined by ^{1}HNMR and GC analysis of the corresponding Mosher amides.

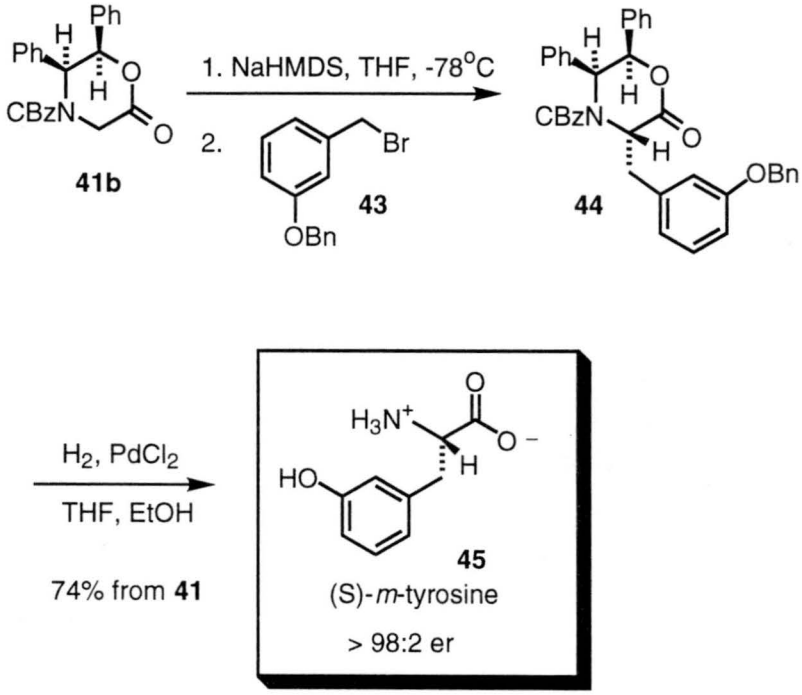


Figure 18 Asymmetric Synthesis of (S)-*m*-Tyrosine

4. Synthesis of 2-amino-3-N-methylaminobutyric acid

Sankyo reported the presence of the unnatural amino acid 2-methyl-3-N-methylaminobutyric acid (AMBA) in their structural elucidation of the mureidomycins.[17b] The assignment of the structure of this compound was

made following spectral analysis of hydrolysis products from mureidomycin A and verified by chemical synthesis. Commercially available D-threonine was protected as the CBz methyl ester. The β-hydroxyl group was converted to the corresponding chloride, followed by displacement with methylamine and subsequent deprotection to yield AMBA. In the Sankyo report, the product of this synthesis matched the amino acid obained through degradation of the natural product. On the basis of basis of this work, the absolute stereochemistry of AMBA was assigned as depicted by **48**.

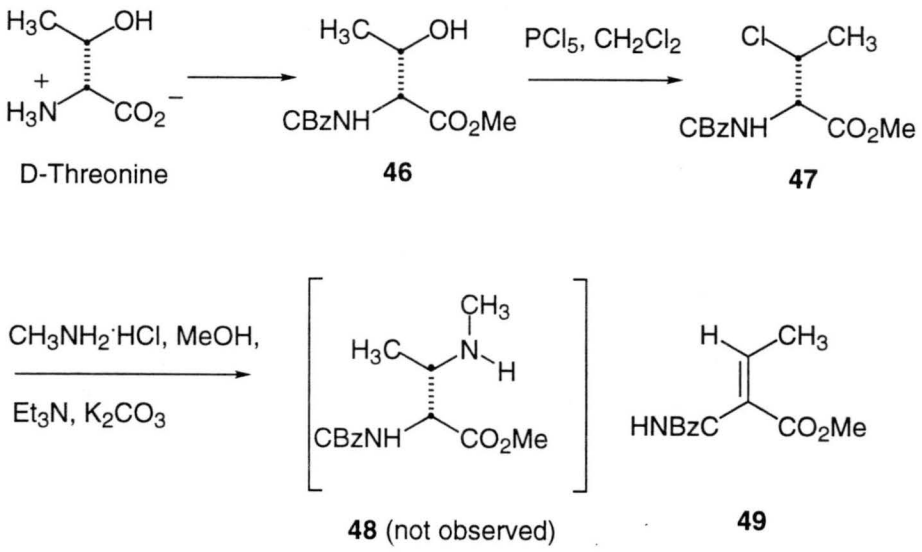


Figure 19 Sankyo Synthesis of AMBA

Accordingly, chloride **47** was synthesized as reported. However, reaction of the chlorinated amino acid with methylamine did not produce any of the desired product, but instead gave only the dehydro derivative **49**. Inspection of the Sankyo synthesis revealed that approximately 20 grams of D-threonine were used to synthesize less than 500 mg of AMBA, which corresponds to a 2% overall yield. The nitrogen protecting group was varied

(CBz, BOC, Phth) in order to change the steric environment proximal to the α-position, thereby reducing the amount of elimination. However, in all cases, dehydrothreonine was the sole reaction product, except when the phthalimide protecting group was used, in which case an 8% yield of the desired β-amino acid was observed.

The Sankyo synthesis of AMBA requires that the amino acid have the 2R, 3S absolute configuration, which is identical to the starting material D-threonine. Chlorination with PCl_5[50] proceeds with inversion of configuration, and displacement with methylamine should result in a second inversion, leading to a net retention of stereochemistry. It has since been discovered that Sankyo's stereochemical assignment was incorrect, and that AMBA has the 2R, 3R configuration.[51] With this new data, alternative syntheses were explored.

In order to remove the possibility of elimination, the N-Boc-Thr-OMe (**50**) was reduced to the amino alcohol using established procedures (**Figure 20**).[52] The primary alcohol was selectively masked as the *tert*-butyl-diphenylsilyl ether, and the secondary alcohol was converted to the mesylate. Reaction with methylamine hydrochloride did not proceed as expected, yielding only starting material after 24 hours at room temperature, and when the temperature was elevated, only aziridine formation was observed. As a result, the amine functionality was introduced in multiple steps. Reaction of the mesylated derivative of **51** with azide ion furnished **52** which was then subjected to catalytic hydrogenation to provide amine **53**. Alkylation with

methyl iodide did not produce the N-methyl amino acid as desired. Instead, the over-alkylated product was obtained, along with a large quanity of starting material. Additional efforts to introduce the methyl group have proven unsuccessful thus far. To explore the remaining methodology required for the synthesis of AMBA, the silyl protecting group was removed with acetic acid, followed by oxidation to the amino acid (**54**). This sequence involves a single inversion of stereochemistry at the β center as required to produce the compound having the 2R, 3R configuration.

In a recent report, diaminobutyric acids were synthesized from threonine with the carboxcylic acid masked as the *tert*-butyl carbazate[53] which is easily removed at the end of the synthesis by heating to reflux in 6 M HCl (**Figure 21**). The azido intermediate **56** has been synthesized in high yield, and further elaboration should generate Fmoc-protected AMBA. Additional efforts are required to N-methylate the products of these syntheses or to develop methodology that allows the introduction of the N-methyl group in a single transformation.

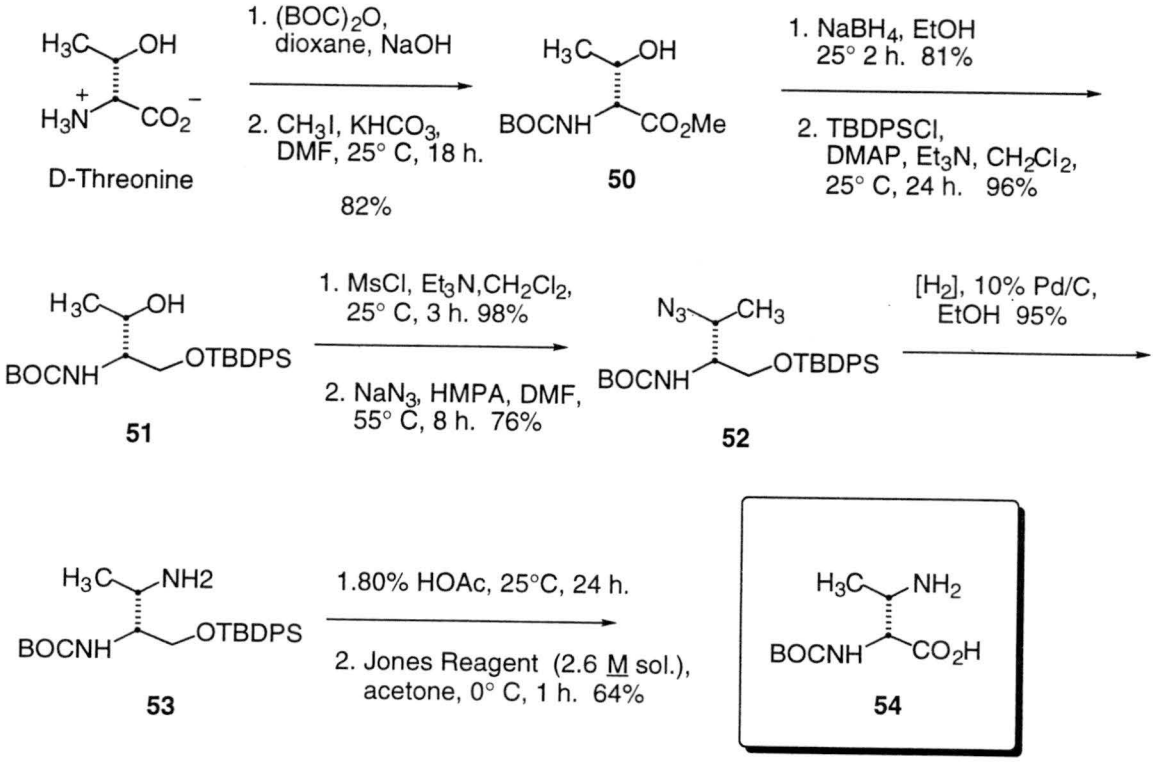


Figure 20 **Synthesis of 2,3-diaminoacid *via* the Amino Alcohol**

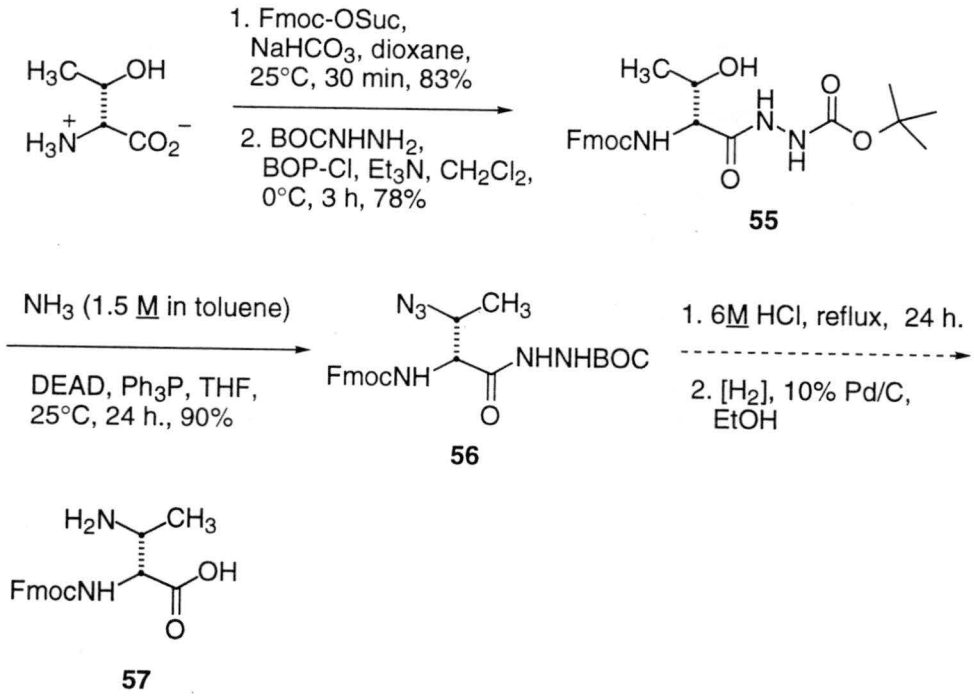


Figure 21 **Synthesis of 2,3-diaminoacid *via* the *t*-butyl Carbazate Derivative**

5. Conclusions

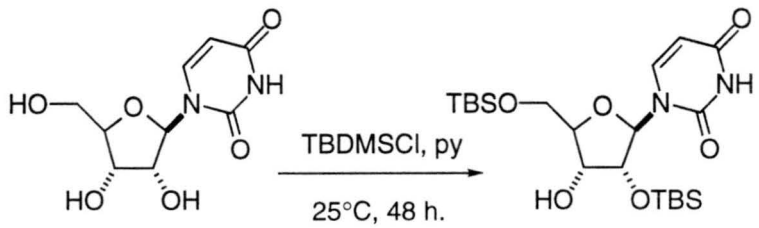
Significant progress has been made toward the total synthesis of the mureidomycin antibiotics. Methodology has been developed that allows the large-scale synthesis of 3'-deoxyuridine, which was previously difficult to obtain. At this time, the unusual enamine moiety has not been produced. However, two different routes to this compound have been explored, and upon further study may prove effective . Progress has also been made toward the generation of the unnatural amino acids in this molecule. A short, efficient synthesis of (S)-*m*-tyrosine has been completed, and a derivative of AMBA lacking the N-methyl group has been synthesized.

C. Experimental

General Procedures

^{1}H NMR spectra were obtained on a Bruker AC 300 MHz spectrometer and chemical shifts were reported in parts per million relative to TMS (0.00), deuterated chloroform (7.24), deuterated dimethylsulfoxide (2.54), or deuterium oxide (4.80). The numbers in parentheses were specified by Cambridge Isotope Labs, andover, MA. Infrared spectra were recorded on a Perkin-Elmer 1600 Series FTIR as KBr pellets or thin films from methylene chloride on NaCl plates. Optical rotations were determined on a Rudolph Research Autopol III automatic polarimeter at a wavelength of 589 nm (sodium D line) with a 1.0 dm cell and a volume of 1 mL. Specific rotations, $[\alpha]_D$, are reported in degrees per decimeter at a specified temperature and concentration of grams per 100 mL. Melting points were obtained using a Mel-Temp aparatus and are uncorrected. Elemental analyses were performed by M-H-W Laboratories in Phoenix, AZ and have an error of $\pm$0.4%. Mass spectra were obtained on a 1992 Fisions VG Autospec at the Chemistry Department at Colorado State University. Column chromatography was performed with Merck silica gel grade 60, 230-400 mesh, 60 Å. Analytical preparatory thin layer chromatography was performed with Merck Kieselgel 60 F_{254} plates. Reagents and solvents were all commercial grade and used without further purification with the rxception of THF (distilled over sodium, benzophenone), methylene chloride (distilled over CaH_2), ether (distilled over sodium, benzophenone),

and DMF and HMPA (dried over 4Å molecular sieves). All air-sensitive reactions were run under an atmosphere of nitrogen or argon. All glassware was oven or flame-dried prior ro use.



15a

2',5'-bis-O-(*tert*-butyldimethylsilyl) uridine (15a)

Uridine (20.0 g, 81.9 mmol) was dissolved in dry pyridine (160 mL) and stirred at room temperature for 10 min. *tert*-butyldimethylsilyl chloride (30.86 g, 204.8 mmol) was added in a single portion and the reaction was stirred at room temperature for 48 h. The reaction mixture was concentrated under vacuum and the resulting crude material was dissolved in CH_2Cl_2 (500 mL), washed with 5% $NaHCO_3$ solution (2 x 100 mL), dried ($MgSO_4$), and concentrated to a white foam. Product was purified by silica gel (800 g) column chromatography (ether/hexane, 2:1 v/v) to yield 26.3 g (68%) of 2',5'-bis-O-(*tert*-butyldimethylsilyl) uridine as a white solid.

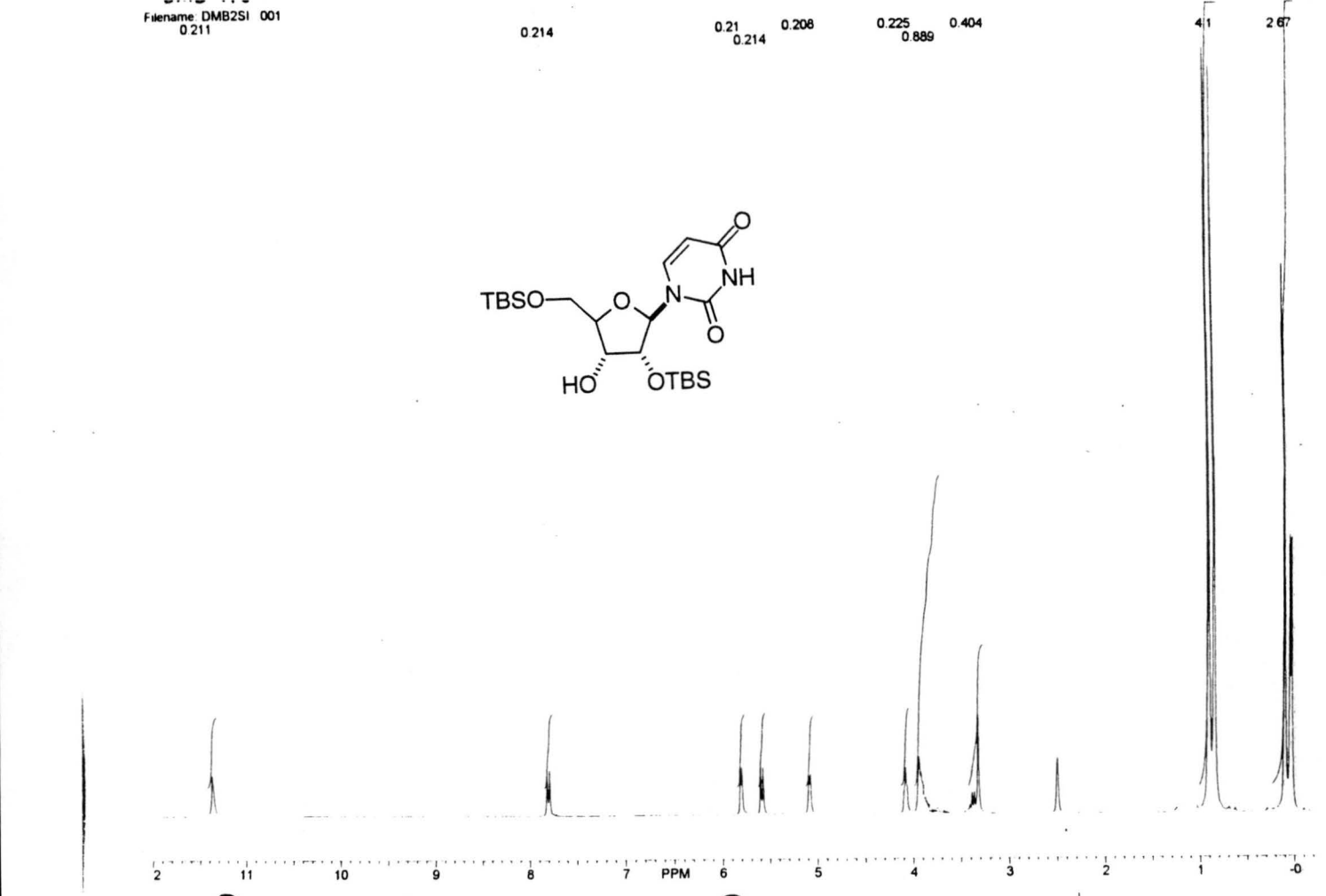
R_f=0.59 (ether/hexane, 2:1, v/v);

m.p. 122 °C (lit[27] 122 °C);

^1H NMR (300 MHz) (d_6-DMSO) δ 0.07 (12H, m), δ 0.86 (18H, d), δ 3.83 - 3.95 (3H, m), δ 4.08 (1H, t), δ 5.08 (1H, d, J=4.8 Hz), δ 5.58 (1H, d, J=8.1 Hz), δ 5.81 (1H, d, J=4.8 Hz), δ 7.79 (1H, d, J=8.1 Hz), δ 11.33 (1H, s, D_2O exchangeable).

^{13}C NMR (75.48 MHz) ($CDCl_3$) δ -5.49, -5.22, -4.70, 18.01, 25.66, 25.89, 62.51, 70.28, 76.47, 84.72, 88.42, 102.26, 140.03, 150.27, 163.36.

IR (NaCl, neat) 3452, 3190, 3052, 2987, 2953, 2929, 2885, 2857, 1683, 1489, 1463, 1430, 1388, 1338, 1259, 1221, 1133, 1099, 1021, 1003 cm -1.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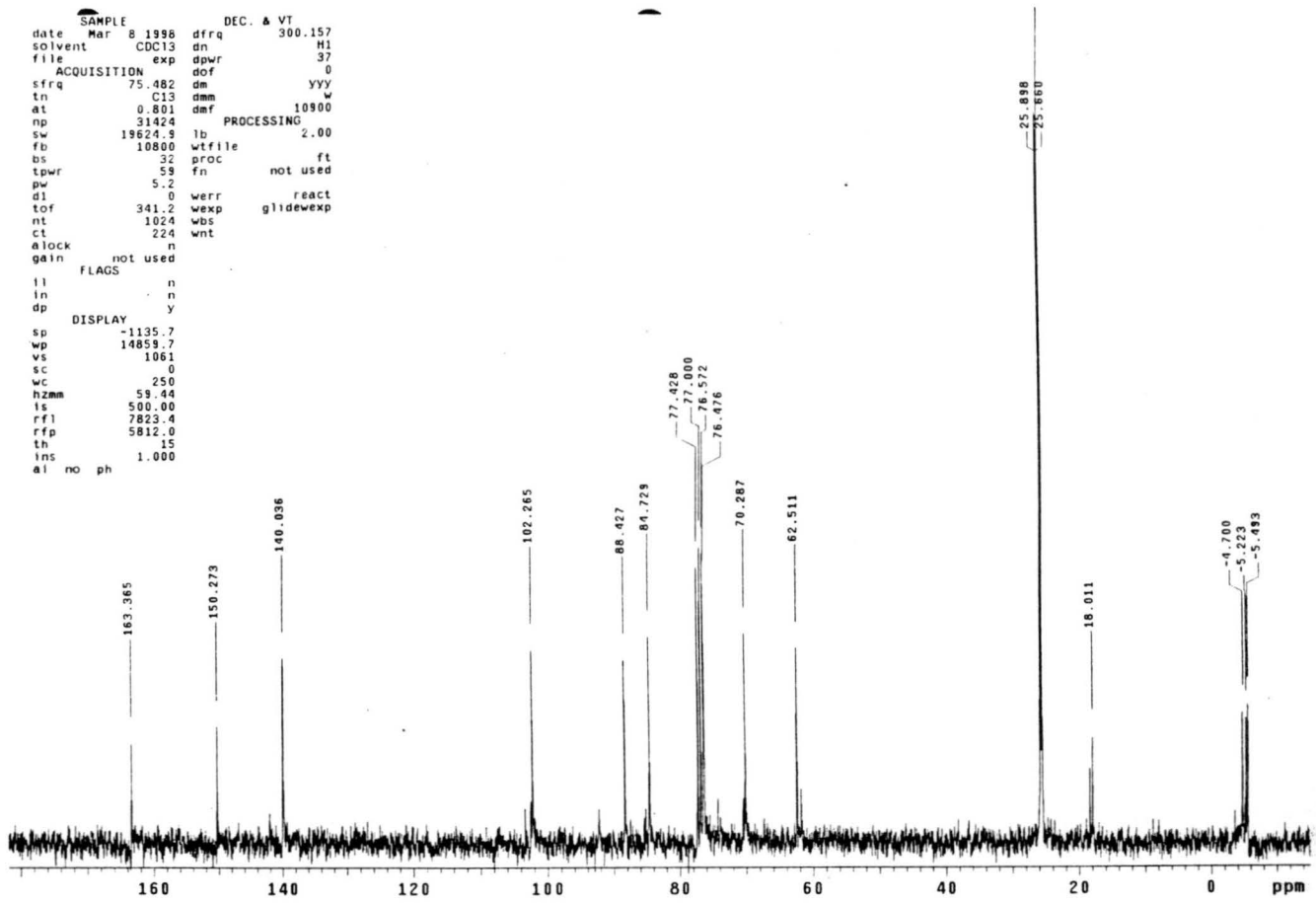
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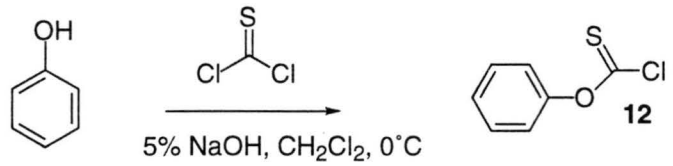
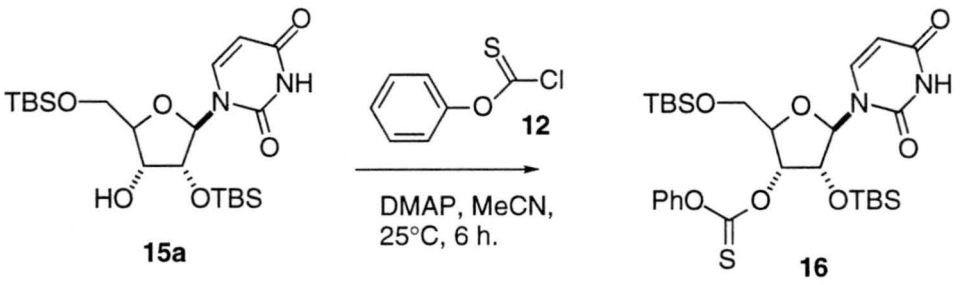


Phenylchlorothionocarbonate (12)

Phenol (10.0 g, 106.3 mmol) was dissolved in $CHCl_3$ (64 mL) and 5% NaOH solution (95 mL) and cooled to 0°C in an ice bath. Thiophosgene (8.1 mL, 106.3 mmol) was added dropwise and the reaction was warmed to 25°C and stirred for 2 hours. Layers were separated and the organic phase was washed with 0.1 M HCl and H_2O, dried ($MgSO_4$) and concentrated to a yellow oil. Kugelrhor distillation (b.p. 78-81°C, 8 mm Hg) gave 15.6 g (85%) of phenylchlorothionocarbonate as a bright yellow oil.



2',5'-bis-O-(*tert*-butyldimethylsilyl)-3'-O-(phenoxythiocarbonyl) uridine (16)

To a stirred solution of 2',5'-O-di-*tert*-butyldimethylsilyl uridine (5.00 g, 10.58 mmol) and 4-(dimethylamino)pyridine (6.50 g, 52.90 mmol) in dry acetonitrile (100 mL) was added dropwise phenyl chlorothiocarbonate (2.20 mL, 15.87 mmol) under nitrogen at room temperature. After 24 h. all starting material had been consumed. Solvent was removed and the crude solid was dissolved in CH_2Cl_2 (150 mL) and extracted with cold 1.0 M HCl (2 X 50 mL), saturated $NaHCO_3$ (2 X 50 mL), and saturated NaCl (2 X 50 mL). The organic layer was dried ($MgSO_4$) and concentrated to give a yellow oil which was purified by silica gel (200 g) column chromatography (CH_2Cl_2,MeOH, 95:5, v/v) to yield 4.58 g (74%) of 2',5'-bis-O-(*tert*-butyldimethylsilyl)-3'-O-(phenoxythiocarbonyl) uridine as a light yellow crystalline solid.

R_f =0.62 (CH_2Cl_2,MeOH, 95:5, v/v);

m.p. 54-56°C

^{1}H NMR (300 MHz) ($CDCl_3$) δ 0.12 (12H, m), 0.91 (18H, d), 3.81 (1H, 1/2ABq, J=15.3 Hz), 3.97 (1H, 1/2 ABq, J=12.8 Hz), 4.36 (1H, m), 4.42 (1H, t), 5.49 (1H, t),5.61 (1H, d, J=8.1 Hz), 5.92 (1H, d, J=3.8 Hz), 6.92 - 7.39 (5H, m), 7.88 (1H, d, J=8.1 Hz), 8.78 (1H, s, D_2O exchangeable).

^{13}C NMR (75.48 MHz) ($CDCl_3$) -5.45, -5.10, -4.85, 17.99, 18.37, 25.59, 25.91, 62.06, 74.22, 77.47, 79.61, 81.92, 88.99, 102.42, 121.59, 126.72, 129.57, 139.57, 150.32, 153.16, 162.47, 189.17.

IR (NaCl, neat) 3413, 2954, 2929, 2857, 1682, 1592, 1490, 1462, 1379, 1255, 1221, 1134, 1067, 1023, 1004 cm -1.

$[\alpha]_D^{25}$= +46.2 (c=1.0, CH_2Cl_2).

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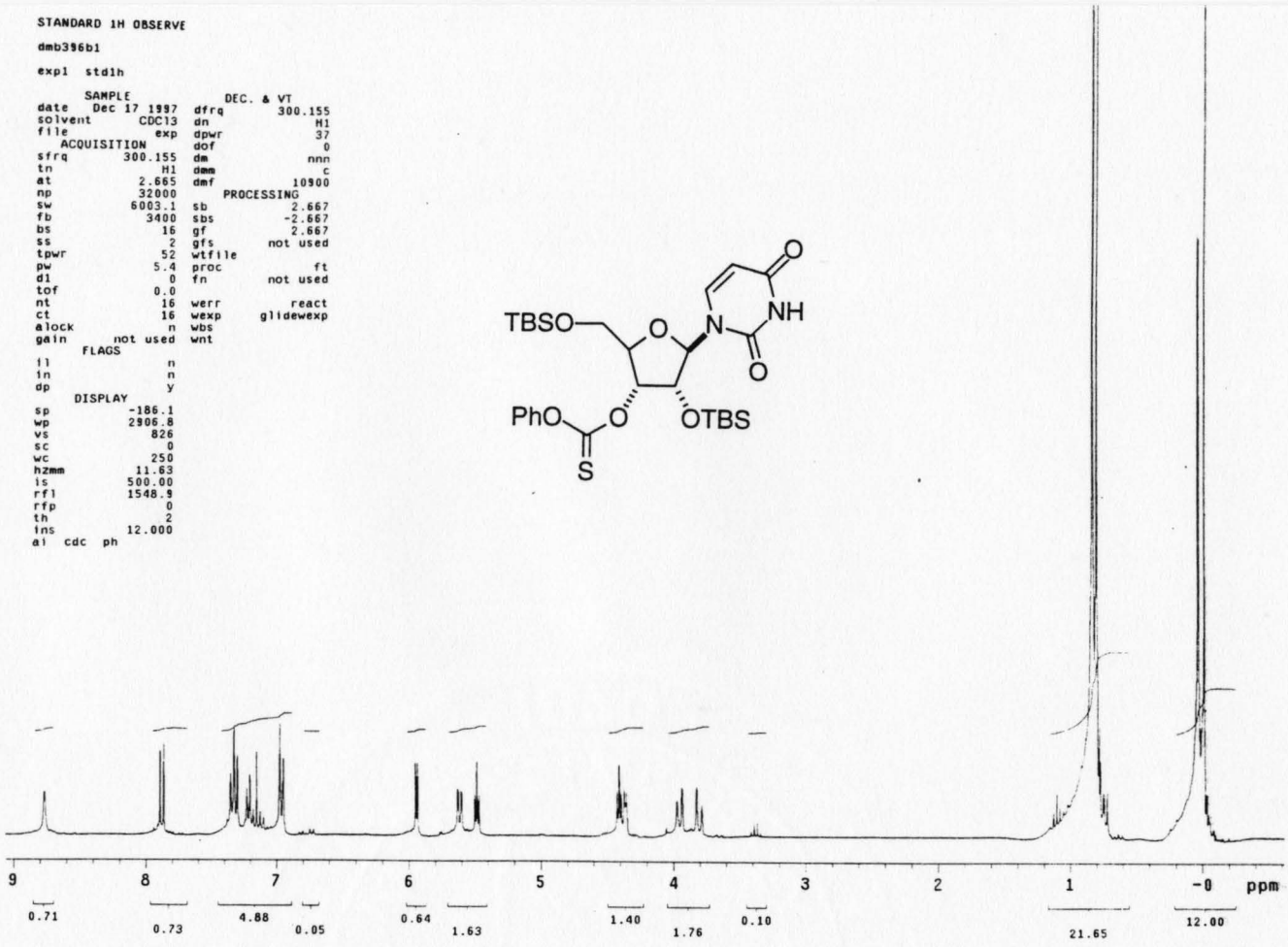
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wbs
wnt
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NH
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PhO
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4
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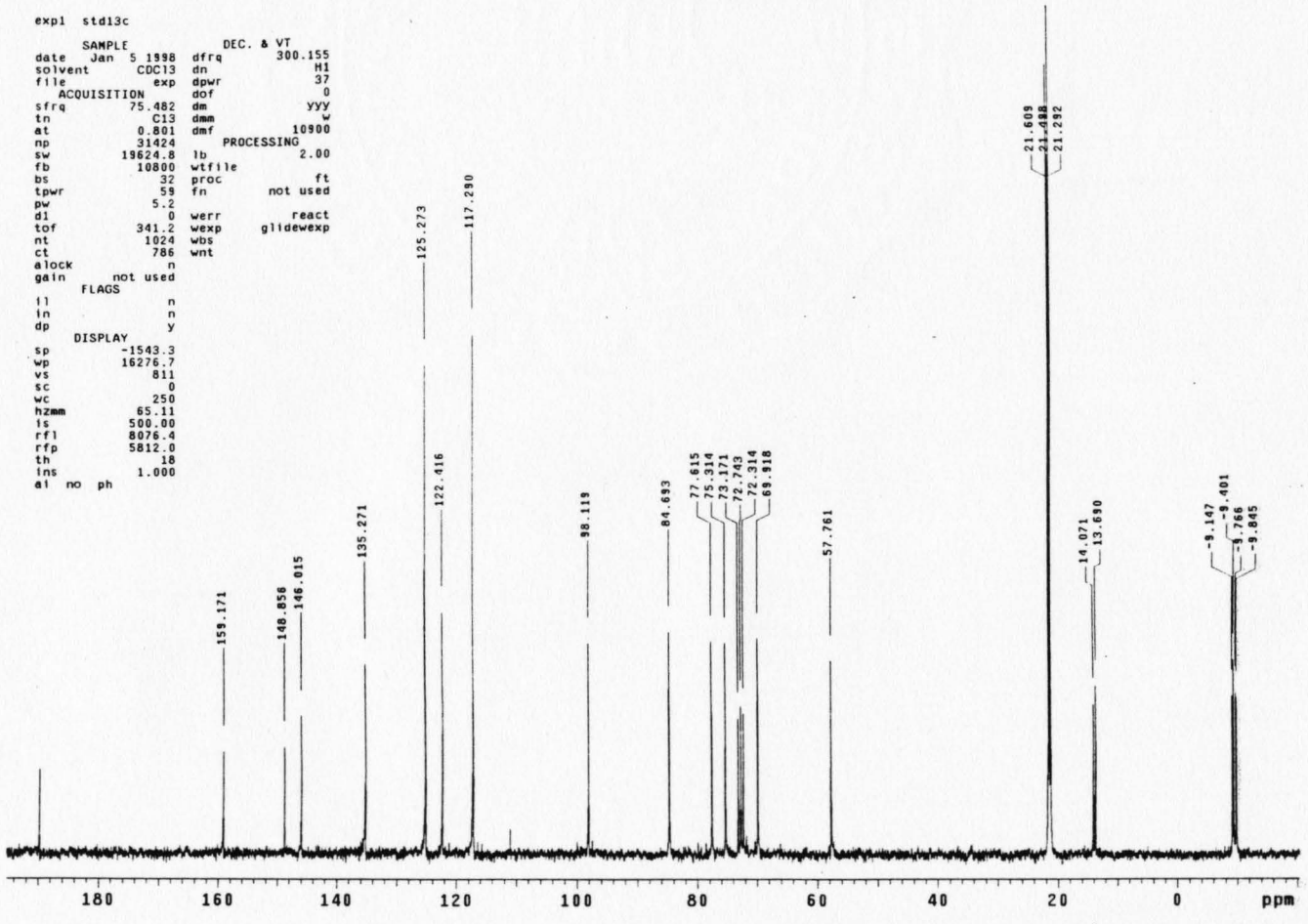
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date Jan 5 1998
solvent CDCl3
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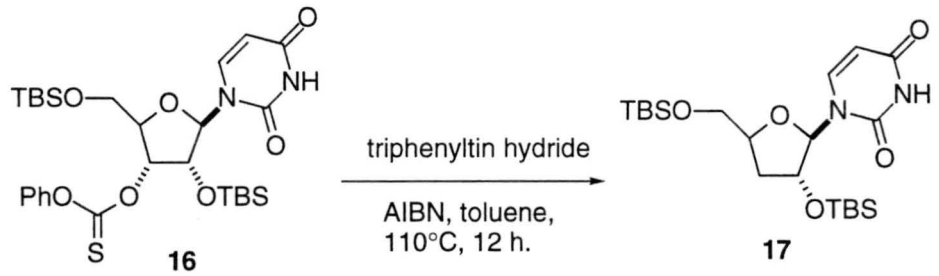


2',5'-bis-O-(*tert*-butyldimethylsilyl)-3'-deoxyuridine (17)

2',5'-O-di-*tert*-butyldimethylsilyl-3'-O-(phenoxythiocarbonyl) uridine (3.00 g, 5.17 mmol) was dissolved in dry toluene (50 mL). 2,2'-azobis(2-methylpropionitrile) (AIBN, 0.896 g, 5.46 mmol) was added and the solution was degassed with N_2 for 10 min. Triphenyltin hydride (7.26 g, 20.68 mmol) was added in a single portion and the solution was refluxed for 8 h. Solvent was removed in vacuo and the crude material was purified by silica gel (100 g) column chromatography (CH_2Cl_2,MeOH, 97:3) to give 1.84 g (83%) of 2',5'-bis-O-(*tert*-butyldimethylsilyl)-3'-deoxyuridine as a clear oil.

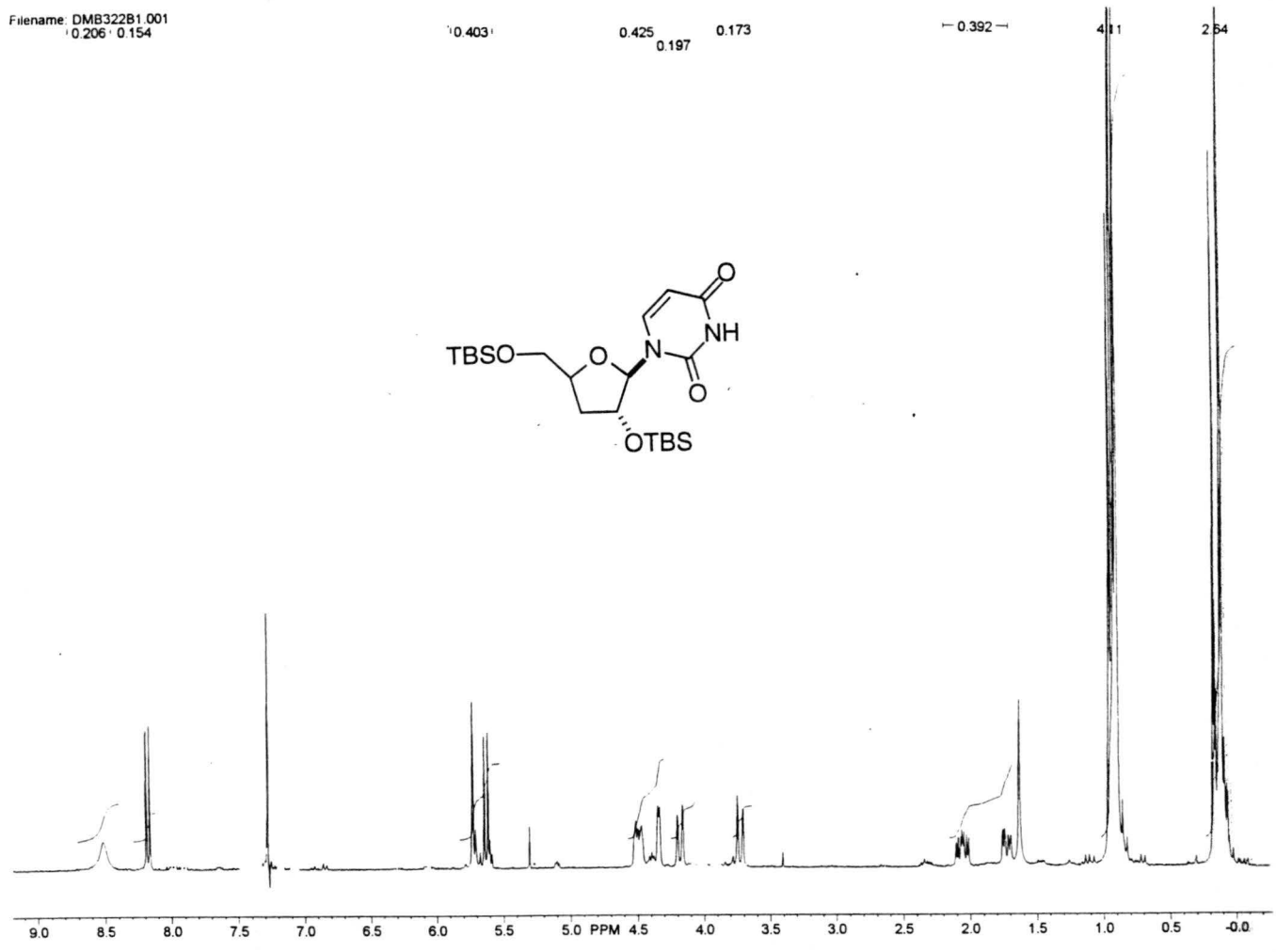
R_f =0.51 (CH_2Cl_2,MeOH, 97:3);

^{1}H NMR ($CDCl_3$) δ 0.13 (12H, m), 0.92 (18H, m), 1.70 (1H, dd, J=5.1, 1.8 Hz), 1.75 (1H, dd, J=5.1, 1.5 Hz), 2.01 - 2.09 (1H, m), 3.72 (1H, 1/2 ABq, J=12.1 Hz), 4.18 (1H, 1/2 ABq, J=12.0 Hz), 4.35 (1H, d, J=3.9 Hz), 4.47 - 4.53 (1H, m), 5.62 (1H, d, J=8.1 Hz), 5.73 (1H, s), 8.16 (1H, d, J=8.1 Hz), 8.50 (1H, s, D_2O exchangeable).

^{13}C NMR (75.48 MHz) ($CDCl_3$) -5.60, -5.49, -5.25, -4.71, 17.85, 18.40, 23.34, 32.69, 39.26, 62.70, 81.55, 92.31, 100.91, 136.30, 140.32, 150.14, 163.73.

IR (NaCl, neat) 3171, 3053, 2989, 2928, 2856, 1956, 1682, 1490, 1462, 1430, 1387, 1338, 1256, 1221, 1132, 1021, 1003 cm -1.

HRMS (FAB+) m/e (M+H) 457.2561 ($C_{21}H_{40}N_2O_5Si_2$ + H requires 457.2554).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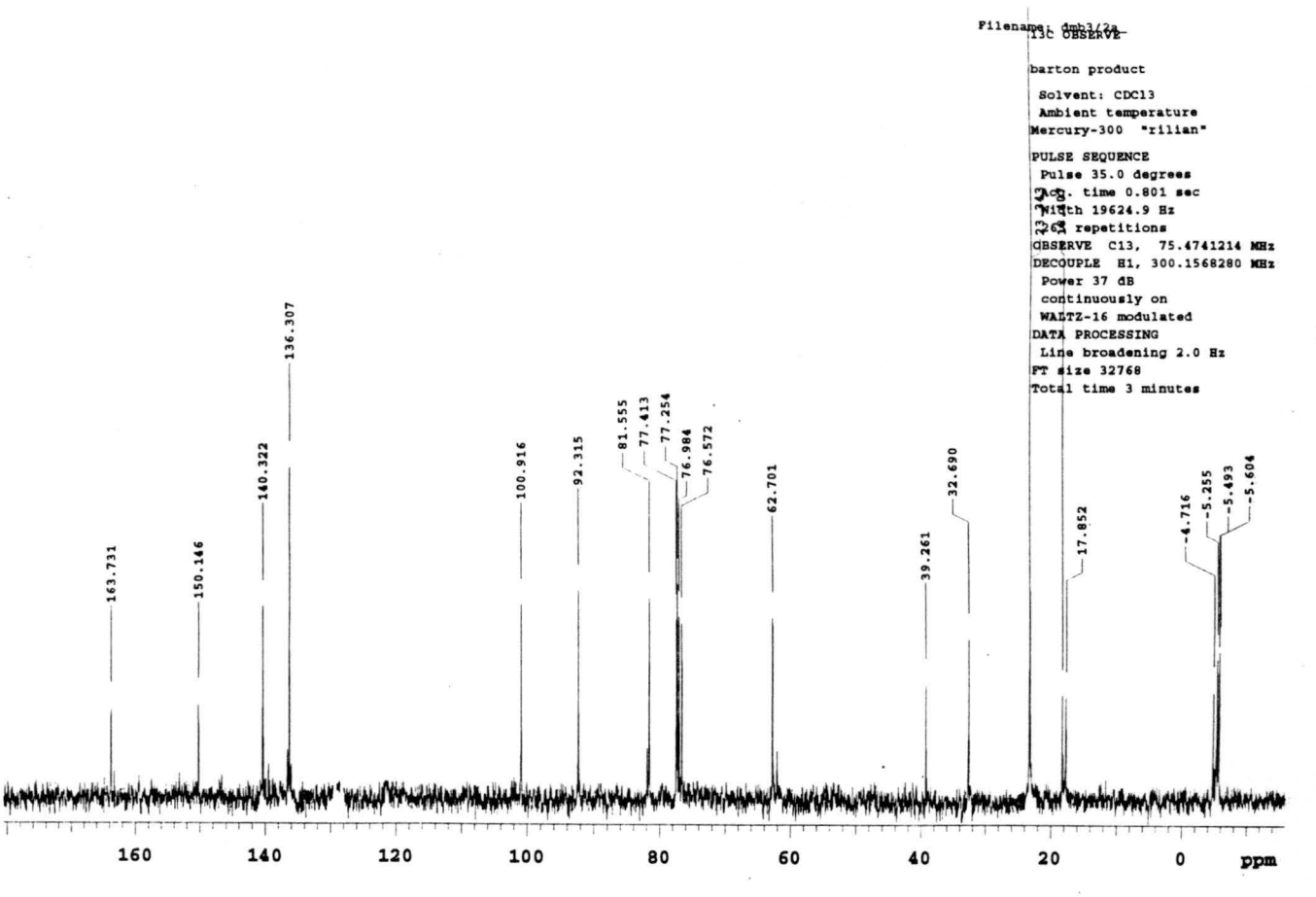
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barton product
Solvent: CDCl3
Ambient temperature
Mercury-300 "rilian"
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Acq. time 0.801 sec
Width 19624.9 Hz
263 repetitions
OBSERVE C13, 75.4741214 MHz
DECOUPLE H1, 300.1568280 MHz
Power 37 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 2.0 Hz
FT size 32768
Total time 3 minutes
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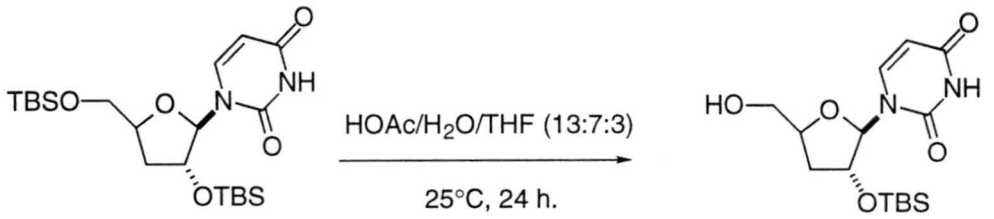


17 18

2'-O-(*tert*-butyldimethylsilyl)-3'-deoxyuridine (18)

2',5'-O-di-(*tert*-butyldimethylsilyl)-3'-deoxyuridine (3.00 g, 6.57 mmol) was dissolved in a solution of 13:7:3 HOAc/H_2O/THF and stirred at room temperature for 24 h. The reaction mixture was concentrated in vacuo and the residue was chromatographed over silica gel (100 g) (CH_2Cl_2,MeOH, 95:5, v/v) to yield 1.76 g (78%) of 2'-O-(*tert*-butyldimethylsilyl)-3'-deoxyuridine as a white foam.

R_f=0.33 (CH_2Cl_2,MeOH, 95:5, v/v).

m.p. 58-60°C.

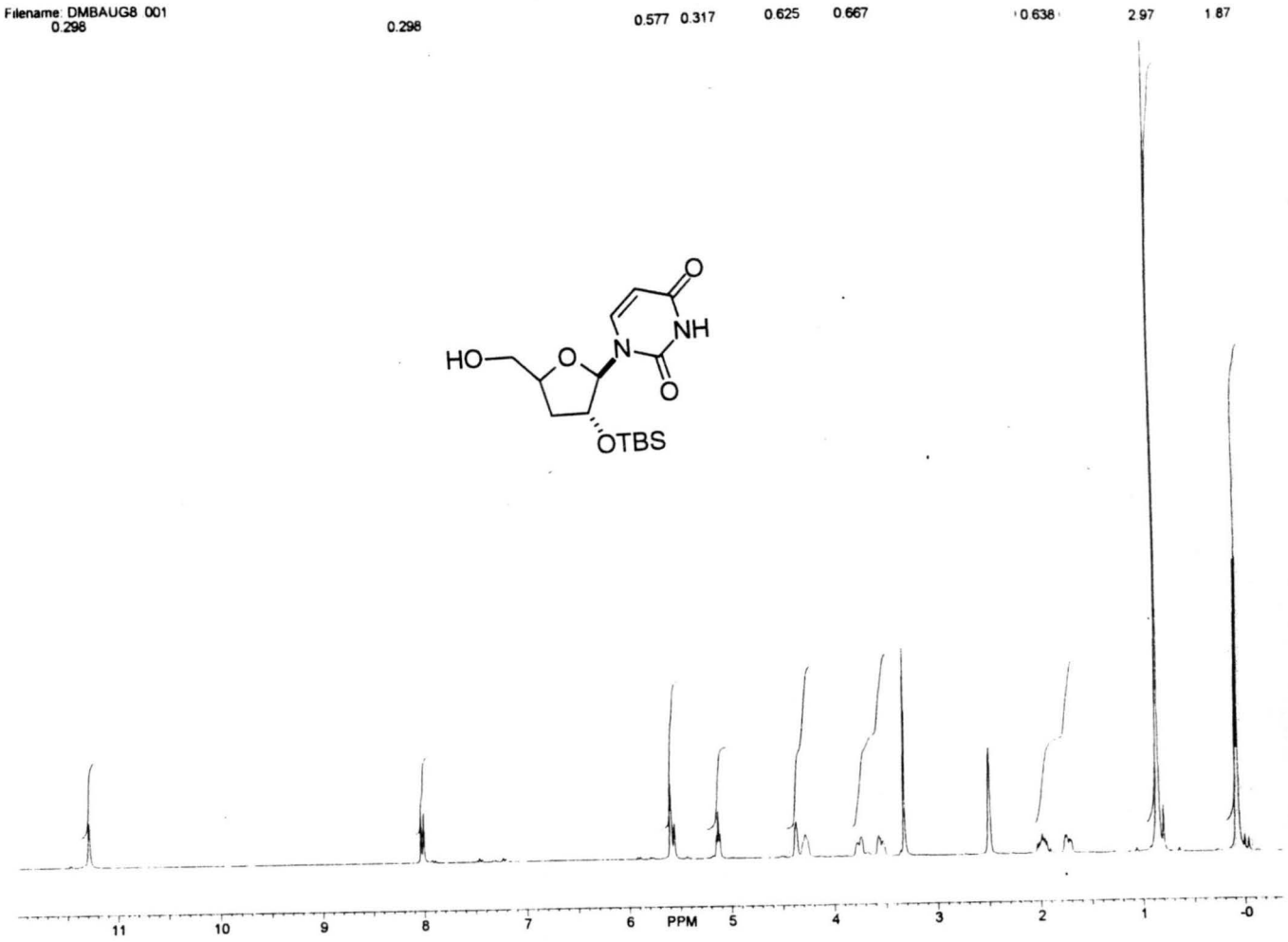
^{1}H NMR (300 MHz) (d_6-DMSO) δ 0.09 (6H, d), 0.88 (9H, s), 1.71 (1H, dd, J=5.7, 3.3 Hz), 1.75 (1H, dd, J=5.4, 2.1 Hz), 1.94 - 2.01 (1H, m), 3.52 - 3.58 (1H, m), 3.73 – 3.78 (1H, m), 4.28 (1H, m), 4.37 (1H, m), 5.57 (2H, m), 8.02 (1H, d, J=8.1 Hz), 11.28 (1H, s, D_2O exchangeable).

^{13}C NMR (75.48 MHz) ($CDCl_3$) δ -5.04, -4.68, 17.94, 25.69, 33.56, 62.60, 76.51, 81.17, 93.98, 101.34, 141.03, 150.06, 163.60.

IR (NaCl, neat) 3399, 3053, 2929, 2856, 1684, 1558, 1540, 1463, 1388, 1261, 1122, 1076, 1001 cm -1.

$[\alpha]_D^{25}$= -9.8 (c=1.0, CH_2Cl_2).

HRMS (FAB+) m/e (M+H) 343.1697 ($C_{15}H_{26}N_2O_5Si$ + H requires 343.1689). Anal. Calcd for $C_{15}H_{26}N_2O_5Si$: C, 52.61; H, 7.65; N, 8.18. Found: C, 52.73; H, 7.70, N, 8.23.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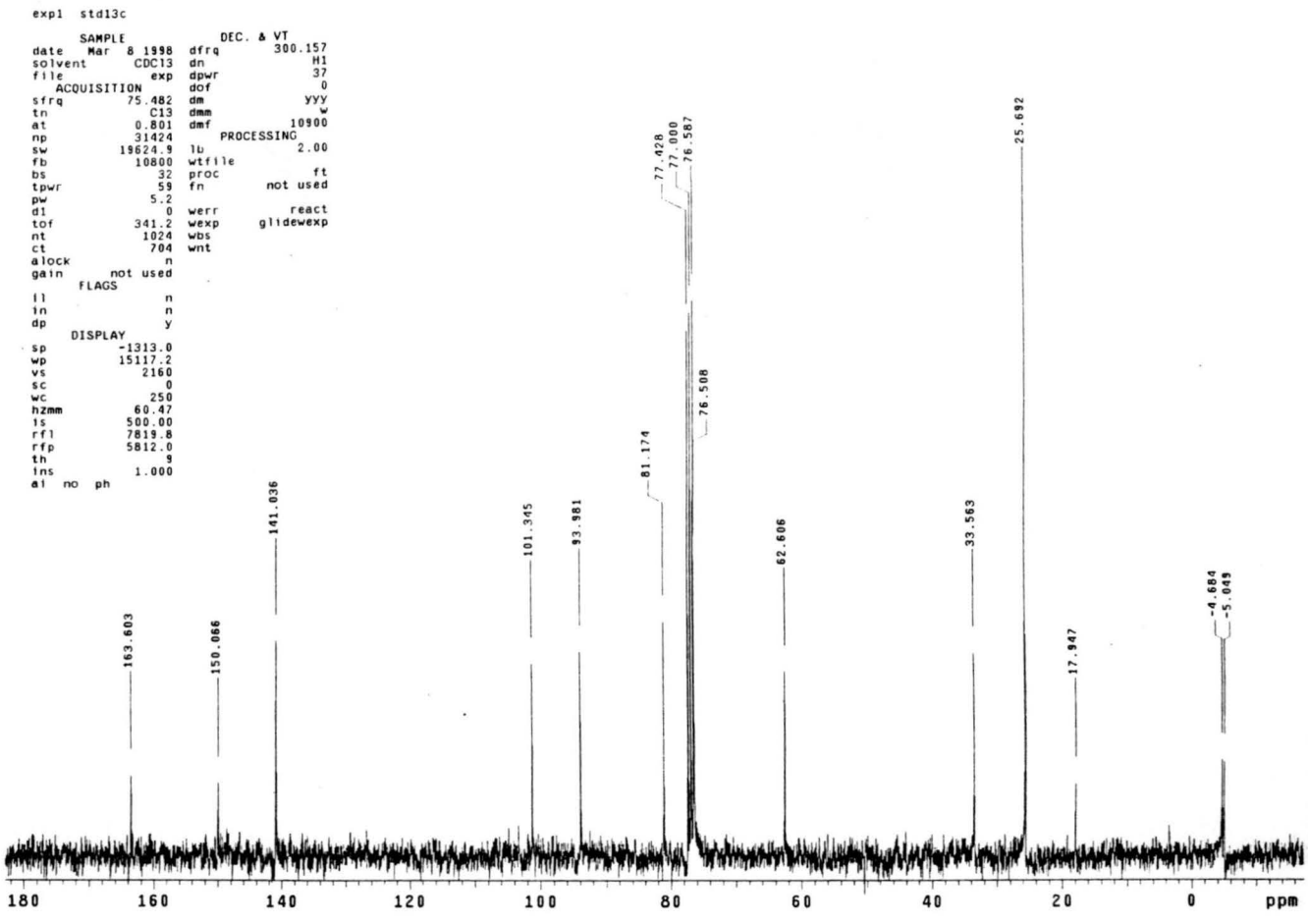
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proc ft
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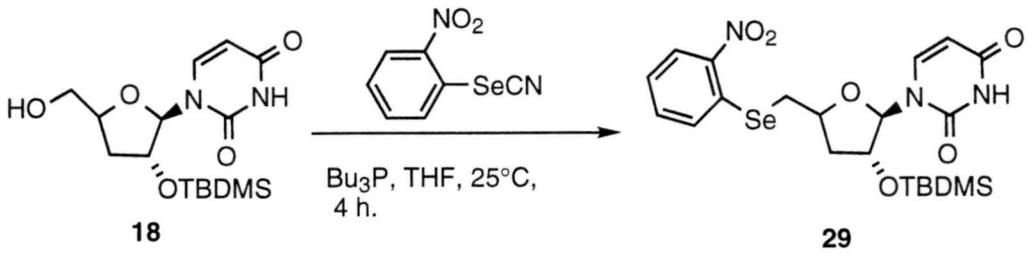


18 **29**

2′-(O-*tert*butyldimethylsilyl)-5′-(*o*-nitrophenyl)-seleno-3′5′-dideoxyuridine (29)

Alcohol **18** (0.85 g, 2.48 mmol) was dissolved in THF (20 mL). *O*-nitrophenylseleno cyanate (1.12 g, 4.97 mmol) was added followed by tributylphosphine (1.24 mL, 4.97 mmol) and the reaction was stirred at 25°C under argon for 1 h. The mixture was concentrated to a crude red solid which was purified by column chromatography (CH_2Cl_2,MeOH, 95:5, v/v) to yield 1.26 g (96%) of **29** as a bright yellow solid.

R_f=0.39 (CH_2Cl_2,MeOH, 95:5, v/v).

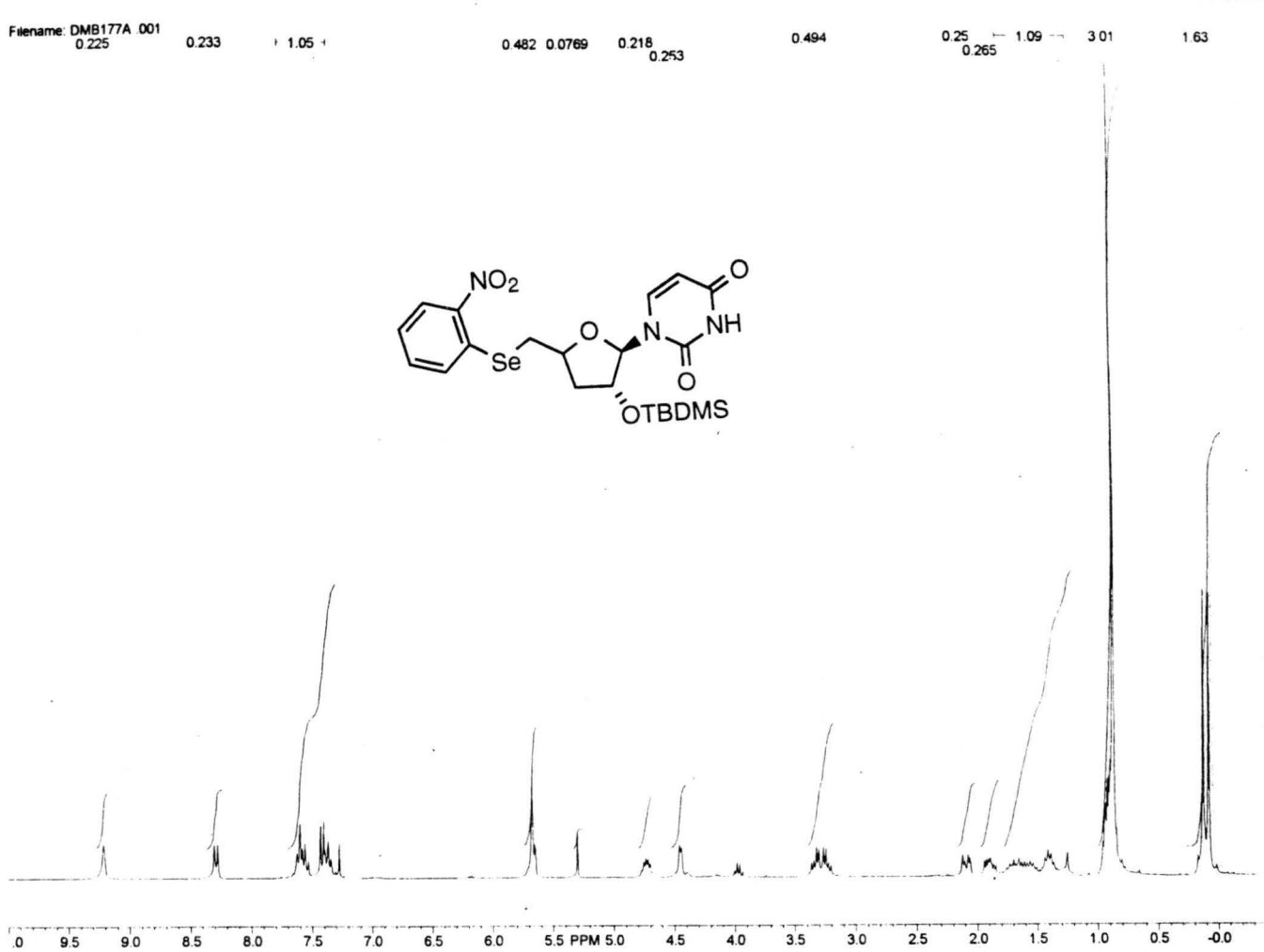
m.p. 86-88°C.

^{1}H NMR (300 MHz) ($CDCl_3$) δ 0.11 (6H, d), 0.89 (9H, s), 1.89-1.92 (1H, m), 2.06-2.12 (1H, m), 3.21 - 3.36 (2H, ddd, J=16.8, 12.6, 5.7 Hz), 4.44 (1H, d, J=4.8 Hz), 4.72 (1H, m), 5.67 (2H, m), 7.33-7.62 (4H, m), 8.29 (1H, d, J=8.4 Hz), 9.22 (1H, s, D_2O exchangeable).

^{13}C NMR (75.48 MHz) ($CDCl_3$) δ -5.51, -5.14, 17.47, 25.23, 29.46, 39.23, 76.15, 78.41, 93.72, 101.42, 125.53, 125.95, 128.73, 133.32, 139.24, 149.51, 162.95.

IR (NaCl, neat) 3419, 2954, 2929, 2856, 1683, 1590, 1331, 1304, 1259, 1152 cm -1.

HRMS (FAB+) m/e (M+H) 528.1059 ($C_{21}H_{29}N_3O_6SiSe$ + H requires 528.1069).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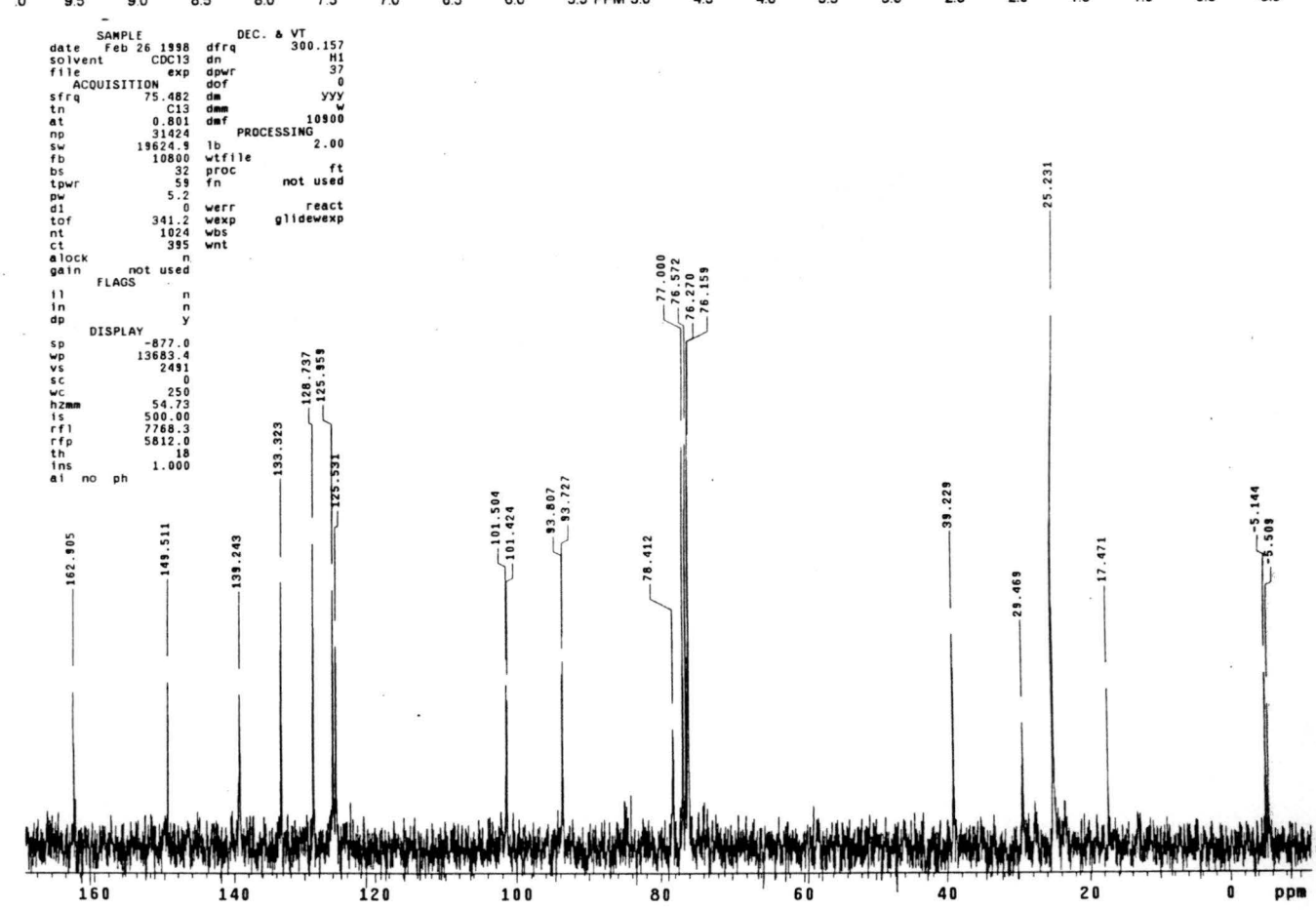
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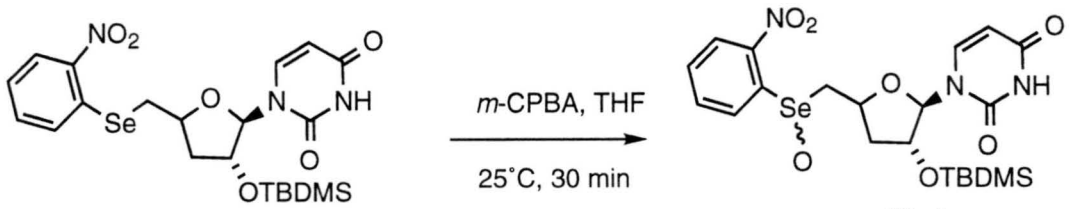


29 **30a,b**

2′-(O-*tert*butyldimethylsilyl)-5′-(*o*-nitrophenyl)-selenoxy-3′5′-dideoxyuridine (30a,b)

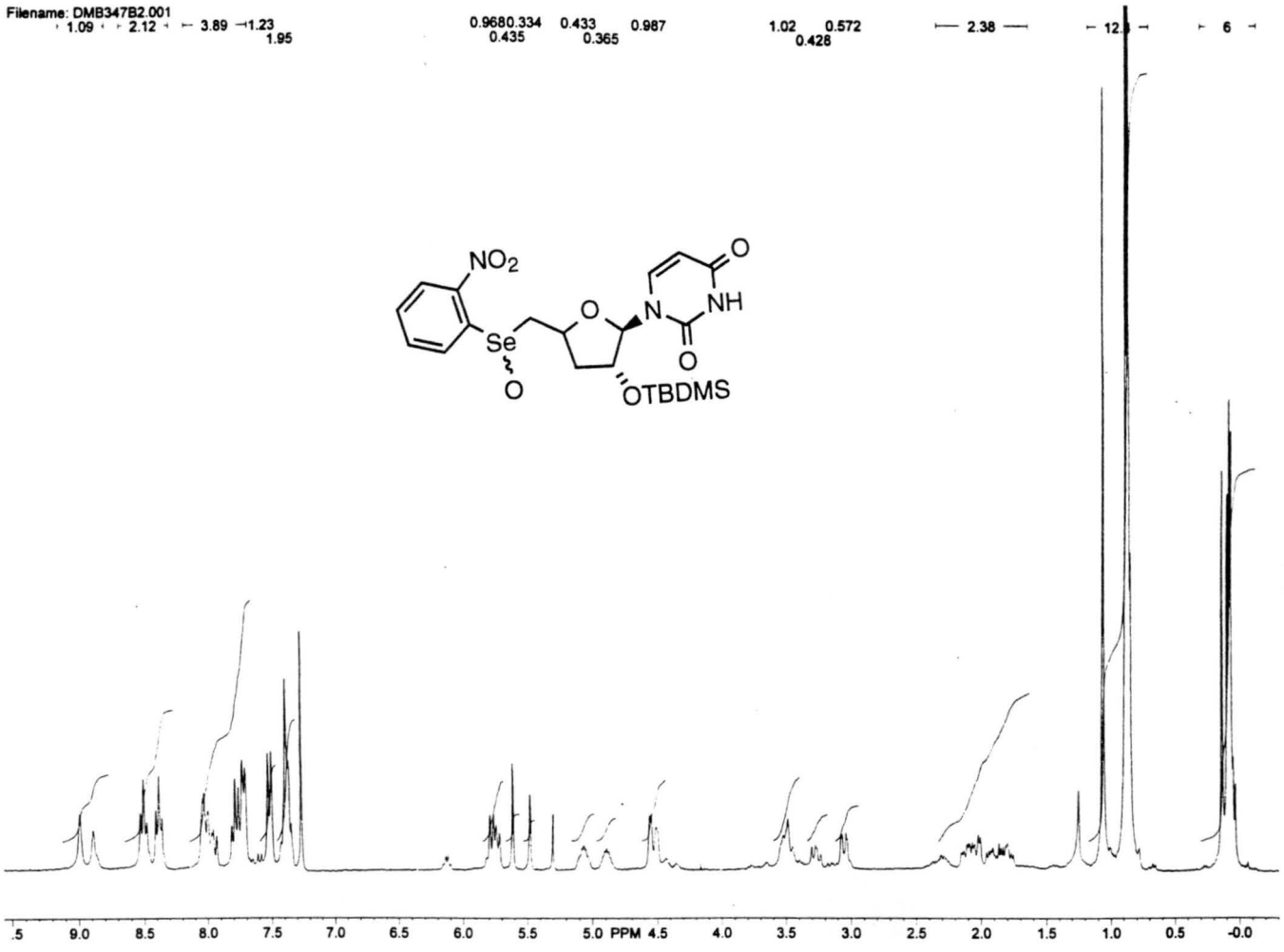
Compound **29** (0.10 g, 0.19 mmol) was combined with *m*-CPBA (0.072 g, 0.209 mmol) in THF (4 mL) and stirred at 25°C under argon for 30 min. The reaction was concentrated to a yellow solid which was purified by silica gel chromatography (CH_2Cl_2,MeOH, 95:5, v/v) to yield 100 mg (99%) of a white solid.

R_f=0.19 (CH_2Cl_2,MeOH, 95:5, v/v).

^{1}H NMR (300 MHz) ($CDCl_3$) δ 0.08 (6H, m), 0.87 (9H, d), 1.75-2.37 (2H, m), 3.01 - 3.52 (2H, m), 4.50-4.56 (1H, m), 4.89 (1H, m), 5.07 (1H, m), 5.48 (1/2H, s), 5.61 (1/2H, s), 5.72 (1/2H, d, J=8.1 Hz), 5.78 (1/2H, d, J=8.1 Hz), 7.34-8.04 (4H, m), 8.36-8.53 (1H, m), 8.89 (1/2H, s, D_2O exchangeable), 8.99 (1/2H, s, D_2O exchangeable).

IR (NaCl, neat) 3271, 2926, 2857, 1748, 1699, 1596, 1575, 1529, 1472, 1427, 1337, 1287, 1245, 1100 cm -1.

LRMS (FAB+) m/e (M+H) 543.2 ($C_{21}H_{29}N_3O_6SiSe$ + H requires 543.5).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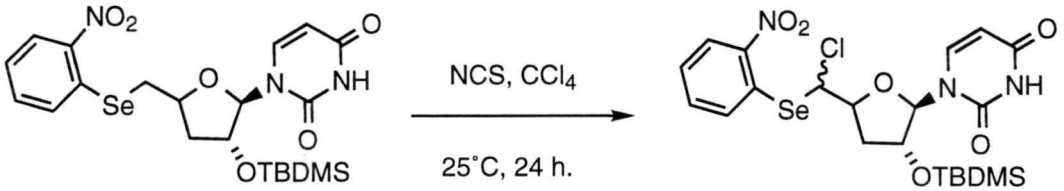


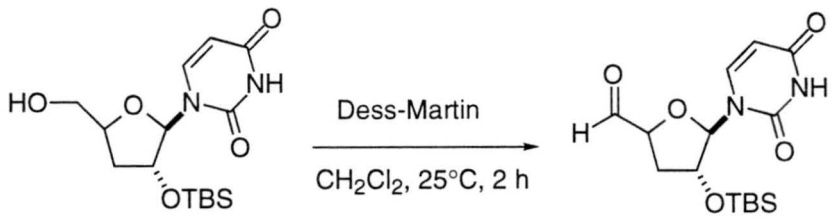
29 **32a,b**

2′-(O-*tert*butyldimethylsilyl)-5′-(*o*-nitrophenyl)-seleno-5′chloro-3′5′-dideoxyuridine (32a,b)

Selenide **27** (0.10 g, 0.19 mmol) was dissolved in carbon tetrachloride (2 mL). N-chlorosuccinimide (0.076 g, 0.569 mmol) was added and the reaction was stirred at 25°C under argon. The reaction was monitored by TLC. When starting material had been comsumed, the succinimide was filtered off and the solvent was evaportated to yield a white solid. This compound was analyzed by ^{1}H NMR and LRMS and used without purification.

R_f=0.61 (CH_2Cl_2,MeOH, 95:5, v/v). ^{1}H NMR (300 MHz) ($CDCl_3$) δ 0.08 (6H, m), 0.88 (9H, d), 1.67-2.31 (2H, m), 3.06 - 3.14 (1H, m), 4.38-4.42 (1H, m), 4.72 (1H, m), 5.11 (1H, m), 5.51 (1/2H, s), 5.56 (1/2H, s), 5.81 (1/2H, m), 5.88 (1/2H, m), 7.22-8.19 (4H, m), 8.13-8.34 (1H, m).

LRMS (FAB+) m/e (M+H) 579.5 ($C_{21}H_{28}N_3O_6SiSeCl$ + H requires 579.0).



18 **37**

2'-O-(*tert*-butyldimethylsilyl)-3'-deoxyuridine-5′-aldehyde (37)

Alcohol **18** (0.019 g, 0.057 mmol) was dissolved in methylene chloride (3 mL). Dess-Martin periodinane (0.031 g, 0.086 mmol) was added and the reaction was capped with a glass stopper and stirred at 25°C for 3 h. A solution of sodium thiosulfate pentahydrate (0.142 g, 0.573 mmol) in saturated $NaHCO_3$ (2 mL) was added and the reaction was stirred for an additional 2 h. The mixture was diluted with methylene chloride (2 mL) and the layers were separated. The organic phase was extracted with $NaHCO_3$ and brine, dried ($MgSO_4$) and concentrated to a white solid. Chromatography (CH_2Cl_2,MeOH, 9:1, v/v) gave aldehyde **37** as a colorless oil in 64% yield.

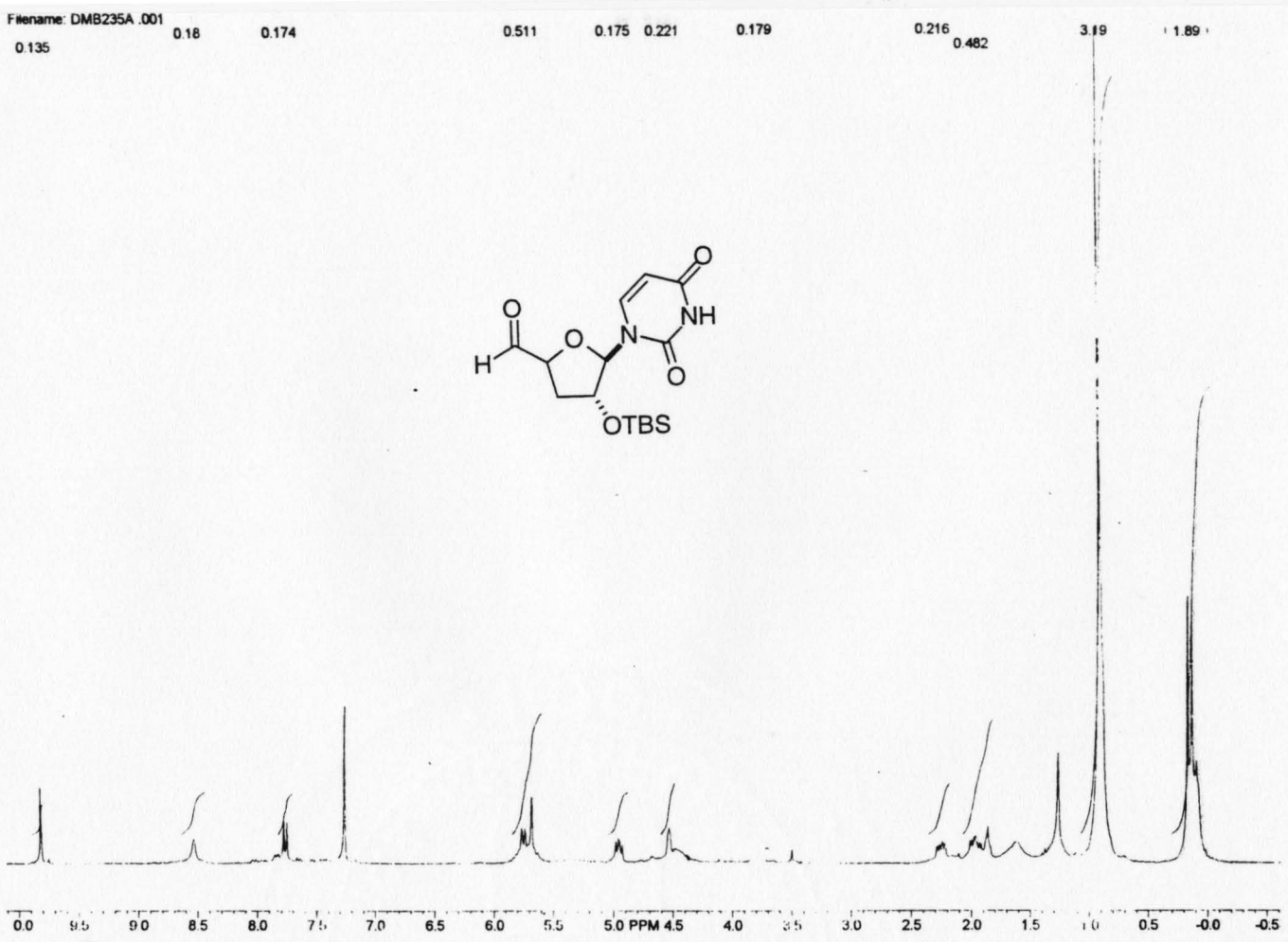
R_f=0.57 (CH_2Cl_2,MeOH, 9:1, v/v).

^{1}H NMR (300 MHz) ($CDCl_3$) δ 0.12 (6H, d), 0.90 (9H, s), 1.87-2.01 (1H, m), 2.22-2.24 (1H, m), 4.54 (1H, m), 4.95 (1H, dd, J=6.6, 3.2 Hz), 5.69 (1H, s), 5.77 (1H, d, J=8.1), 7.76 (1H, d, J=8.1 Hz), 8.54 (1H, s, D_2O exchangeable), 9.82 (1H, s).

^{13}C NMR (75.48 MHz) ($CDCl_3$) -5.12, -4.71, 17.86, 25.59, 34.48, 45.70, 75.50, 94.72, 101.94, 139.76, 150.05, 163.50, 198.63.

IR (NaCl, neat) 3194, 3062, 2953, 2929, 2886, 2857, 1684, 140, 1462, 1379, 1261, 1101, 1006 cm -1.

LRMS (FAB+) m/e (M+H) 341.2 ($C_{15}H_{26}N_2O_5Si$ + H requires 341.4).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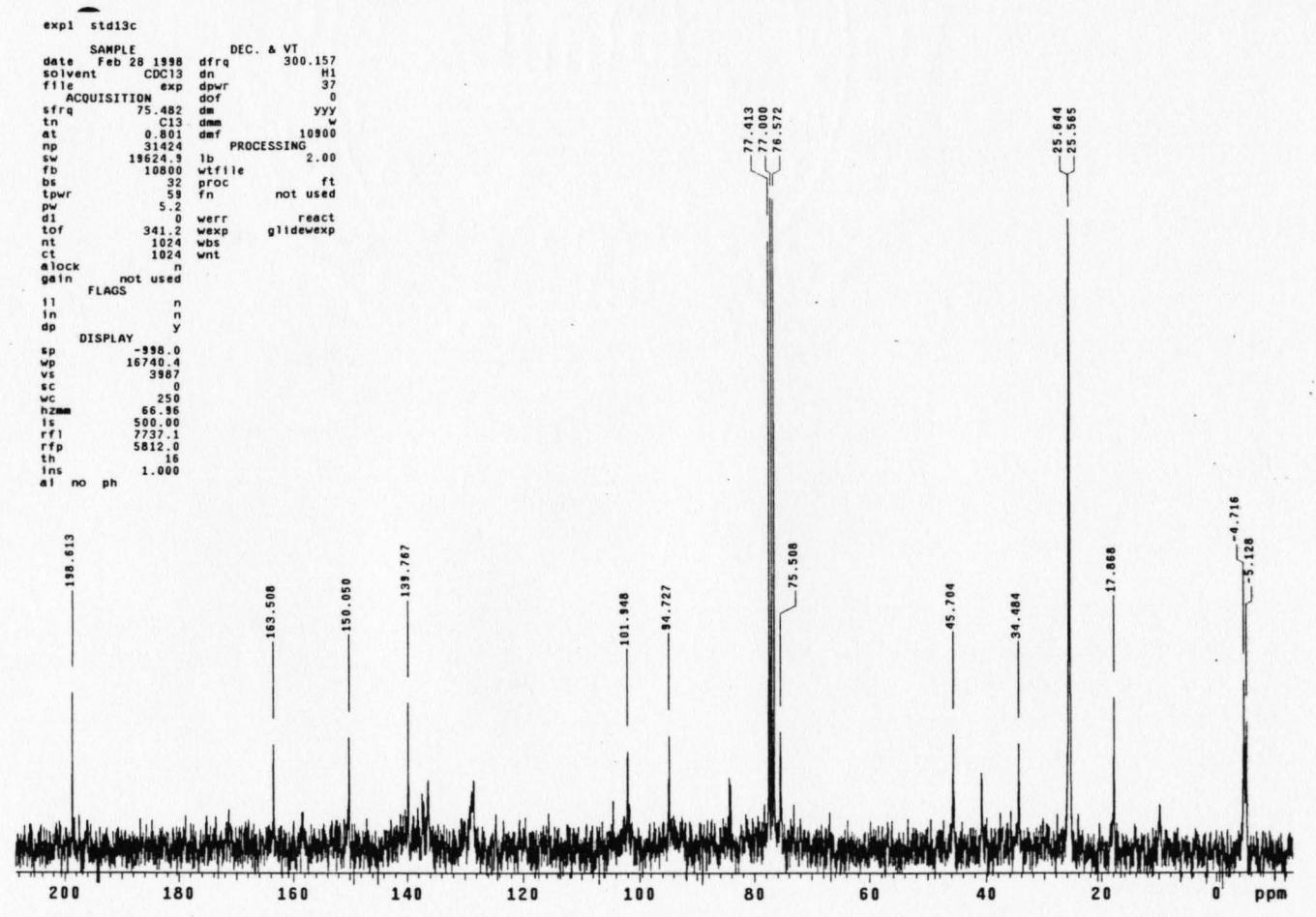
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date Feb 28 1998
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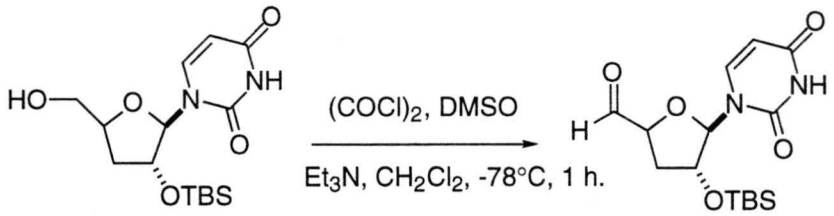
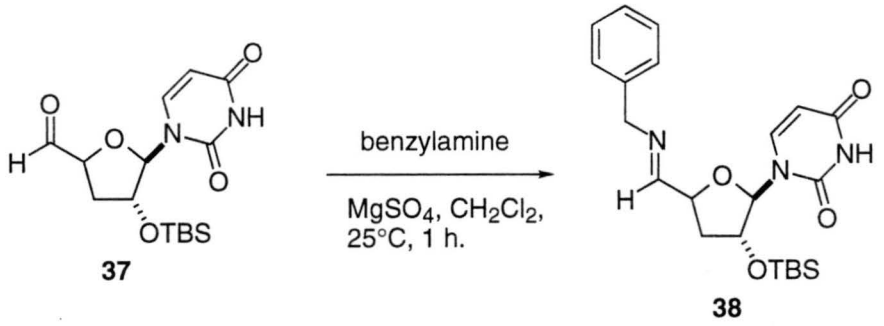


18 **37**

2'-O-(*tert*-butyldimethylsilyl)-3'-deoxyuridine-5'-aldehyde. (37)

Oxalyl chloride (28.0 μL, 0.32 mmol) was dissolved in methylene chloride (2 mL) and cooled to -78°C while stirring under argon. Dimethyl sulfoxide (45.6 μL, 0.642 mmol) in methylene chloride (1 mL) was added dropwise and the reaction was stirred for 5 min. Alcohol **18** in methylene chloride (1 mL) was added and the reaction was stirred at -78°C for 15 min. Triethylamine (.20 mL, 1.46 mmol) was added and the reaction was stirred at -78° for 10 min. then allowed to warm to room temperature. The mixture was concentrated and the crude residue was applied to a column of silica gel (CH_2Cl_2,MeOH, 9:1, v/v) to give 90 mg (91%) of aldehyde **37** as a colorless oil.

(data for this compound matches that reported on the previous page)



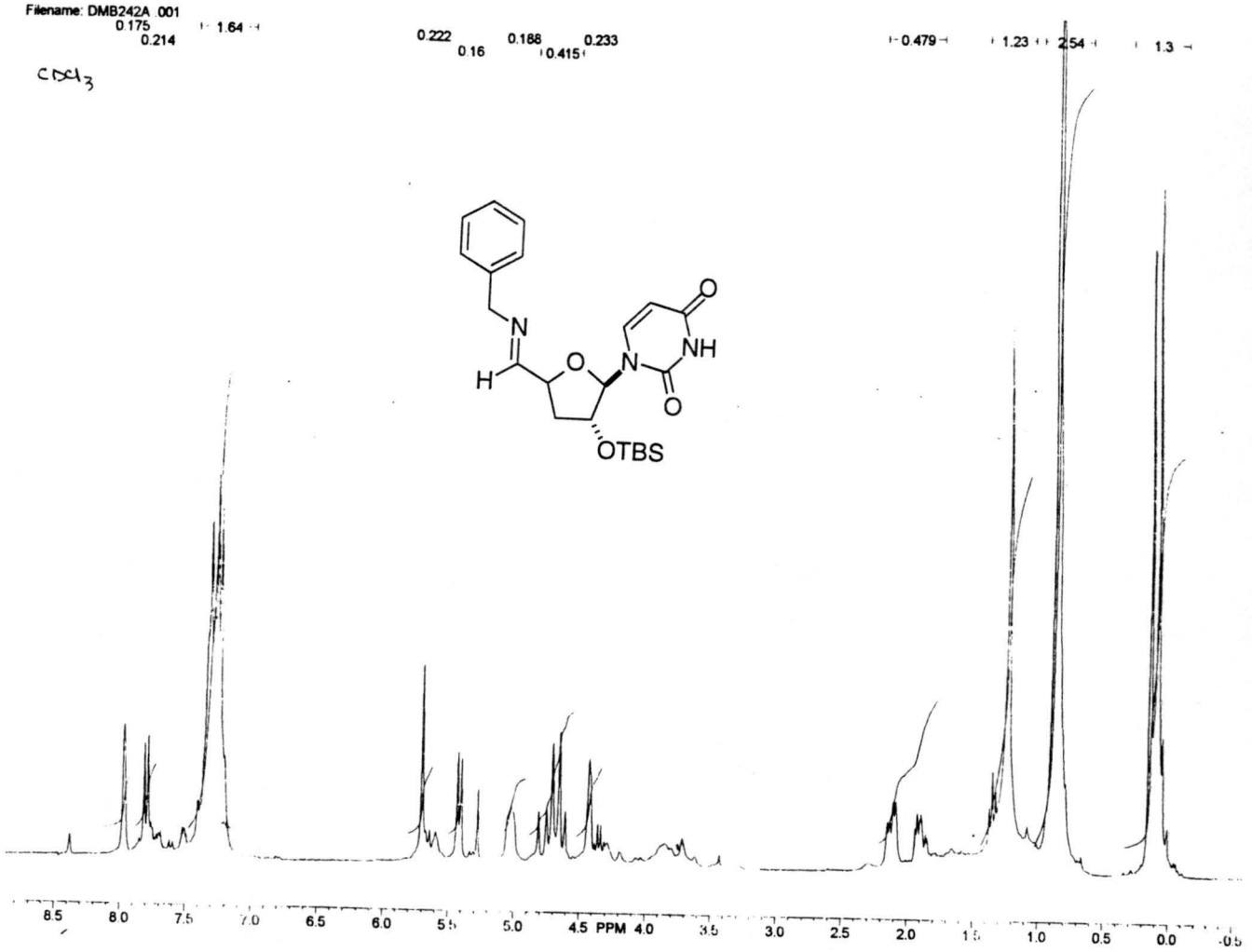
37 38

2'-O-(*tert*-butyldimethylsilyl)-3'-deoxy-5'-benziminouridine (38)

$MgSO_4$ (0.018 g, 0.147 mmol) was placed in a flame-dried round bottom flask. Aldehyde **37** (0.005 g, 0.015 mmol) was dissolved in 200 µL of methylene chloride and added to the reaction followed by benzylamine (12.7 µL, 0.073 mmol). The reaction was stirred under argon at 25°C overnight. The $MgSO_4$ was removed by filtration and the mixture was concentrated to a light yellow oil.

R_f=0.28 (CH_2Cl_2,MeOH, 95:5, v/v).

^{1}H NMR (300 MHz) ($CDCl_3$) δ 0.11 (6H, d), 0.87 (9H, s), 1.84-1.94 (1H, m), 2.09-2.15 (1H, m), 4.42 (1H, m), 4.68 (2H, m), 5.02 (1H, m), 5.41 (1H, d, J=8.4 Hz), 5.70 (1H, s), 7.20-7.41 (5H, m), 7.79 (1H, d, J=8.1 Hz), 7.97 (1H, s).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CDCl3
N
H
O
N
NH
O
OTBS
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
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3.0
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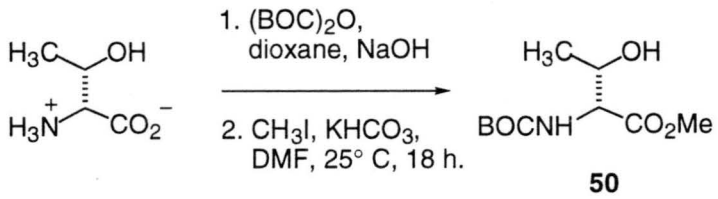
1.5
1.0
0.5
0.0
-0.5



50

(2R,3S)-N-(*tert*-butoxycarbonyl)-D-threonine methyl ester (50)

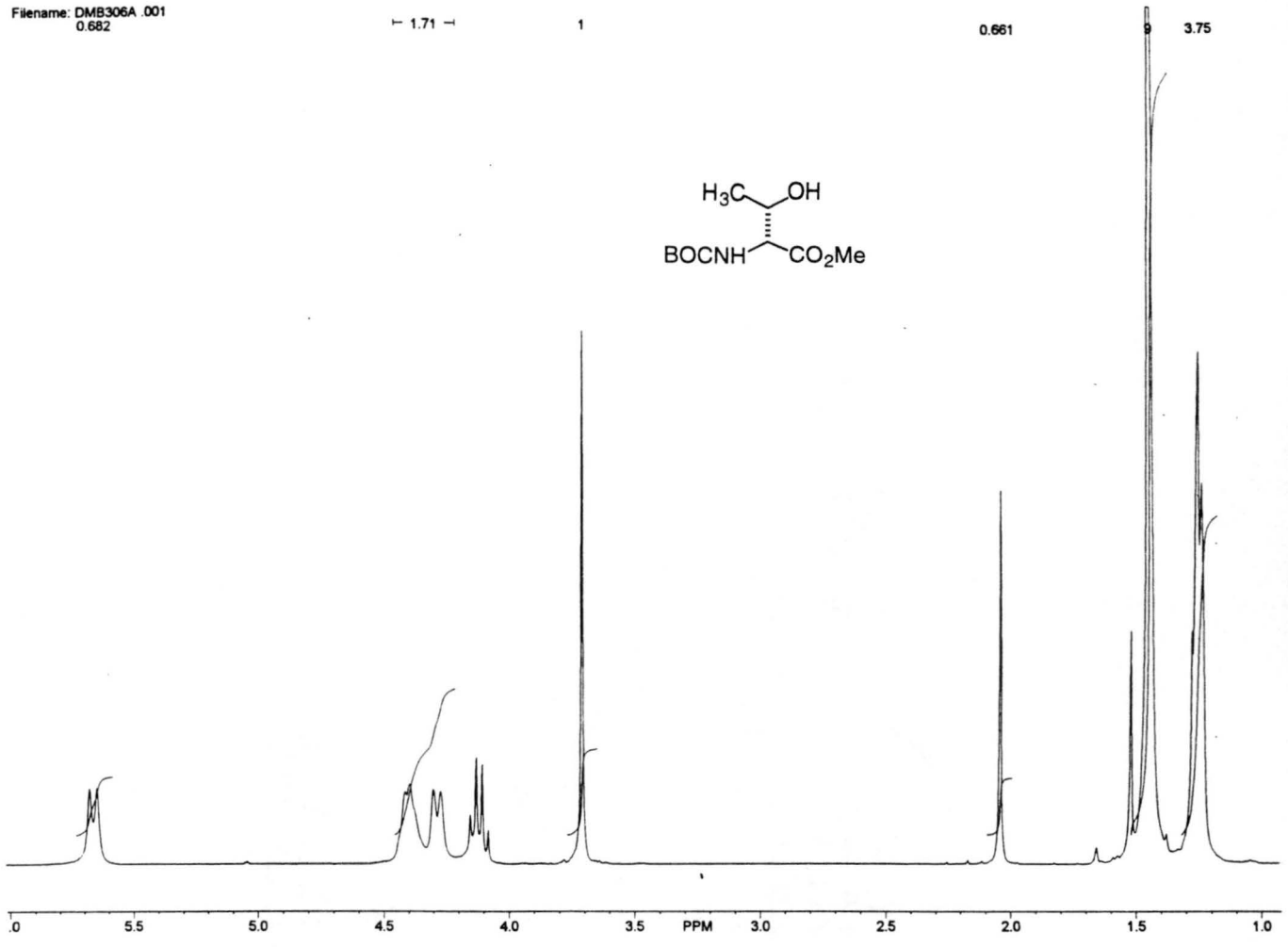
To a suspension of D-threonine (10.0 g, 83.9 mmol) in dioxane (160 mL) and H_2O (80 mL) at 0°C was added 1 M NaOH (83.9 mL, 83.9 mmol) and $(BOC)_2$ (20.0 g, 92.3 mmol). The reaction was stirred for 30 min. then concentrated to a volume of approximately 100 mL. Ethyl acetate was added and the solution was cooled to 0°C and acidified to pH 2 with 10% $KHSO_4$. The layers were separated and this sequence was repeated three times. The organic layers were combined, washed with H_2O, dried ($MgSO_4$) and concentrated to give a white solid. 17.1 g (93%) of N-*tert*-butoxycarbonyl-D-threonine were isolated in this manner. This compound was used without further purification.

^{1}HNMR (300 MHz, $CDCl_3$): δ 1.26 (3H, d, J=6.4 Hz), 1.46 (9H, s), 4.11 (1H, m), 4.28 (1H, d, J=8.7 Hz), 4.40 (1H, m), 5.66 (1H, d, J=8.7 Hz).

N-(*tert*-butroxycarbonyl)-D-threonine (5.0 g, 22.8 mmol) was dissolved in DMF (150 mL). $KHCO_3$ (4.6 g, 45.6 mmol) and methyl iodide (2.3 mL, 36.5 mmol) were added and the reaction was stirred at 25°C for 10 min. The reaction mixture was poured into 200 mL of H_2O and extracted three times

with ethyl acetate. The organic layers were combined, washed with brine, dried ($MgSO_4$) and concentrated to a light yellow oil. Chromatography (ethyl acetate/hexanes, 1:1, v/v) gave **50** as a colorless oil.

^1H NMR (300 MHz, $CDCl_3$): δ 1.23 (3H, d, J=6.4 Hz), 1.43 (9H, s), 2.33 (1H, d, J=5.1 Hz), 3.76 (3H, s), 4.21 (2H, m), 5.36 (1H, d, J=8.5 Hz). ^{13}C NMR (75.47 MHz, $CDCl_3$): δ 20.1, 28.5, 52.5, 58.9, 68.1, 156.5, 172.7.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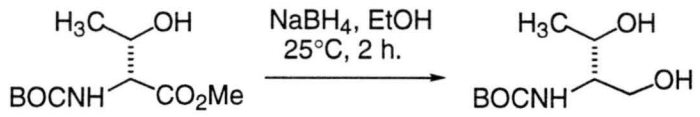
2.0
1.5
1.0



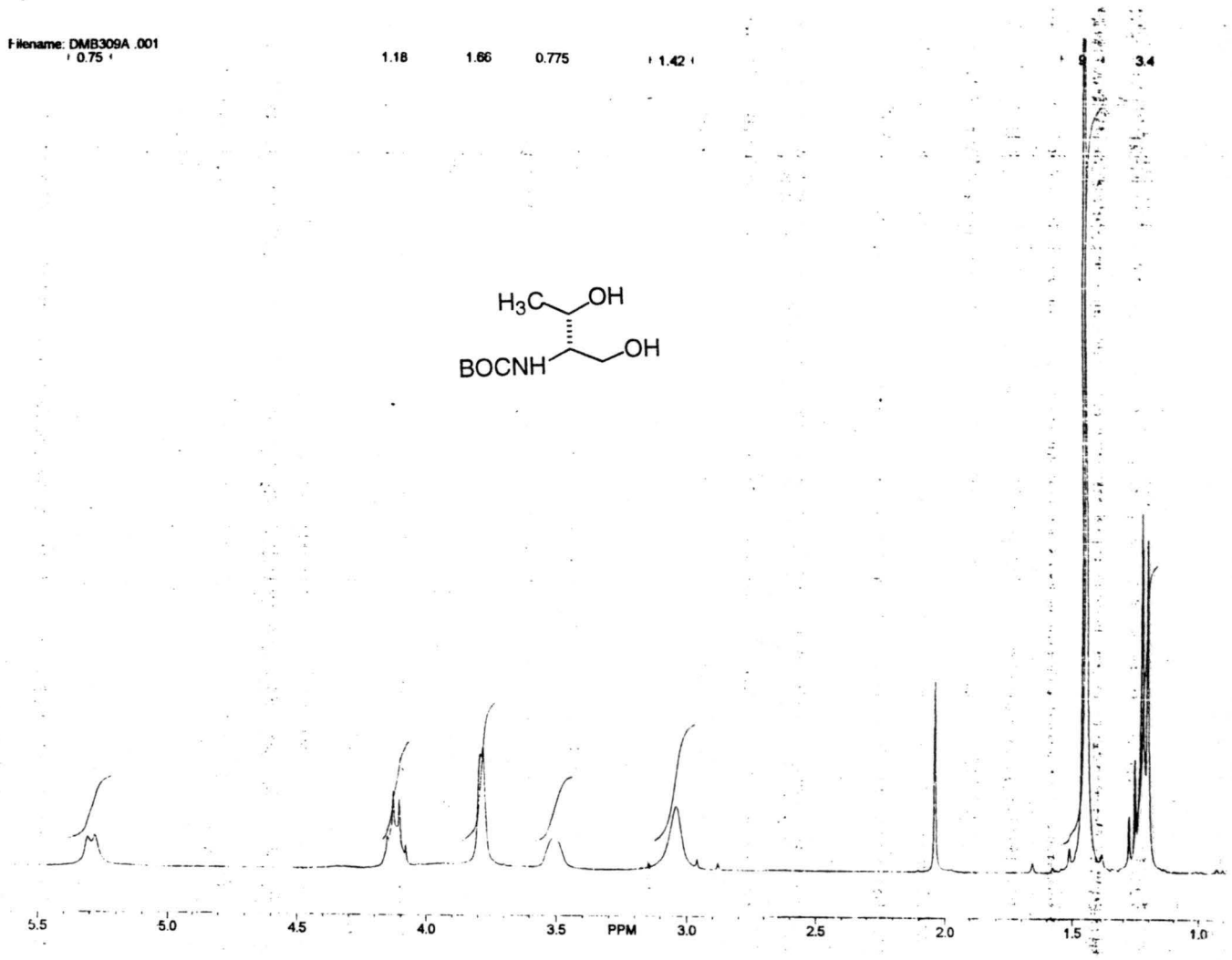
50

(2R,3S)-2-(*tert*-butoxycarbonyl)amino-1,3-butanediol

(2R,3S)-N-(*tert*-butoxycarbonyl)-D-threonine methyl ester **(50)** (1.3 g, 5.53 mmol) was dissolved in absolute ethanol (10 mL) and cooled to 0°C while stirring under argon. Sodium borohydride (0.42 g, 11.2 mmol) was added in small portions over 10 min. as the reaction was strongly exothermic. The reaction was stirred at 0° for 2 h. then at 25°C for 2 h. followed by quenching with sat'd NH_4Cl (25 mL). The mixture was concentrated to a volume of 20 mL and extracted with ethyl acetate (3 X 30 mL). The organics were washed with brine, dried ($MgSO_4$) and concentrated to a yellow oil. This compound was sufficiently pure to be used without purification. A total of 0.92 g (81%) of the title compound was isolated as a clear oil.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.21 (3H, d, J=6.3 Hz), 1.45 (9H, s), 3.04 (1H, s), 3.51 (1H, m), 3.78 (2H, m), 4.11 (1H, m), 5.28 (1H, d, J=8.1 Hz).

IR (NaCl, neat) 3452, 3101, 3071, 2987, 2868, 1683, 1637, 1585, 1479, 1388, 1325, 1240, 1121 cm^{-1}.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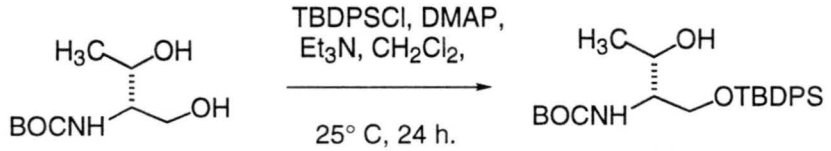
1.0



51

(2R,3S)-2-(*tert*-butoxycarbonyl)-amino-1-(*tert*-butyldiphenyl)-siloxy-3-butanol (51)

(2R,3S)-2-(*tert*-butoxycarbonyl)amino-1,3-butanediol was dissolved in CH_2Cl_2 (10 mL) followed by DMAP (0.05 g, 0.50 mmol), *tert*-butyldiphenylsilyl chloride (1.16 mL, 4.46 mmol), and triethylamine (746 µL, 5.35 mmol). The reaction was stirred at 25°C under argon for 24 h. The mixture was poured into chloroform (20 mL), washed with H_2O (2 X 20 mL), dried ($MgSO_4$) and concentrated to a yellow oil. The residue was purified by chromatography (ethyl acetate/hexanes, 1:3, v/v) to yield 1.90 g (96%) of **51** as a white solid.

R_f=0.56 (hexanes,ethyl acetate, 3:1, v/v).

m.p. 72-75°C.

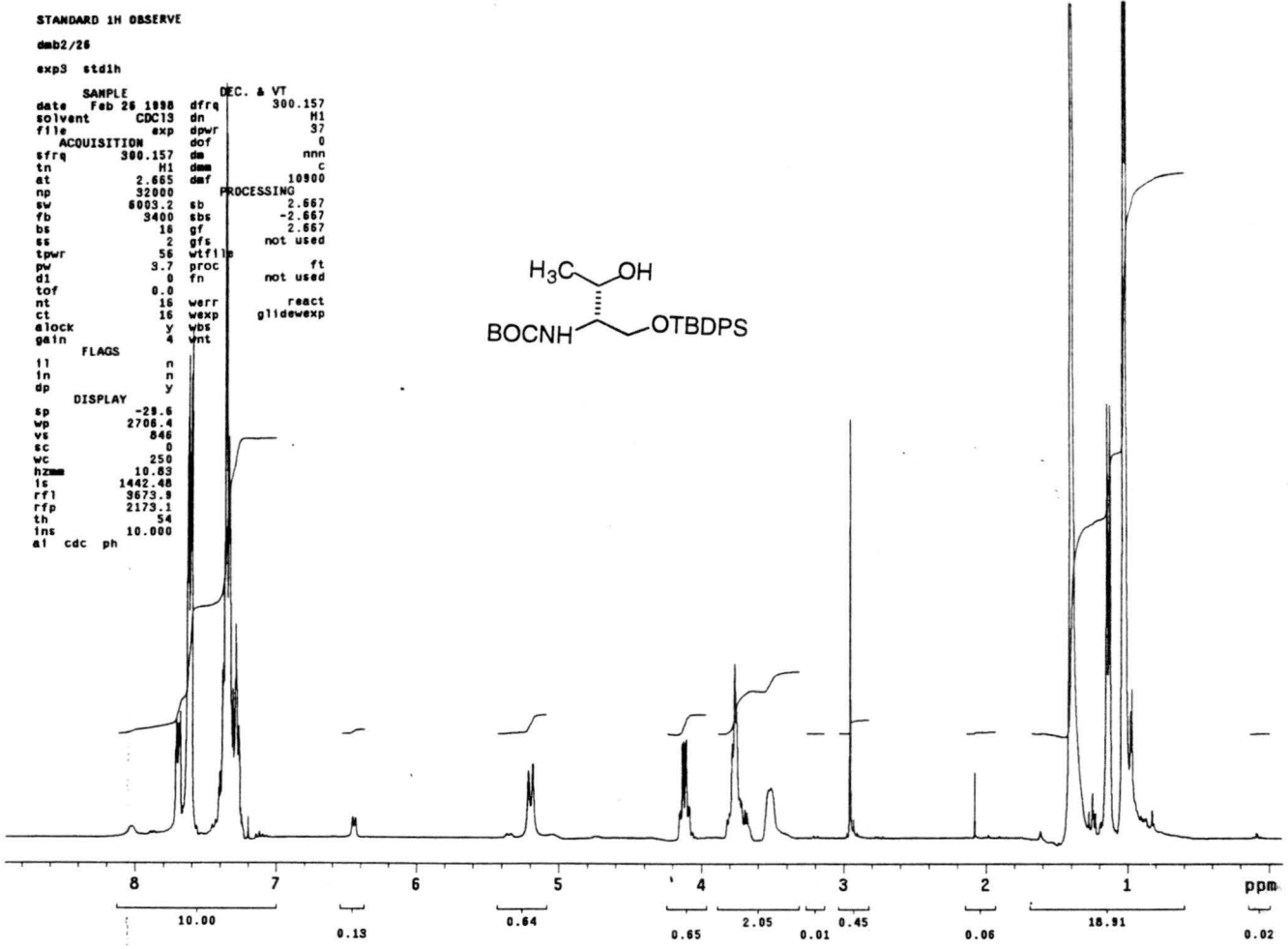
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.08 (9H, s), 1.19 (3H, d, J=6.6 Hz), 1.46 (9H, s), 3.54 (1H, s), 3.83 (2H, m), 4.19 (1H, ddq, J=6.6, 2.4 Hz), 5.17 (1H, d, J=8.4 Hz), 7.33-7.62 (10H, m).

^{13}C NMR (75.47 MHz, $CDCl_3$): δ 18.94, 19.86, 26.73, 28.26, 55.44, 65.92, 68.09, 79.12, 127.29, 127.64, 129.07, 134.62, 135.29, 135.68, 156.01.

IR (NaCl, neat) 3440, 3134, 3071, 3049, 2931, 2857, 2711, 1959, 1890, 1824, 1694, 1647, 1605, 1589, 1502, 1472, 1391, 1366, 1325, 1252, 1170, 1109 cm^{-1}.

$[\alpha]_D^{25}$= +11.2 (c=1.0, CH_2Cl_2).

LRMS (ES+) m/e (M+H) 444.7 ($C_{25}H_{37}NO_4Si$ + H requires 444.7).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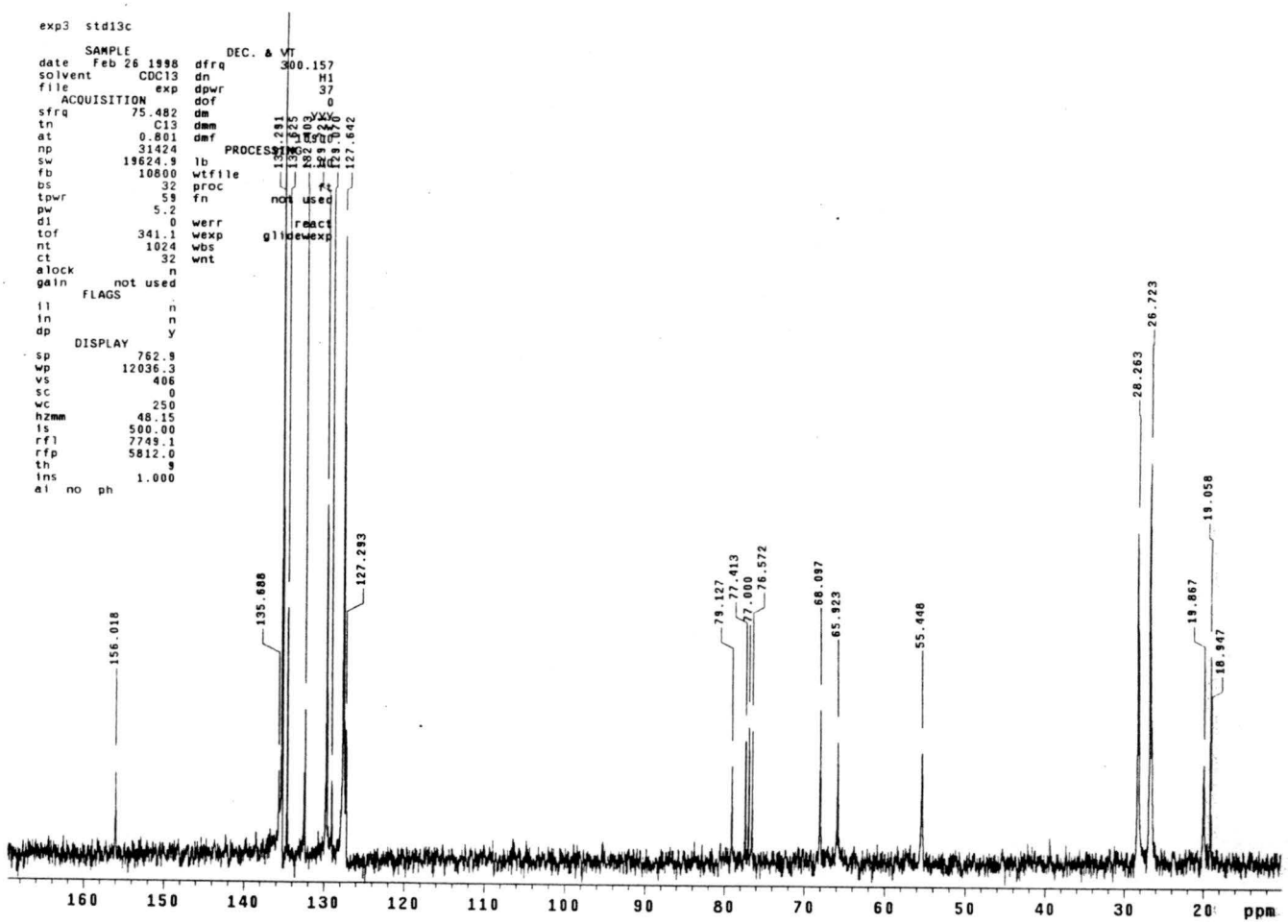
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ct 32
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gain not used
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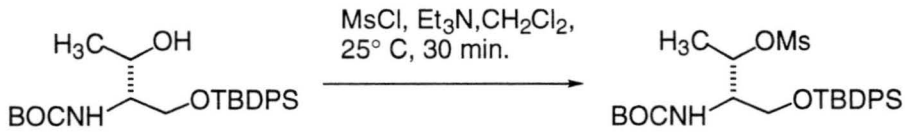


51

(2R,3S)-2-(*tert*-butoxycarbonyl)-amino-1-(*tert*-butyldiphenyl)-siloxy-3-(methanesulfonyloxy)-butane

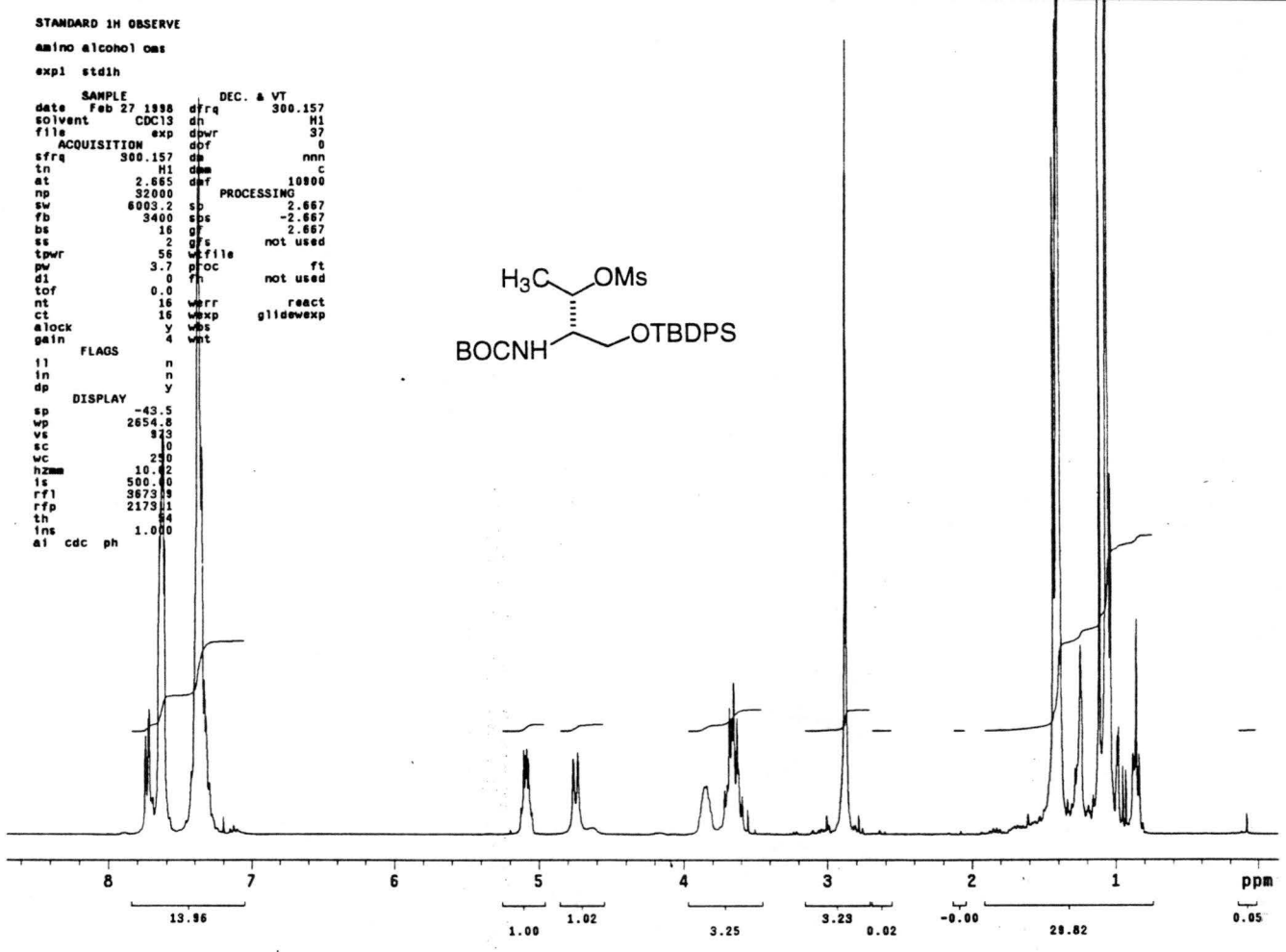
(2R,3S)-2-(*tert*-butoxycarbonyl)amino-1-(*tert*-butyldiphenyl)siloxy-3-butanol **(51)** (1.0 g, 2.25 mmol) was dissolved in CH_2Cl_2. Methanesulfonyl chloride (0.192 mL, 2.48 mmol) was added followed by triethylamine (.376 mL, 2.70 mmol) and the reaction was stirred under argon at 25°C for 30 min. TLC showed complete consumption of starting materials. The mixture was concentrated and dried *in vacuo* to give a yellow solid. The residue was dissolved in hexanes and the undissolved solids were removed by filtration. Concentration of the hexane solution gave a light yellow oil. This compound was used without further purification.

R_f=0.68 (hexanes,ethyl acetate, 3:1, v/v).

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.09 (9H, s), 1.43 (9H, s), 3.54 (1H, s), 1.46 (3H, d, J=6.3 Hz), 2.93 (3H, s), 3.60-3.74 (2H, m), 3.85 (1H, m), 4.71 (1H, d, J=9.6 Hz), 5.11 (1H, dq, J=6.3, 3.6 Hz), 7.32-7.74 (10H, m).

^{13}C NMR (75.47 MHz, $CDCl_3$): δ 18.28, 18.36, 19.05, 26.32,26.41, 26.71, 28.13, 28.23, 38.18, 38.29, 55.24, 62.44, 79.52, 127.67, 130.03, 132.59, 134.60, 135.32, 155.24.

IR (NaCl, neat) 3384, 3071, 3049, 2960, 2932, 2889, 2858, 1716, 1589, 1505, 1472, 1428, 1391, 1365, 1286, 1253, 1174, 1112 cm^{-1}.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rfp 2173.1
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wnt
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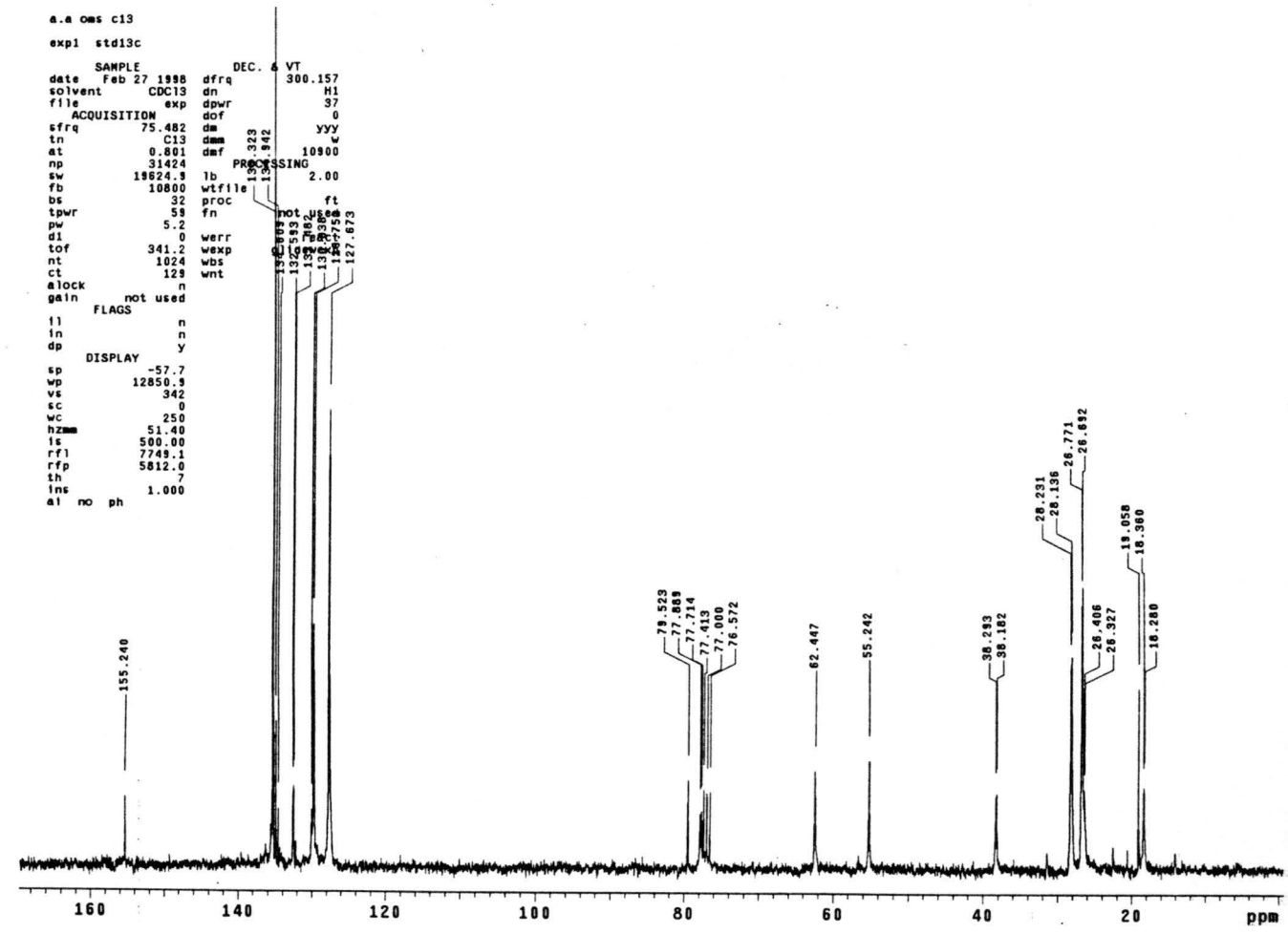
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proc ft
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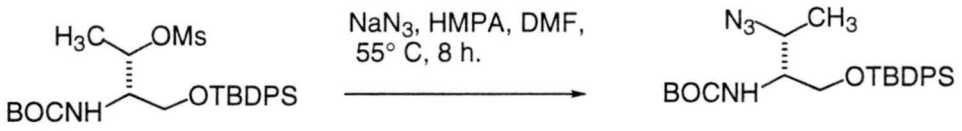


52

(2R,3R)-2-(*tert*-butoxycarbonyl)-amino-1-(*tert*-butyldiphenyl)-siloxy-3-azido-butane (52)

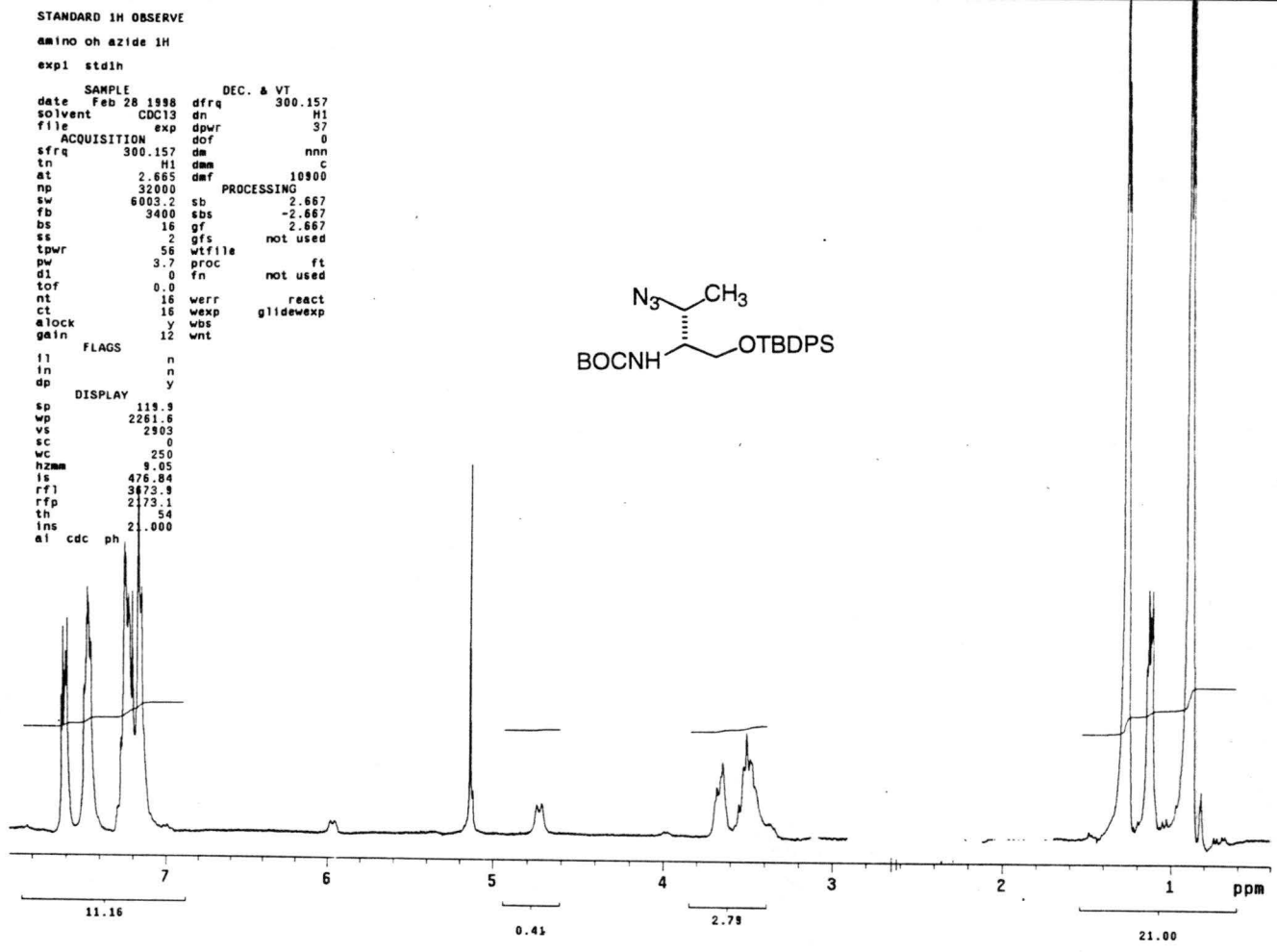
(2R,3S)-2-(*tert*-butoxycarbonyl)amino-1-(*tert*-butyldiphenyl)siloxy-3-(methanesulfonyloxy)-butane (0.900 g, 1.72 mmol) was dissolved in DMF (5 mL) and HMPA (1 mL). Sodium azide (0.56 g, 8.63 mmol) was added and the reaction was heated at 55°C while stirring under argon for 8 h. The mixture was concentrated *in vacuo* and dissolved in CH_2Cl_2 (25 mL), washed with brine (2 X 10 mL), dried ($MgSO_4$) and concentrated to a yellow oil. Chromatography (hexanes/ethyl acetate, 3:1, v/v) gave 0.61 mg (76%) of **52** as a colorless oil.

R_f=0.47 (hexanes,ethyl acetate, 3:1, v/v).

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.03 (9H, s), 1.24 (3H, d, J=6.3 Hz), 1.39 (9H, s), 3.54 (1H, s), 3.58-3.81 (3H, m), 4.79-4.87 (1H, m), 7.27-7.61 (10H, m).

^{13}C NMR (75.47 MHz, $CDCl_3$): δ 18.03, 18.45, 26.08, 26.15, 27.69, 36.06, 48.21, 55.61, 62.46, 78.13, 126.34, 126.94, 127.94, 128.98, 132.13, 134.14, 134.64, 136.24, 155.19.

IR (NaCl, neat) 3676, 3438, 3228, 3069, 3047, 2996, 2883, 2804, 2802, , 2206, 2090, 1923, 1710, 1652, 1589, 1540, 1459, 1429, 1389, 1363, 1297, 1205, 1112, 1067 cm^{-1}.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alock y
gain 12
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dp y
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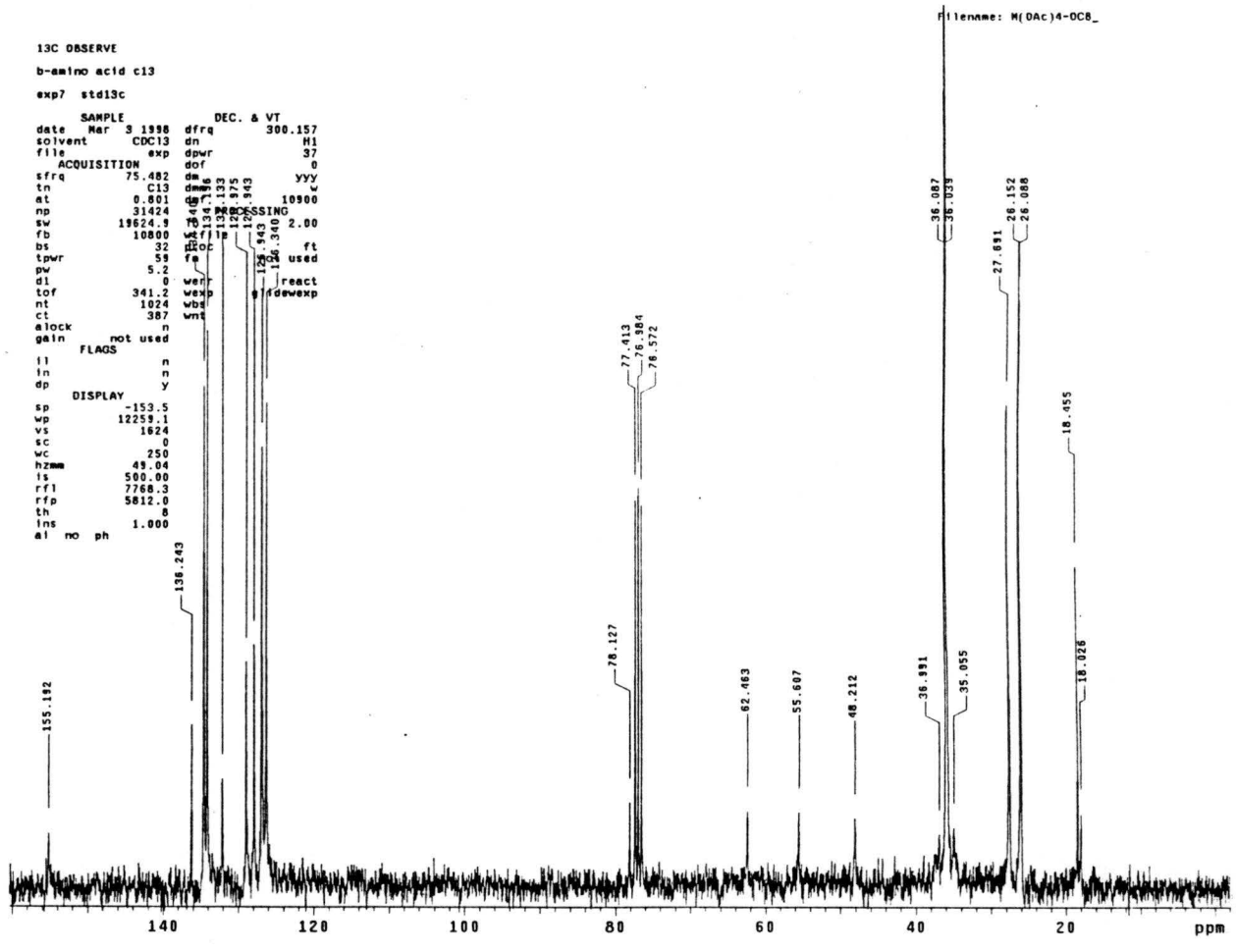
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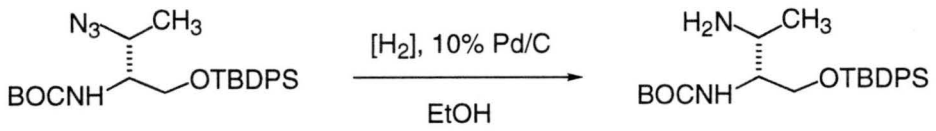


52

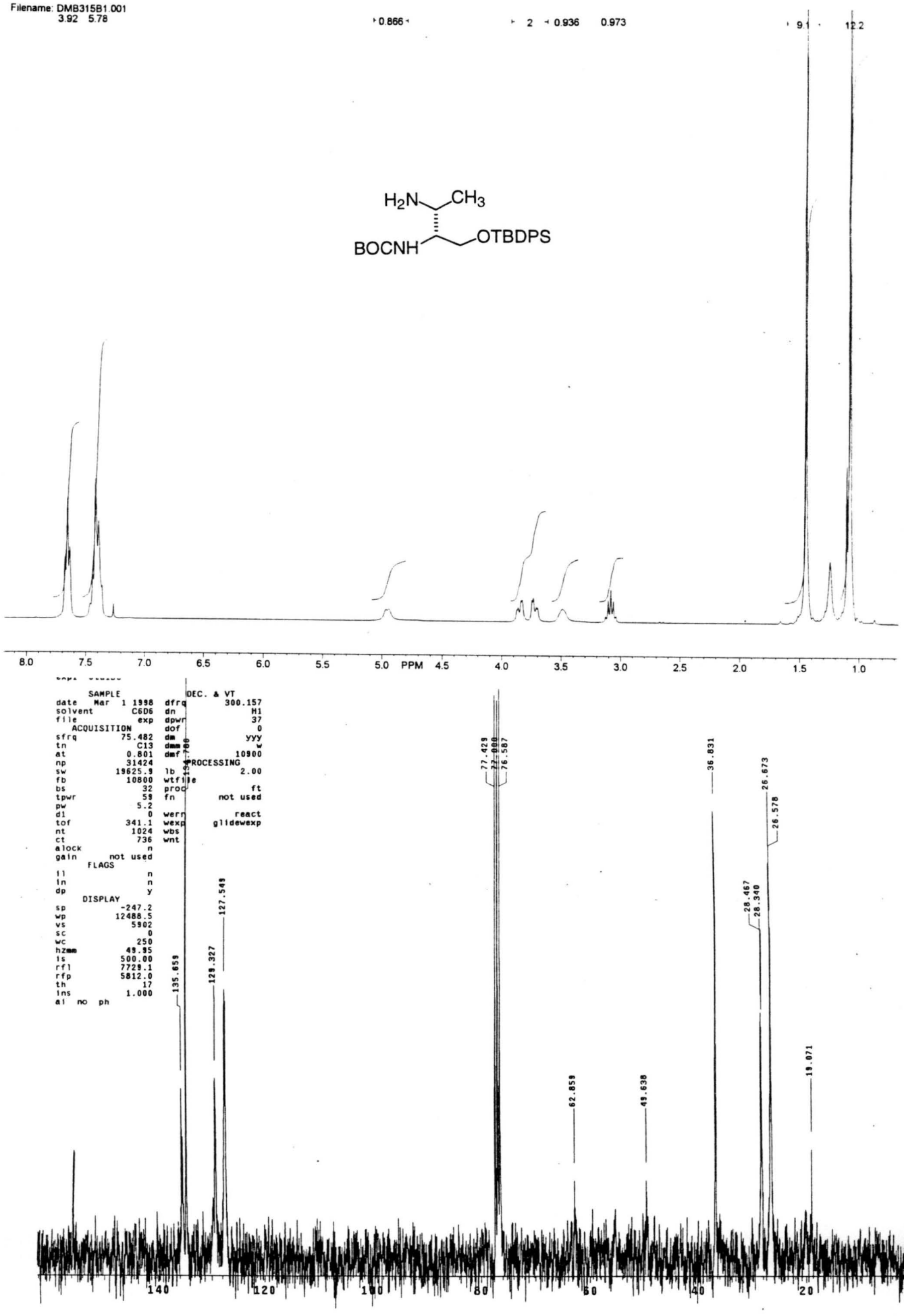
(2R,3R)-2-(*tert*-butoxycarbonyl)-amino-1-(*tert*-butyldiphenyl)-siloxy-3-amino-butane

(2R,3R)-2-(*tert*-butoxycarbonyl)-amino-1-(*tert*-butyldiphenyl)-siloxy-3-azido-butane **(52)** (0.80 g, 1.71 mmol) was dissolved in absolute ethanol (5 mL) and hydrogenated under 4 atm of H_2 in the presence of 10% Pd/C (0.085 g). After 4 h. the reaction was filtered through Celite to remove the catalyst. The filtrate was concentrated to give a clear oil which was purified by flash chromatography ($MeOH/CH_2Cl_2$, 1:9, v/v) to yield 0.72 g (95%) of (2R,3R)-2-(*tert*-butoxycarbonyl)-amino-1-(*tert*-butyldiphenyl)-siloxy-3-amino-butane as a clear oil.

^1H NMR (300 MHz, $CDCl_3$): δ 1.08 (12H, s), 1.25 (2H, br s), 1.45 (9H, s), 3.08 (1H, quint), 3,94 (1H, m), 3.71 (1H, dd, J=10.2, 3.3 Hz), 3.85 (1H, dd, J=13.8, 3.3 Hz), 4.96 (1H, m), 7.37-7.68 (10H, m).

^{13}C NMR (75.47 MHz, $CDCl_3$): δ 19.07, 26.57, 26.67, 28.34, 28.47, 36.83, 49.63, 62.86, 127.54, 129.33, 134.78, 135.66, 151.87.

IR (NaCl, neat) 3284, 3069, 2928, 2888, 2805, 1710, 1588, 1488, 1472, 1457, 1428, 1389, 1364, 1297, 1247, 1199, 1174, 1111, 1067 cm^{-1}.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9.1 12.2
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wbs
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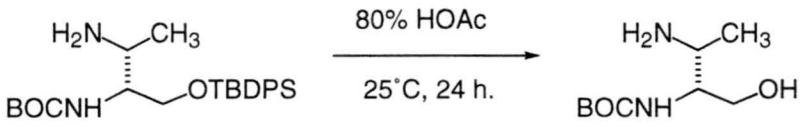
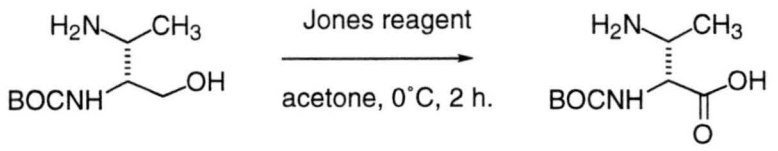


(2R,3R)-2-(*tert*-butoxycarbonyl)-amino-3-amino-1-butanol

(2R,3R)-2-(*tert*-butoxycarbonyl)-amino-1-(*tert*-butyldiphenyl)-siloxy-3-amino-butane (0.50 g, 1.13 mmol) was dissolved in 80% acetic acid solution and stirred at 25°C for 24 h. Solvent was removed *in vacuo* and the crude residue was taken on without purification.



53

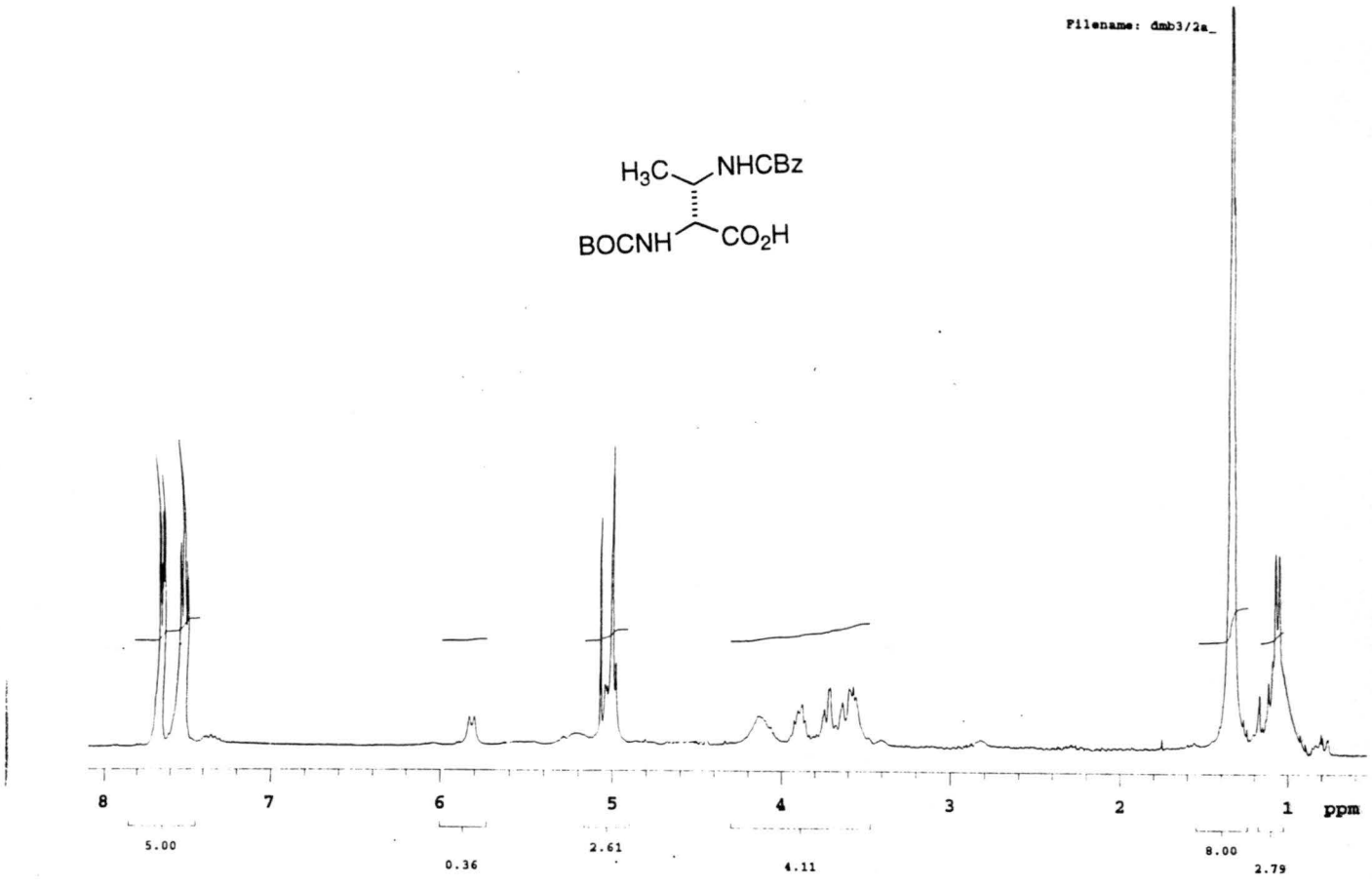
(2R,3R)-2-(*tert*-butoxycarbonyl)-amino-3-amino-1-butanoic acid (53)

(2R,3R)-2-(*tert*-butoxycarbonyl)-amino-3-amino-1-butanol (0.20 g, 0.98 mmol) was dissolved in acetone (5 mL) and cooled to 0°C. Jones reagent (2.67 M sol. in acetone) was added and the reaction was stirred at 0°C for 2 h. Water was added and acetone was evaporated. The aqueous residue was extracted with ethyl acetate, acidified to pH 4 with 10% citric acid solution, and extracted again with ethyl acetate. The organic layers were combined, dried ($MgSO_4$) and concentrated give to a white solid. 0.025 g (0.114 mmol) of the amino acid were immediately dissolved in methylene chloride (2 mL) and benzyl chloroformate (48.7 µL, 0.342 mmol) and triethylamine (47.6 µL, 0.342 mmol) were added. The reaction was stirred at 25°C under argon for 4 h. The solvent was removed and the residue was recrystallized from hexanes/ethyl acetate to give 2R,3R-3-(benzyloxycarbonyl)-2-(*tert*-butoxycarbonyl)aminobutyric acid.

m.p.110°C (Lit.[52] 112-113°C).

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.08 (3H, d, J=6.6 Hz), 1.38 (9H, s), 3.44-4.21 (2H, m), 5.11 (2H, s), 5.88 (1H, br. d), 7.42-7.73 (5H, m).

$[\alpha]_D^{25}$= +22.4 (c=1.0, MeOH) (Lit.[52] $[\alpha]_D^{25}$= +22.1).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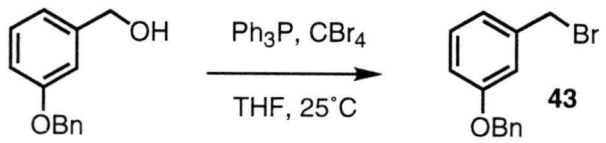
2.79



43

***meta*-(benzyloxy)benzyl bromide (43)**

3-benzyloxy benzyl alcohol (Aldrich) (5.0 g, 23.4 mmol) was reacted with Ph_3P (6.74 g, 25.7 mmol) and CBr_4 (8.50 g, 25.7 mmol) in THF (100 mL) at 25°C for 1 h. Solid material was removed by filtration, and the crude product was purified by flash chromatography (hexanes) to yield **43** (5.89 g, 91%) as a white solid (recryst. hexanes).

m.p. = 37 - 39°C (dec.).

^{1}HNMR (300 MHz, $CDCl_3$): δ 4.39 (2H, s), 4.98 (2H, s), 6.83 - 6.95 (3H, m), 7.17 - 7.39 (6H, m).

^{13}CNMR (300 MHz, $CDCl_3$): δ 33.6, 70.2, 115.1, 115.6, 121.7, 127.7, 128.2, 128.8, 130.0, 136.9, 139.4, 159.1.

IR ($NaCl/CH_2Cl_2$): 3013, 2985 $cm.^{-1}$

HRMS (ES^+): Calculated for $C_{13}H_{13}OBr$: 276.0150; Found: 276.0145

DmB-187

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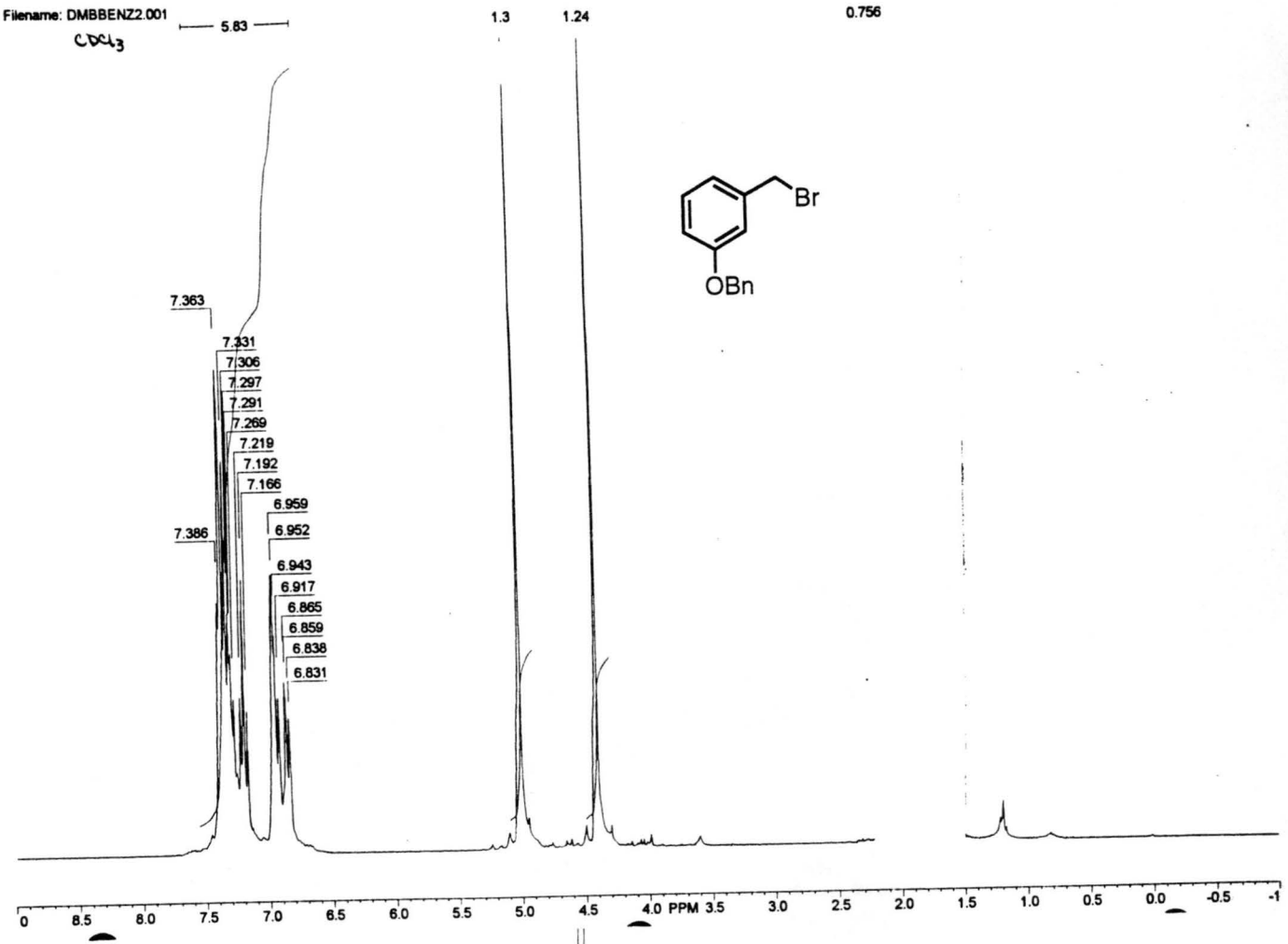
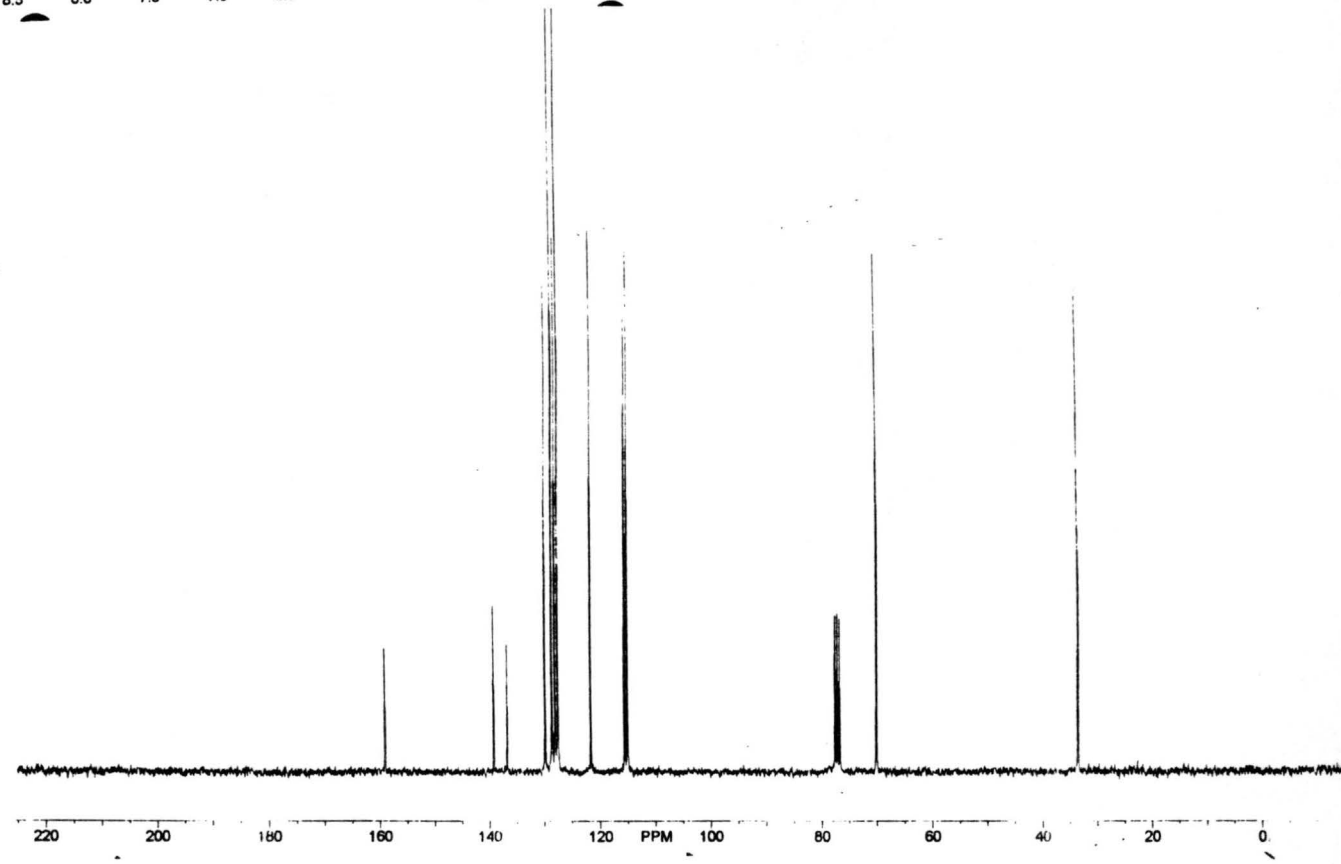
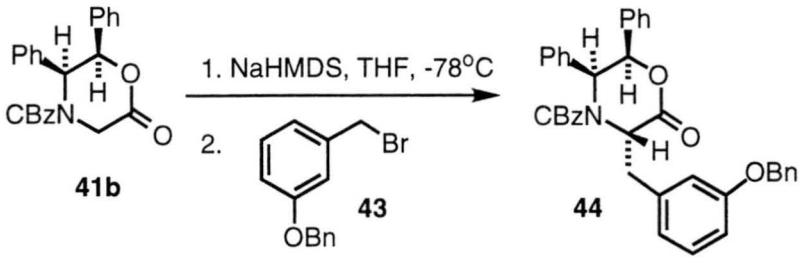
$CDCl_3$







(3S,5S,6R)-4-(Benzyloxycarbonyl)-5,6-diphenyl-3-{[3'-(benzyloxy)phenyl]methyl}-2,3,5,6-tetrahydro-4H-1,4-oxazin-2-one (44).

NaHMDS (12.3 mL, 12.3 mmol, 1 M solution in THF) was added dropwise to a solution of oxazinone **41b** (3.17 g, 8.20 mmol) (Aldrich) and *meta*-(benzyloxy)benzyl bromide **43** (2.50 g, 9.02 mmol) in THF (160 mL) and HMPA (16 mL) at -78°C. After three hours, the reaction mixture was poured into ethyl acetate and extracted with brine and H_2O. The organic extracts were dried ($MgSO_4$) and concentrated to a yellow oil which was purified by flash chromatography (CH_2Cl_2/MeOH, 99:1) to give **44** (4.15 g, 87%) as a white solid. (recryst. CH_2Cl_2/hexanes)

m.p. = 146 - 148°C (dec.).

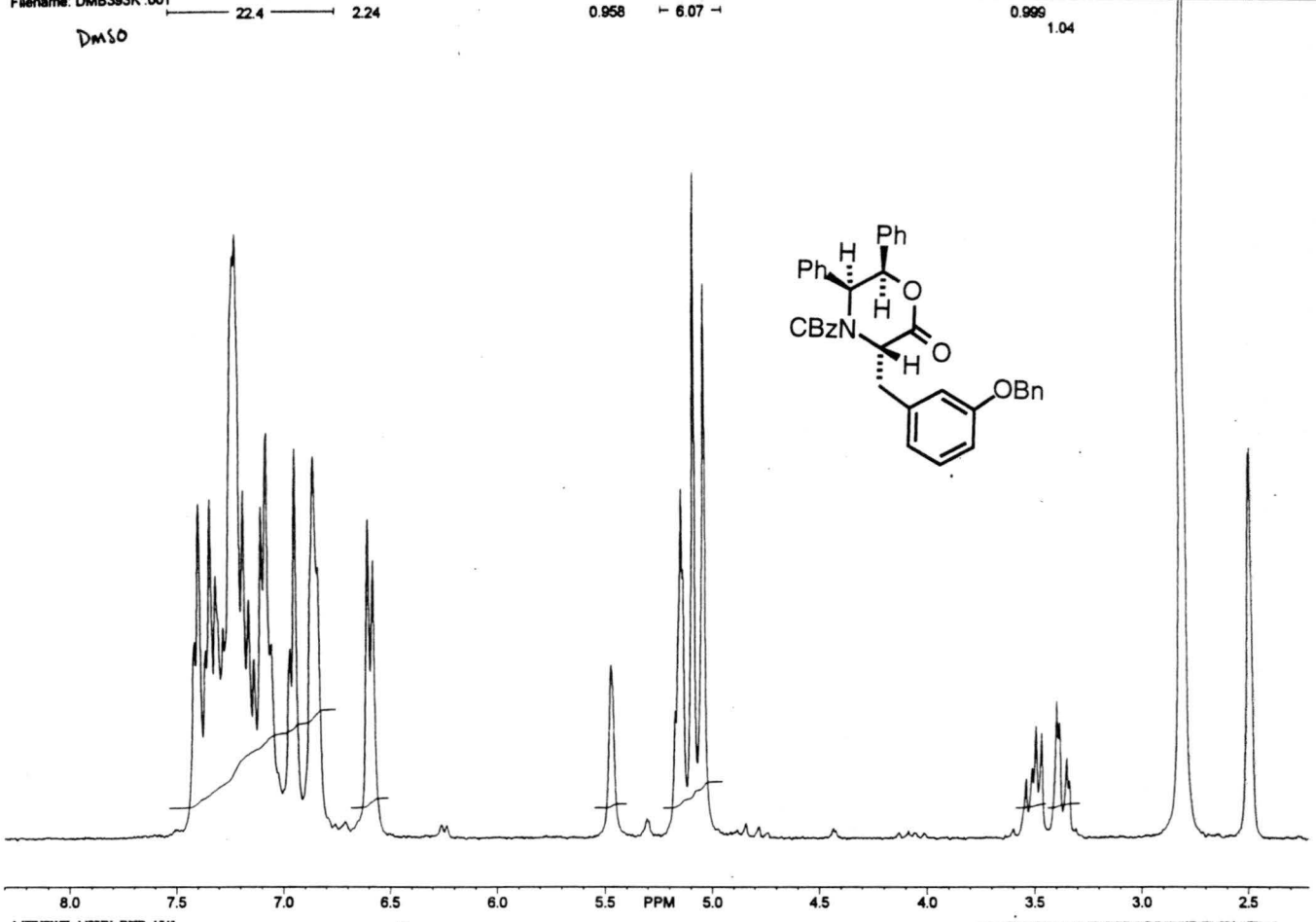
^{1}HNMR (300 MHz, DMSO-d_6, 393 K): δ 3.37 (1H, dd, J = 13.8, 3.9 Hz), 3.49 (1H, dd, J = 13.5, 8.1 Hz), 5.04 (2H, s), 5.09 (2H, s), 5.14 (2H, s), 5.47 (1H, s (br)), 6.59 (2H, d, J = 7.5 Hz), 6.83 - 7.42 (22H, m).

^{13}CNMR (300 MHz, DMSO-d_6): δ 39.3, 59.9, 67.7, 69.9, 78.4, 114.8, 122.7 - 138.6 (unresolved), 154.6, 159.6, 168.6.

IR (KBr): 1698, 1750, 2950, 3030 cm.$^{-1}$

$[\alpha]^{25}_{D}$ = +52.45° (c 2.0, $CHCl_3$).

Anal. Calcd. for $C_{38}H_{33}NO_5$: C: 78.19; H: 5.69; N: 2.39, Found: C: 78.18; H: 5.52; N: 2.19.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Ph
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H
CBzN
O
H
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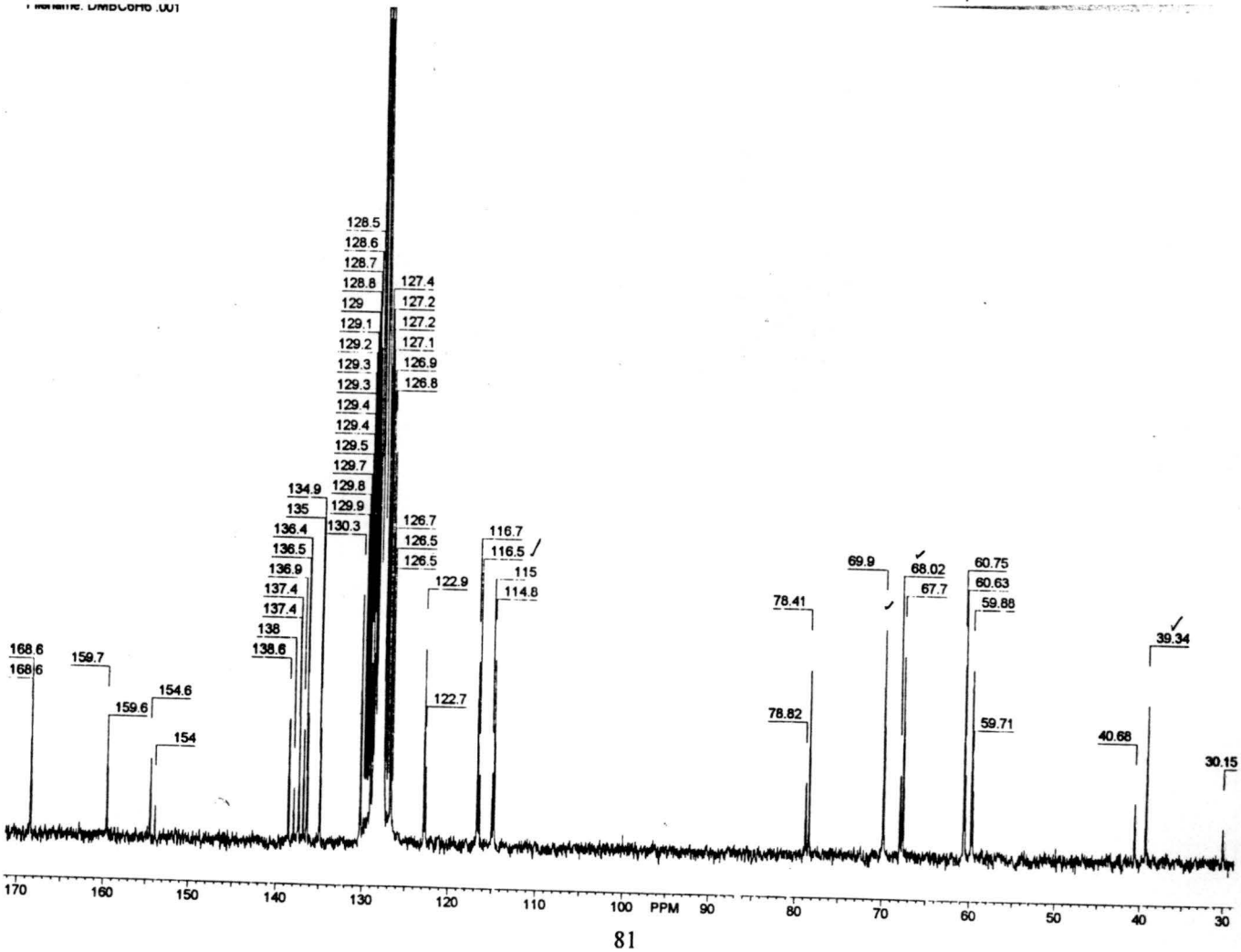
4.0
3.5
3.0
2.5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129.3
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130.3
127.4
127.2
127.2
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126.9
126.8
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137.4
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126.5
126.5
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114.8
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168.6
159.7
159.6
154.6
154
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78.82
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39.34
40.68
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170
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150
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130
120
110
100
PPM
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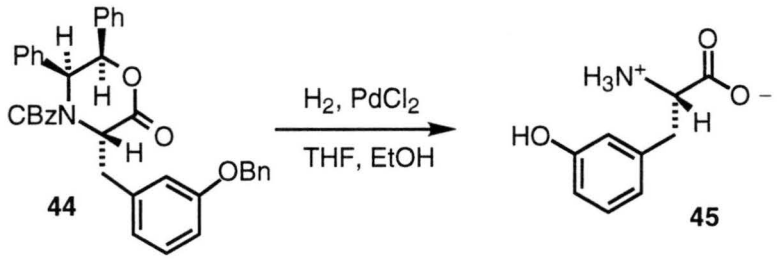
70
60
50
40
30

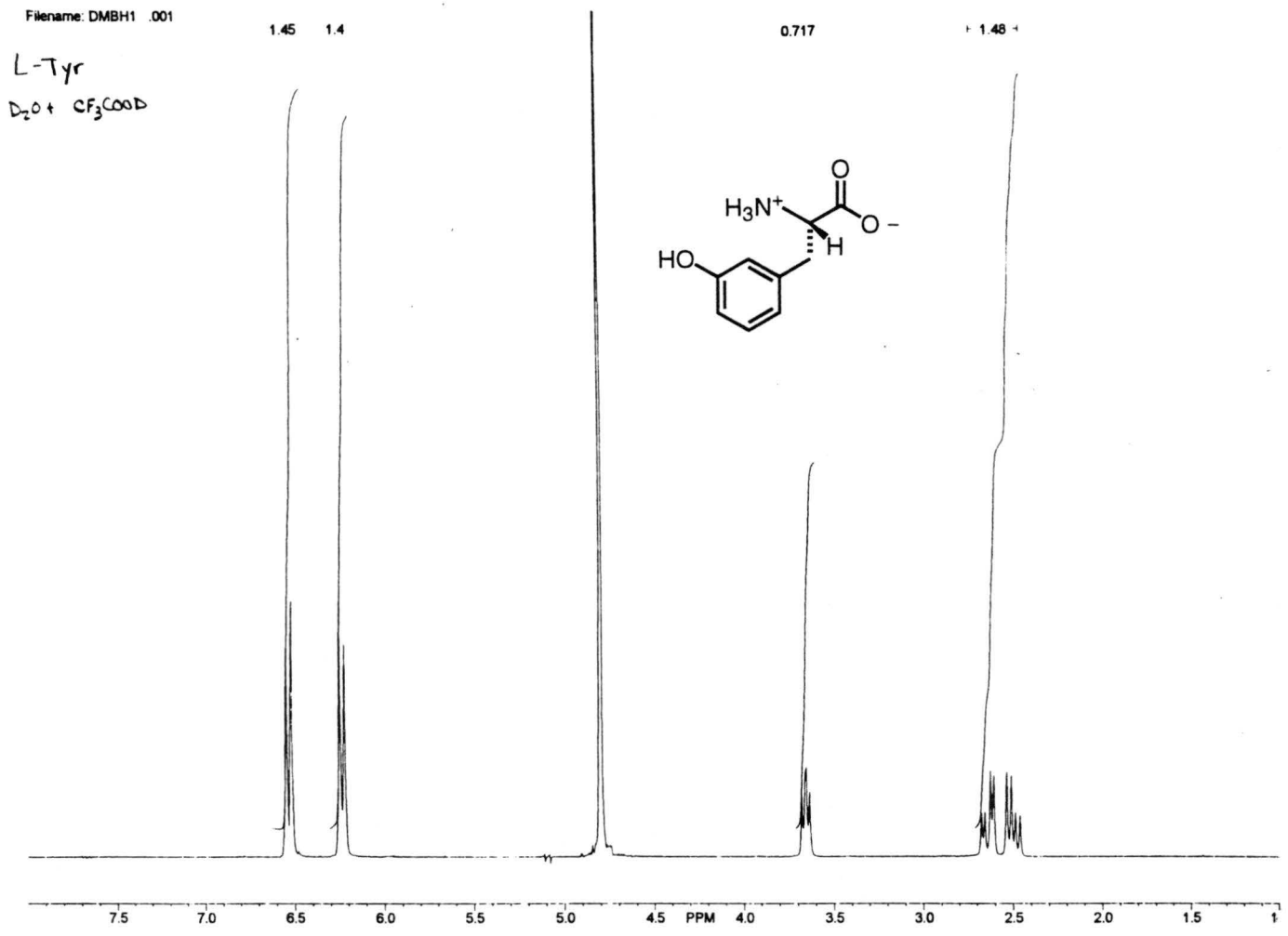


(S)-*meta*-tyrosine hydrochloride (45)

To a solution of compound **44** (0.5 g, 0.857 mmol) in ethanol (5 mL) and THF (5 mL) was added $PdCl_2$ (0.045 g, 0.254 mmol). The reaction mixture was hydrogenated at 50 psi for 18 h. The mixture was purged with nitrogen and filtered through Celite to remove the catalyst. Removal of the solvents *in vacuo*, followed by trituration with Et_2O produced 0.154 g (99%) of *meta*-tyrosine (**45**). This compound was dissolved in 1 N HCl and concentrated, followed by trituration with Et_2O to give (S)-*meta*-tyrosine hydrochloride.

$[\alpha]^{25}_D$ -7.4° (c 2.0, 1 N HCl) (lit.[1b] $[\alpha]^{25}_D$ (S)-*meta*-tyrosine hydrochloride -7.9° (c 2.0, 1 N HCl)).

^{1}HNMR (300 MHz, D_2O vs. HOD): δ 3.11 (1H, dd, J = 14.7, 7.5 Hz), 3.24 (1H, dd, J = 14.4, 5.4 Hz), 4.29 (1H, dd, J = 7.5, 5.7 Hz), 6.71 - 6.85 (3H, m), 7.26 (1H, t).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H
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7.0
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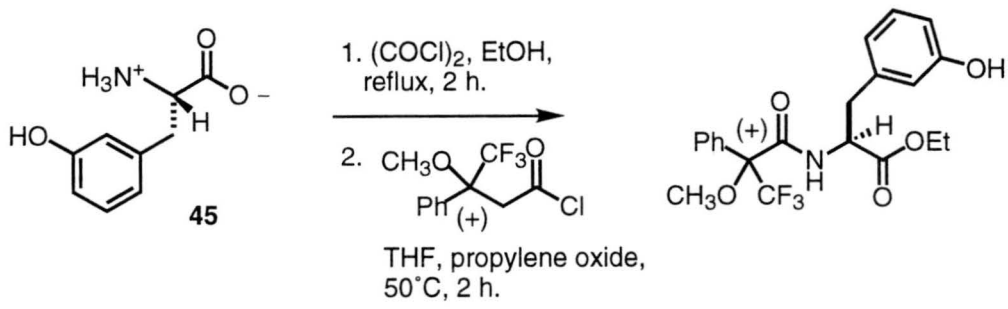
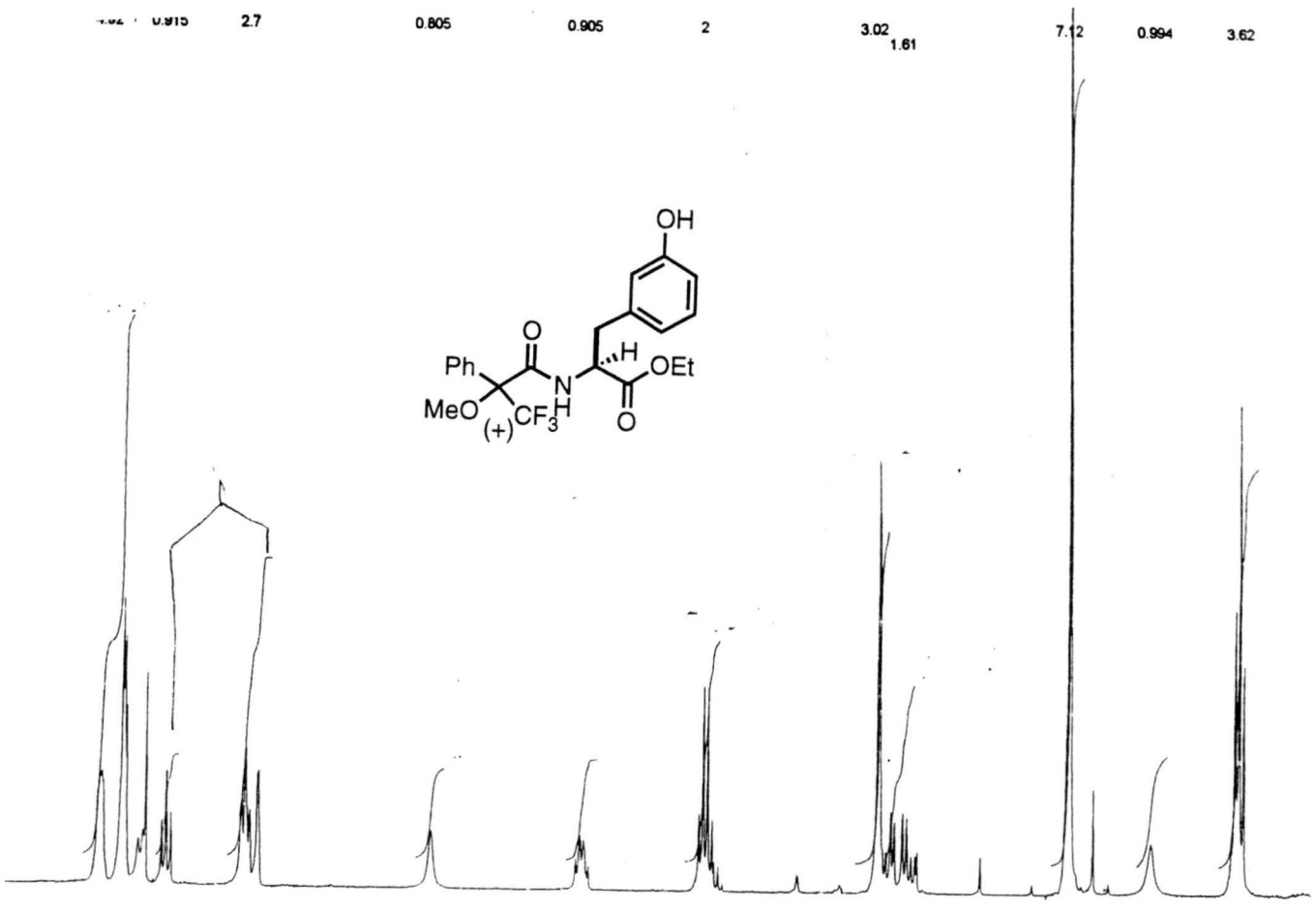
1



Determination of optical purity:

Oxalyl chloride (48.0 μL, 0.550 mmol) was added dropwise to a solution of the amino acid **45** in ethanol (1 mL) at 0°C, followed by refluxing for 2 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. The crude amino ester hydrochloride salt was combined with (+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (24.6 μL, 0.132 mmol) and propylene oxide (40 μL, 0.571 mmol) in THF (1 mL) and heated at 50°C for 2 h. Optical purity was measured by examination of the ^{1}HNMR spectrum of the resulting Mosher amide and glc analysis (Alltech AT-1^{TM}, non-polar polymethylsiloxane) (>96% ee).

Racemic *m*-tyrosine (Aldrich) was subjected to identical conditions.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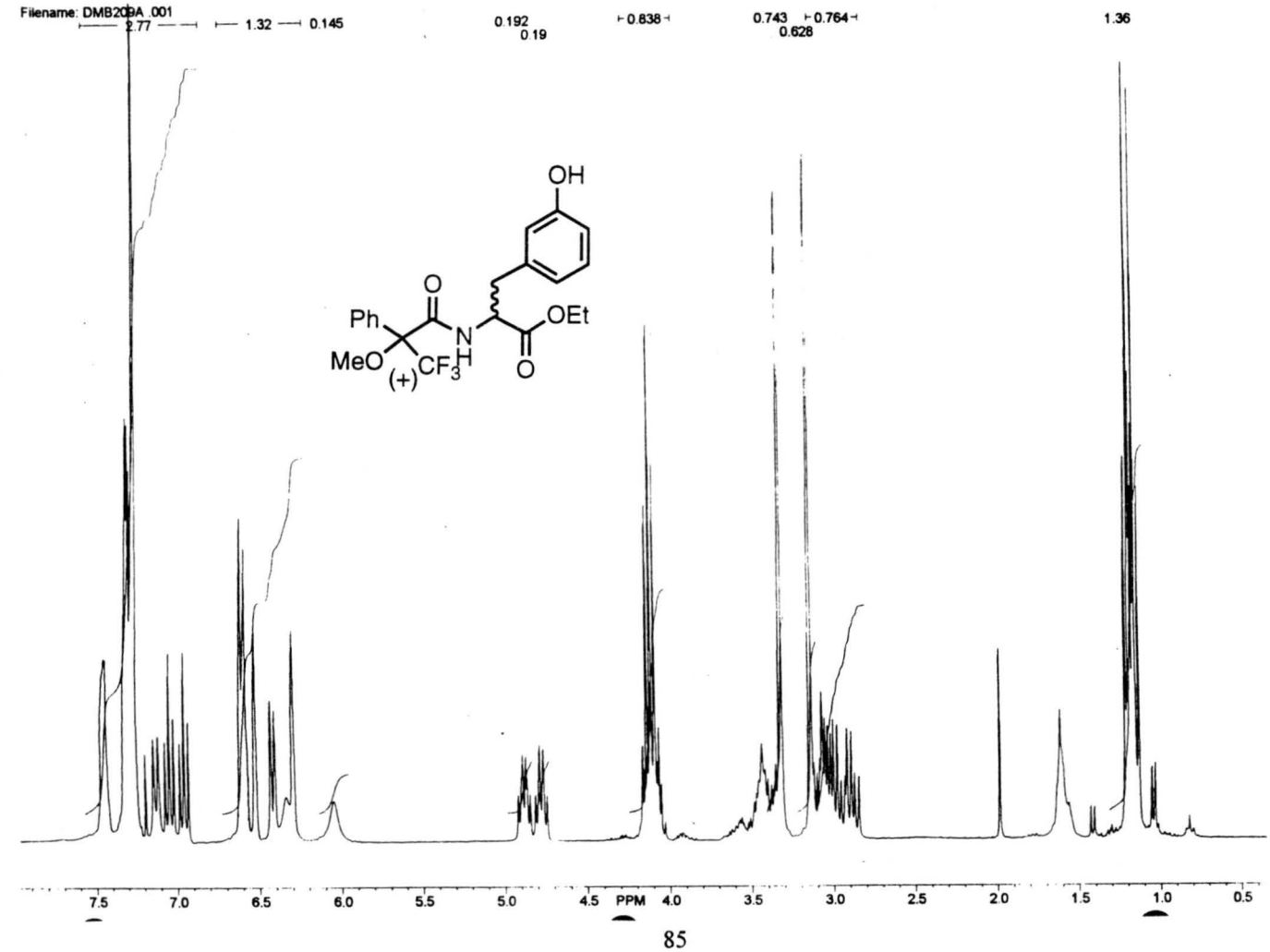
OEt
O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(+)
CF3
N
H
OEt
O
7.5
7.0
6.5
6.0
5.5
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PPM
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2.5
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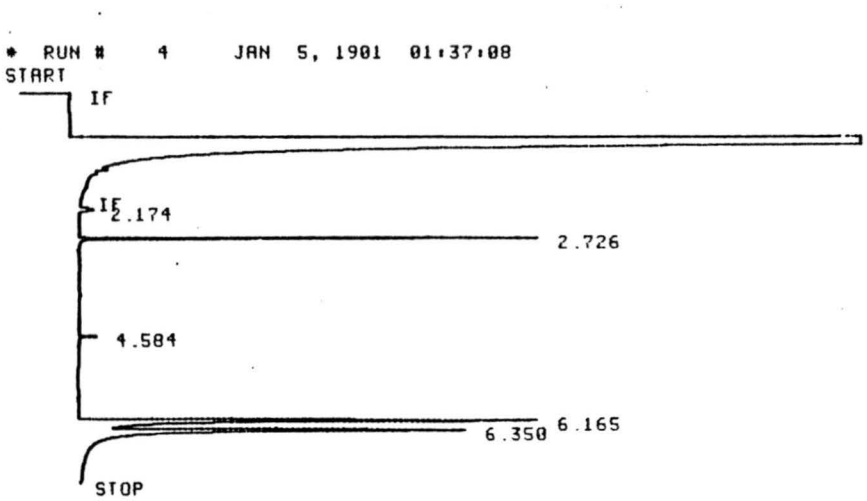

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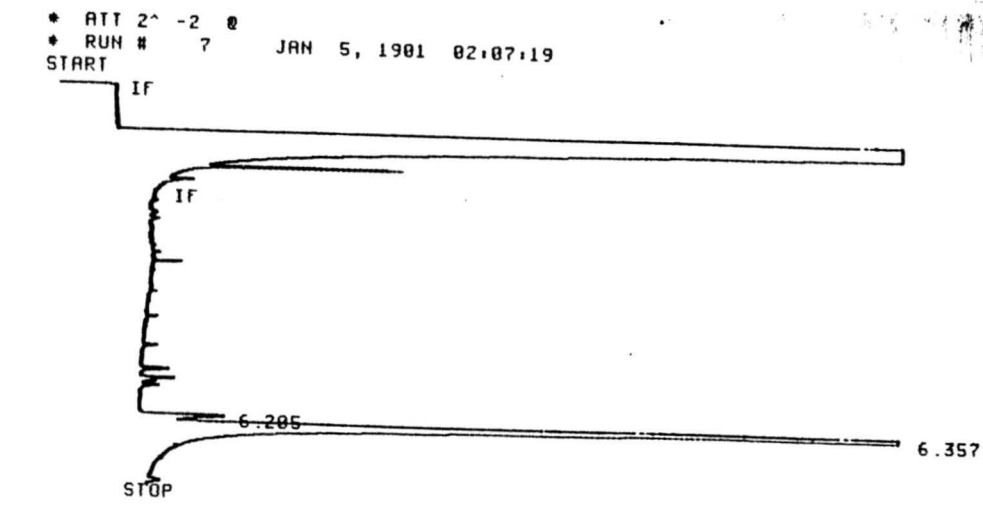

RUN# 7 JAN 5, 1901 02:07:19

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TOTAL AREA= 30002
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Chapter 2

A. Introduction

It is interesting that the mureidomycin antibiotics specifically target *Pseudomonas aeruginosa*. These organisms are often referred to as "oportunistic pathogens," because they typically infect hosptial patients whose immune systems have been depleted.[54] The constant presence of antibiotics in the hospital-care environment has allowed these bacteria to acquire resistance to most commercial antibiotics. One explanation[55,56] for the resistance of *Pseudomonas* is a low permeability of the outer membrane. Drug molecules cross the outer membrane through the water-filled channels of porins, which are bacterial proteins that allow the nonspecific diffusion of hydrophilic solutes. Early studies by Nakae[57] showed that the membrane of *P. aeruginosa* would not allow the diffusion of disaccharides having a molecular weight of 342 daltons. However, the mureidomycins range in molecular weight from 840 to 899 daltons. Nikaido has offered an explanation for the observed diffusion of large molecules across the *Pseudomonas* membrane through a detailed study of the individual porin proteins.[56] Porin F was identified as the major membrane protein associated with the uptake of large solutes such as antibiotics. The size of this porin was compared to a similar protein in *E. coli*. Measurements were made as M_r,

which indicates the molecular weight of the saccharide that diffuses at 10% of the rate of diffusion of the simple sugar L-arabinose. The results showed that *P. aeruginosa* had an M_r of 610 while M_r for *E. coli* was only 210. Nikaido proposed two possible explanations for the discrepancy between the large size of the *Pseudomonas* porins and the low permeability of the outer membrane.[56,58]

(i) Electron diffraction experiments indicate the apparent smaller size of *E. coli* porins is a result of non-uniformity of the channel size. Occasional narrowing of the porin channels exists, but does not provide a sufficient barrier to drug molecules. In contrast, the porins of *P. aeruginosa* are very uniform in size, resulting in substantial drag for solute penetration.

(ii) Highly efficient efflux systems exist which effectively pump drug molecules out of the bacterial cell. The operon *mexA-meB-oprK* has been proposed[59] to function in an efflux system in *Pseudomonas*. Inactivation of this operon by mutagenesis caused *P. aeruginosa* to become susceptible to several common antibiotics which had previously been ineffective.

No explanation has been offered as to why the mureidomycin antibiotics show activity against this generally resistant type of bacteria. The studies cited previously suggest that the drug molecules may slowly diffuse through the porin channels to reach their ultimate target, phospho-N-

acetylmuramyl-pentapeptide translocase. It may be possible to design mureidomycin analogs of lower molecular weight that might diffuse more readily through the outer membranes of *P. aeruginosa* as well as other types of bacteria.

A. Modification of Substrates of Translocase

The exact manner in which the mureidomycin antibiotics interact with translocase is unclear, because very little is known about the structure of this enzyme. As stated previously, the only structural data available is the sequence of translocase from *E. coli*.[5] Because the enzyme is located within the cell membrane, it has not been isolated and purified. As such, it has not been possible to obtain x-ray diffraction data for translocase, which would offer valuable information as to the structural features of the active site of this enzyme. This lack of structural data makes it very difficult to rationally design analogs of the mureidomycins that maintain inhibitory activity against translocase.

Neuhaus has performed substrate specificity studies for translocase using modified UDP-MurNAc-pentapeptides. Replacement of the uracil moiety with with 5-fluorouracil resulted in a 98% decrease in the transfer of the precursor to the undecaprenyl phosphate.[4] Similar results were obtained with the modified nucleoside 6-azauracil. The high specificity of translocase for the uracil moiety suggests that the pyrimidine plays an important role in the transfer reaction.

Modifications of the peptide portion of the natural substrate have been carried out as well.[60,61] The translocase from *S. aureus* has been shown to be highly specific for the L-alanine in position 1 as well as the D-alanine in position 4. Peptides containing glycine at these positions were found to reduce V_{max}/K_M by a factor of 9-15, as determined in a radiolabeling assay. In contrast, substitution of D-alanine at position 5 with glycine resulted in a very slight decrease. Variation between lysine and *meso*-diaminopimelic acid (DAP) at position 4 had very little effect on the transfer reaction. Truncated precursors were also prepared. The UDP-MurNAc-tetrapeptide showed a 4 fold decrease in V_{max}/K_M while the UDP-MurNAc-tripeptide analog exhibited a 77 fold decrease.

The high degree of substrate specificity exhibited by translocase in these studies makes the fact that mureidomycin is recognized even more striking. However, it also suggests that the design of mureidomycin analogs that retain activity will be difficult.

B. Molecular Modeling of Mureidomycin and the UDP-MurNAc-pentapeptide

Molecular modeling studies were performed on the natural substrate, UDP-MurNAc-pentapeptide, as well as mureidomycin A. Comparison of the lowest energy conformations revealed several structural motifs common to both molecules. The minimized structure of Mureidomycin A is shown in **Figure 22**. **Figure 23** is an overlay of the drug molecule with the natural

substrate for translocase, UDP-MurNAc-pentapeptide. By placing the 3'-deoxy nucleoside over uridine, the amide bond of the drug molecule mimics the diphosphate linkage of the natural substrate. More interesting is the fact that the methionine residue of mureidomycin occupies the same region of space as the sugar moiety of N-acetylmuramic acid. The two arms of the mureidomycin peptide assume what appears to be a classical β-turn conformation, formed as a result of hydrogen bonding interactions along the peptide chain.

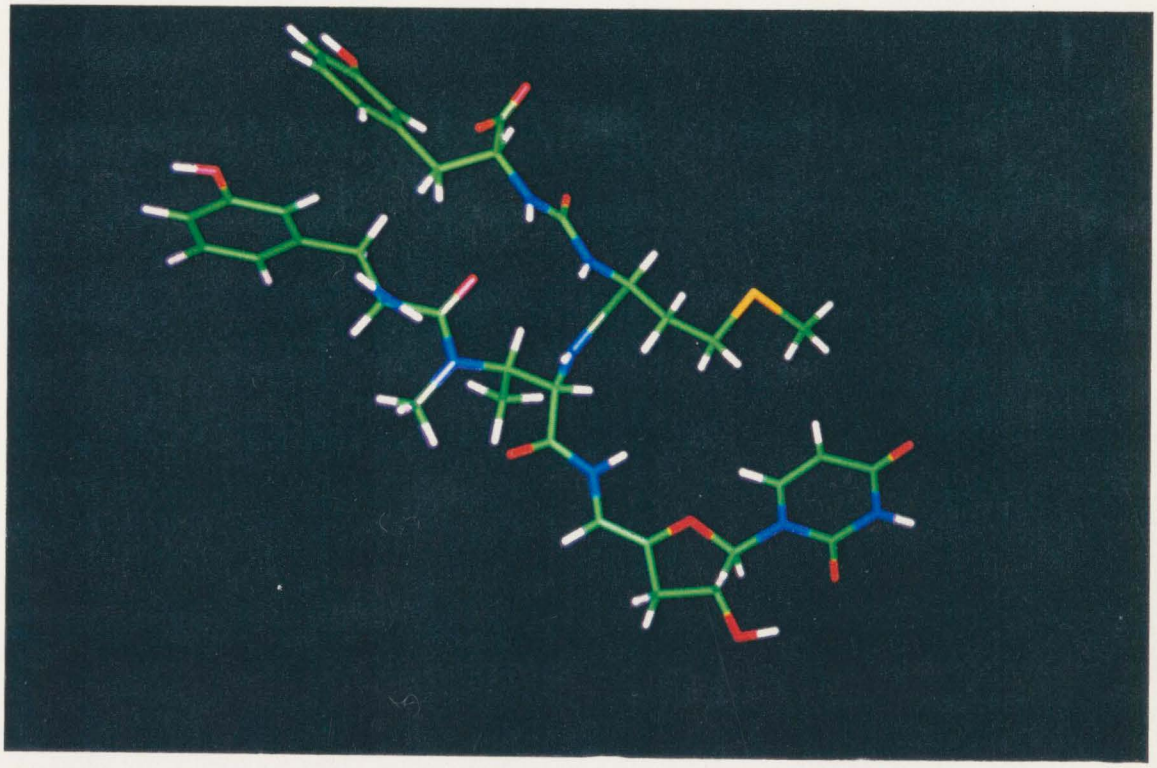


Figure 22 Minimized Structure of Mureidomycin A

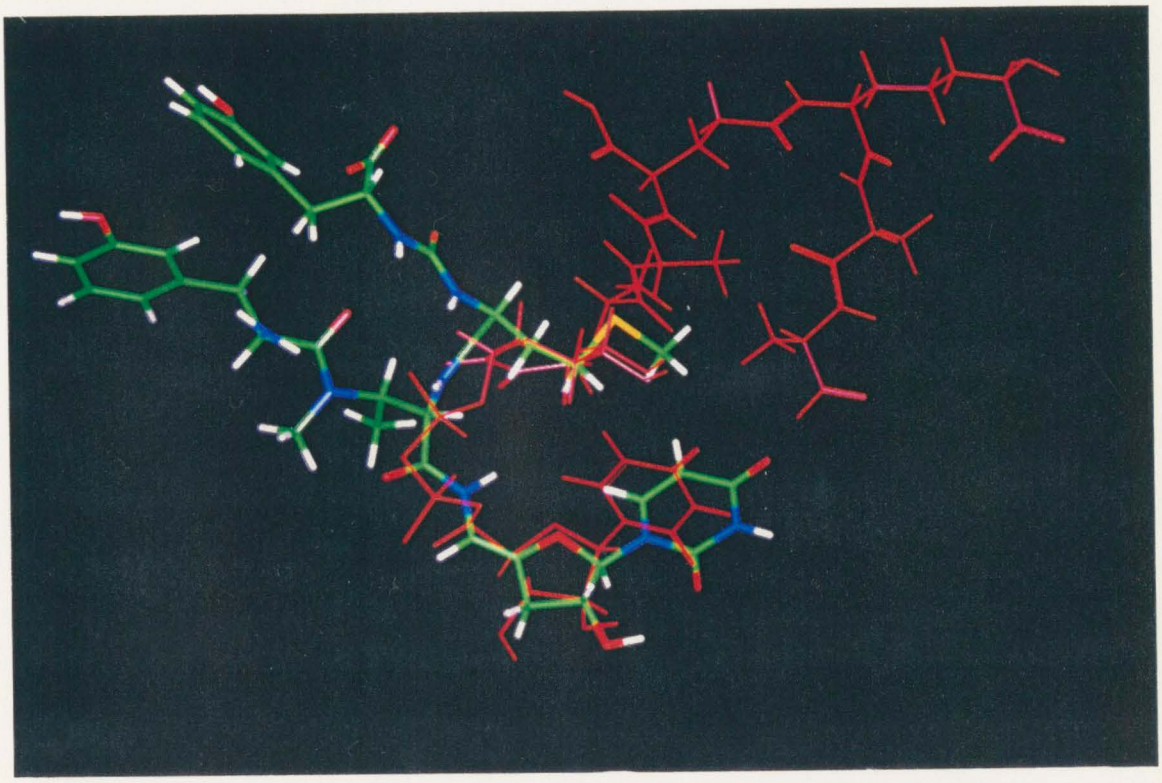


Figure 23 Overlay of Mureidomycin A with UDP-MurNAc-pentapeptide

The conclusions drawn from these experiments are summarized as follows:

(i) Uracil is important for recognition by translocase and therefore should remain unmodified.

(ii) The amide bond linkage between the nucleoside and the peptide is merely a molecular spacer, so the 4′,5′-site of unsaturation may not be necessary for activity, although this is not yet known.

(iii) The peptide sequence of mureidomycin assumes a β-turn in order to force the methionine residue into a restricted conformation.

Several analogs were designed under these restrictions. Each molecule was envisioned as being derived from the nucleoside 3′,5′-dideoxy-5′-aminouridine. Two amino acids were found to provide the desired conformational restriction; proline and sarcosine. Short alkyl chains terminating in an acylated amine were attached to these amino acids. The analog structures were minimized and ovelayed with the natural substrate using the techinque described for mureidomycin A (**Figures 24, 25**). These analogs, shown in **Figure 26**, were predicted to mimic the actions of the mureidomycin antibiotics and act as inhibitors of bacterial translocase.

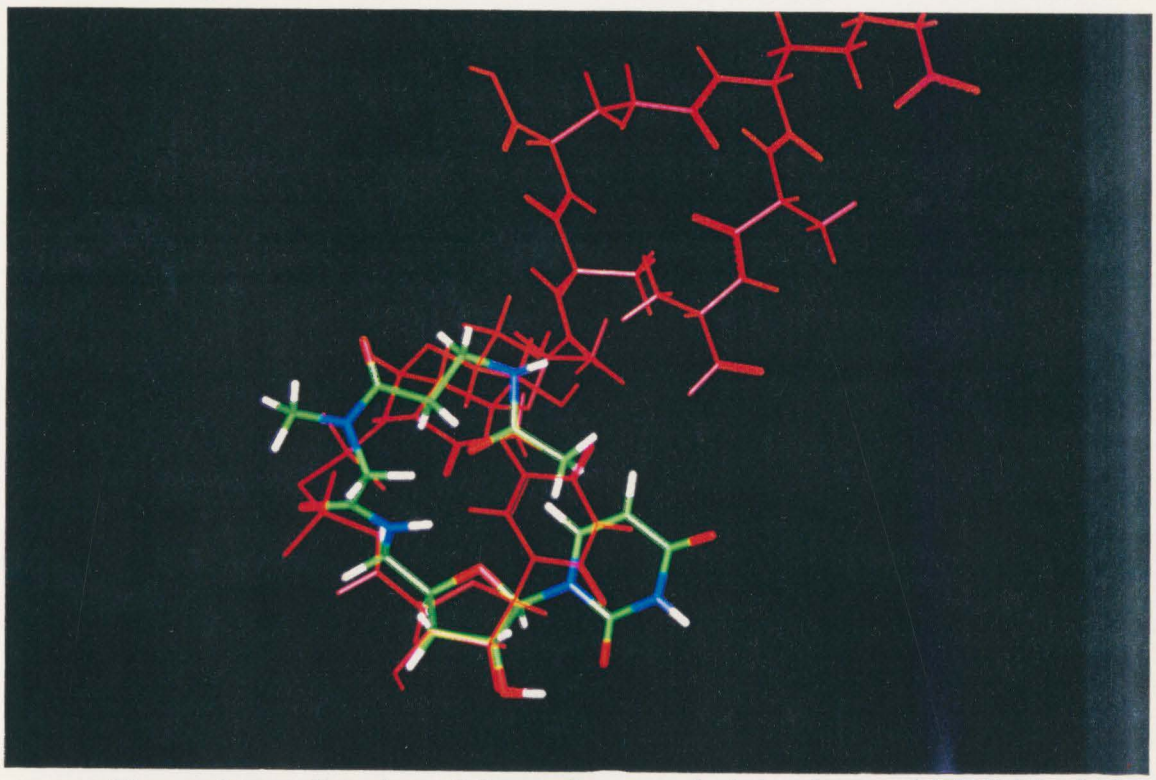


Figure 24 Overlay of Sarcosine-coupled Analog and UDP-MurNAc-pentapaptide

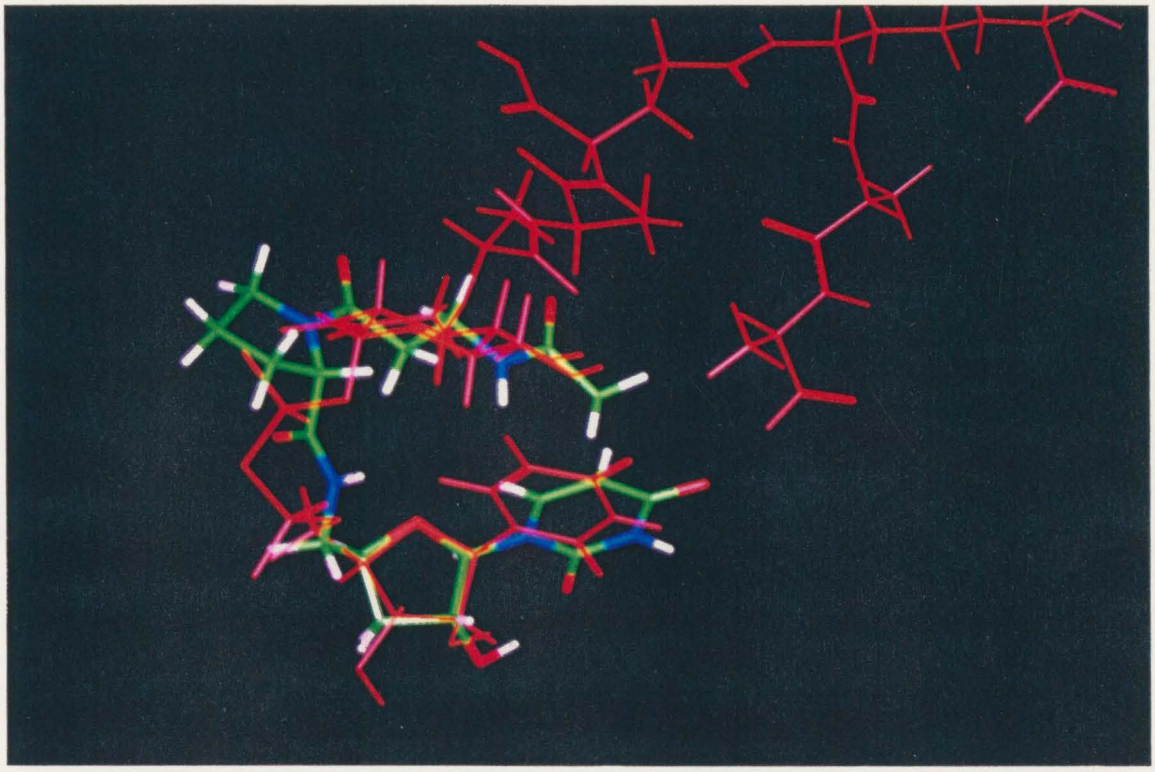
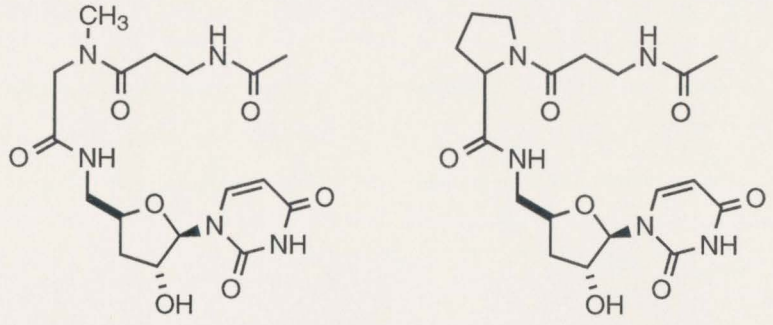


Figure 25 Overlay of Proline-coupled Analog and UDP-MurNAc-pentapaptide



58 59

Figure 26 Truncated Analogs of Mureidomycin

C. Computational Methods

All simulations were carried out on a Silicon Graphics Indigo^2 workstation (Irix 5.3) using the molecular modeling software package

INSIGHT II by Biosym/Molecular Simulations, Inc. Each molecule was constructed and subjected to molecular dynamics simulations using an NVT (constant volume, constant temperature) ensemble (Discover_3). Atom potential types were set as per *cvff* (consistent valence force field) (Biosym/MSI, Inc.). Simulations were run at 298, 373 and 500 K with run times of 5000 fs, time step of 1 fs, and temperature differential of 10 K. The dielectric constant was set at 4.0 to simulate an aqueous environment. In each case, 10 low energy conformations were selected and subjected to 1000 steps of minimization or to an absolute derivative of 0.001 kcal/mol using conjugate gradient and Newton-Raphson techniques. The results of these experiments were used to generate an average structure for each molecule studied. Structures of mureidomycin A and related analogs were manually overlayed with the natural substrate based on overlap of the nucleoside moiety of each molecule.

Chapter 3

A. Introduction

Several structural analogs of the mureidomycin antibiotics were synthesized. The core of these molecules is the modified nucleoside 3′,5′-dideoxy-5′-aminouridine. This compound was envisioned to be an excellent starting point for the synthesis of a number of peptidylnucleoside antibiotics which could be generated *via* peptide coupling reactions with the free 5′-amino group. Biological evaluation of these compounds was performed to test the validity of their design based on molecular modeling data.

B. Results and Discussion

1. Synthesis of 3′,5′-dideoxy-5′-aminouridine

The conversion of the 5′-hydroxy of nucleosides to the corresponding amine was envisioned to be a simple transformation in which the alcohol is first converted to the tosylate or some other appropriate leaving group. Reaction with sodium azide is followed by reduction to the desired aminonucleoside. This methodology was utilized in a model study on the

acetonide protected compound as shown below. The 5′-aminonucleoside **62** was obtained in good yield.

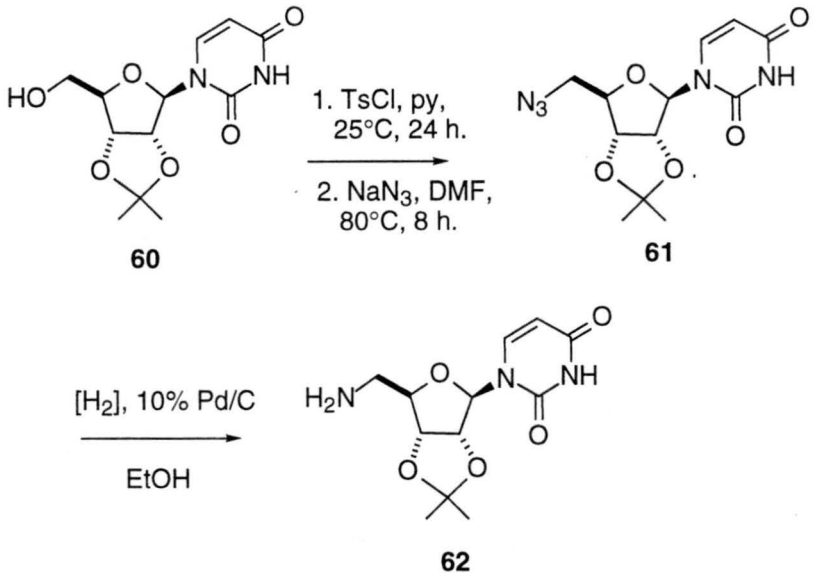


Figure 27 Classical Synthesis of Aminouridine

Yamamoto[62] reported a one-pot conversion of the 5′-hydroxyl function of unprotected purine nucleosides to amines by reaction with $Ph_3P/CBr_4/LiN_3$. When uridine was subjected to these conditions, similar results were obtained.

Figure 28 Yamamoto's Synthesis of Aminouridine

While these reactions seemed very general, they proved ineffective when applied to deoxynucleoside **18**. Attempts to activate the 5'-hydroxyl group of this compound proved unsucessful, and in all cases only starting materials were recovered.

The Mitsunobu reaction is an effective method for the conversion of alcohols to a number of different functional groups including ethers, amines, and esters.[63] This methodology was extended to generate the 5'-azide fom the corresponding alcohol. (**Figure 29**) Deoxyuridine (**18**) was subjected to standard Mitsunobu conditions (DEAD/Ph_3P) to give an excellent yield of 5'-azido uridine (**64**). Two different methods of reduction were carried out on this compound in order to produce the desired aminonucleoside. Initially, the Staudinger reduction was selected, as this method would provide a one-pot transformation from the alcohol to the amine. By modifying the Mitsunobu reaction to include 2 equivalents of Ph_3P, generation of the azide *in situ* would be followed by immediate reduction to the amine. On small scale (~100 mg), this procedure proved very effective. However, removal of triphenylphosphine oxide proved troublesome when >1 gram of the substrate was used. As such, the azide was isolated and catalytically hydrogenated in the presence of 10% Pd/C to generate amine **65** in good yield.

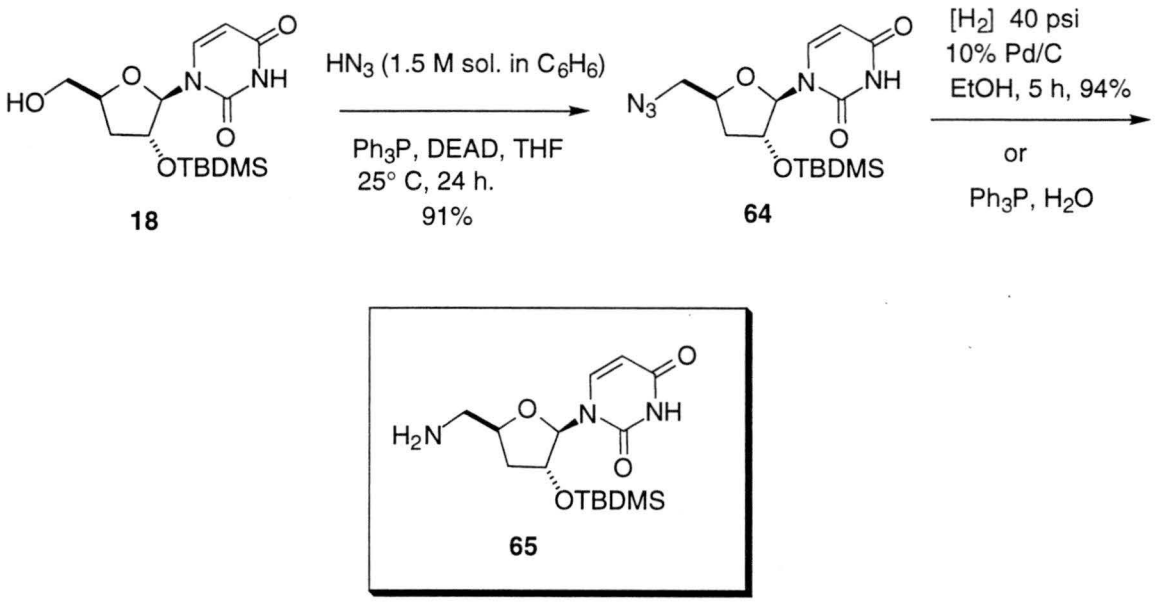


Figure 29 Synthesis of Aminouridine using the Mitsunobu Reaction

2. Synthesis of Simple Peptidylnucleosides

The first analog synthesized for biological evaluation was the acylated amine **66** which was produced by reaction with acetic anhydride in pyridine.

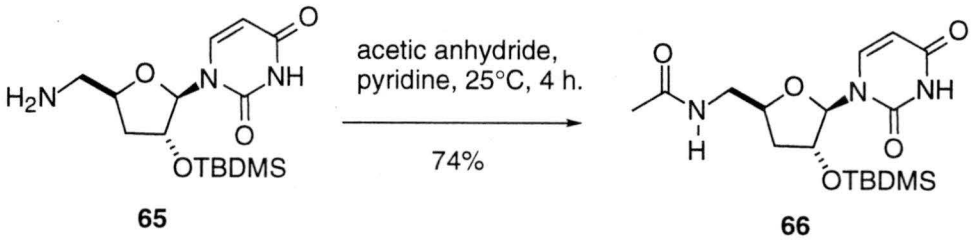


Figure 30 Synthesis of N-Acylated Analog

The analogs designed by molecular modeling were envisioned to be accessible through peptide couplings with the aminonucleoside. The side chains were synthesized as shown in **Figure 31**. 3-aminopropanol was

protected as the TBDPS ether followed by acylation with acetyl chloride. **67** was deprotected and oxidized to the acid with the Jones reagent to give N-acetyl-beta-alananine (**68**).

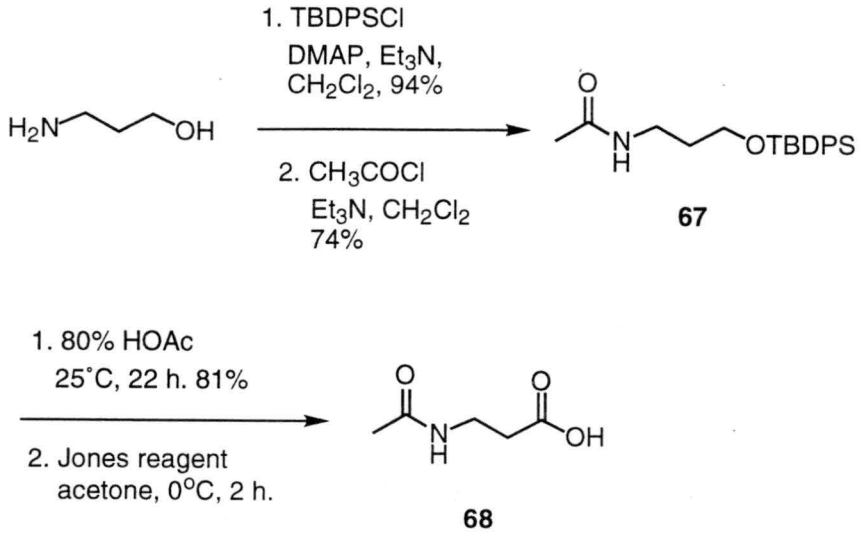


Figure 31 Synthesis of Analogs Side Chains

The appropriately protected sarcosine derivative (**69**) was coupled to the aminonucleoside (**65**) *via* the activated pentafluorophenyl ester. Deprotection with TFA cleaved the BOC protecting group as well as the silyl ether to give **70**. Finally, coupling of the side chain was accomplished with BOP-Cl to give **58**, a structural analog of mureidomycin (**Figure 32**).

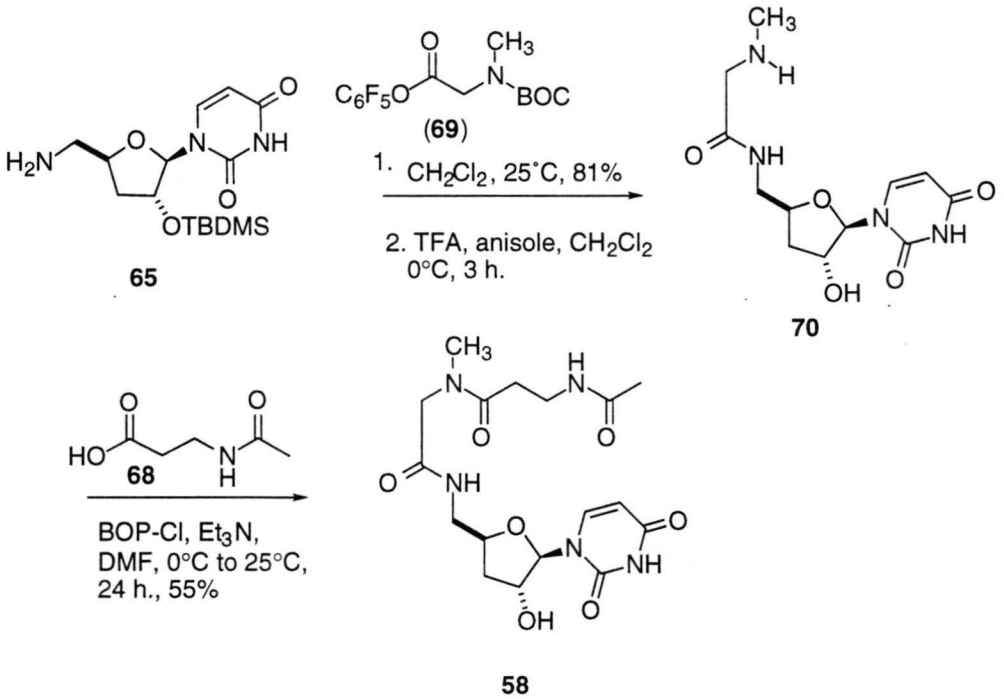


Figure 32 Synthesis of a Mureidomycin Analog

3. Biological Evaulation of Mureidomycin Analogs

The analogs denoted in **Figure 33** were assayed against a number of Gram-(+) and Gram-(-) organisms using an agar diffusion assay. Briefly, agar plates were inoculated with the test organism. Serial dilutions of the drug were prepared on discs and incubated overnight at 37°C.

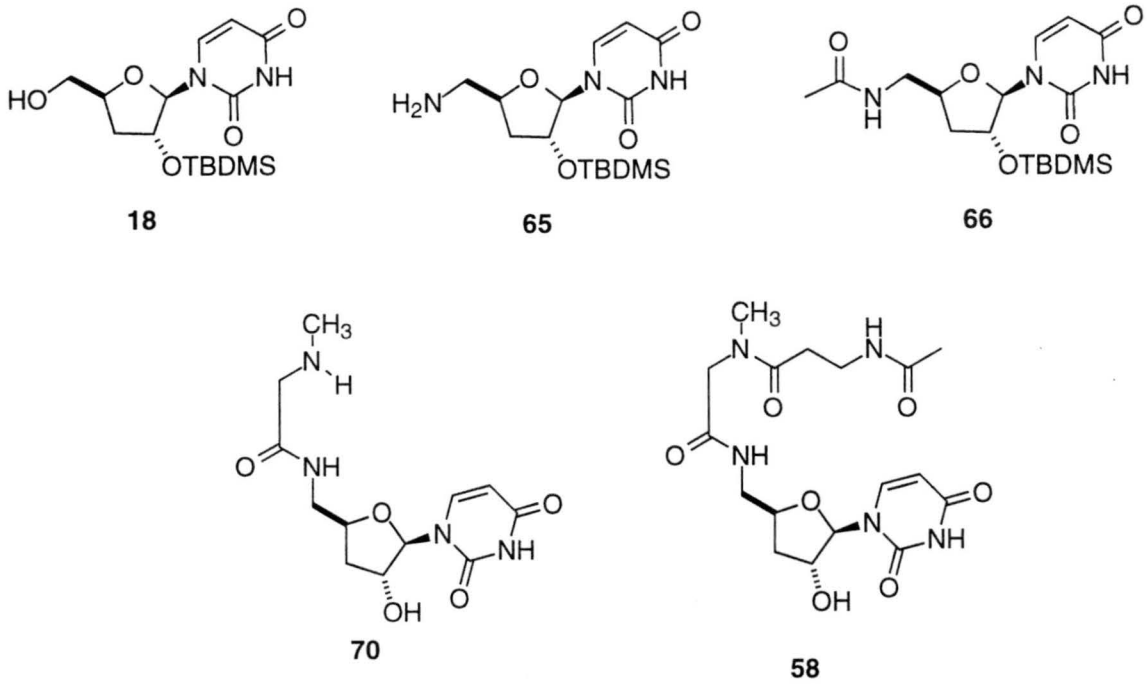


Figure 33 Analogs used in Biological Assay

All compounds tested in these assays were inactive against the Gram-(-) bacterial strains selected (**Table 3**).

Table 3

Test Organism	Zone of Inhibition
E. coli	R
K. pneumonia	R
S. marcescens	R
P. aeruginosa	R

R = resistant; growth up to the disc

4. Conclusions

Methodology was developed that allows the efficient synthesis of 3′-deoxy-5′-aminouridine which was used to generate peptidylnucleoside compounds for biological evaluation. None of the compounds tested showed activity against *P. aeruginosa*. The inactivity of these drugs may be a result of one of the mechanisms of resistance discussed previously. In addition, the molecular model used to design these analogs may be incorrect. In order to proceed with research in this area, the following ideas are proposed:

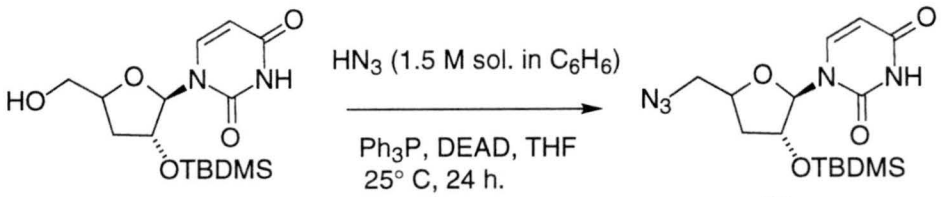
(i) ^{1}H NMR noe experiments on mureidomycin A could be performed in order to better determine the active conformation of this molecule.

(ii) Modifications of the natural substrate, UDP-MurNAc-pentapeptide, would provide further insight as to the types of molecules recognized as substrates by translocase.

(iii) Utilizing the 5′-aminonucleoside, a parallel synthetic approach could be used to generate a large library of compounds for biological evaluation.

(iv) When the synthesis of mureidomycin A is completed, the synthetic strategy could be used to produce analogs which more closely resemble the natural product.

C. Experimental



18 **64**

2'-O-(*tert*-butyldimethylsilyl)-3',5'-dideoxy-5'-azidouridine

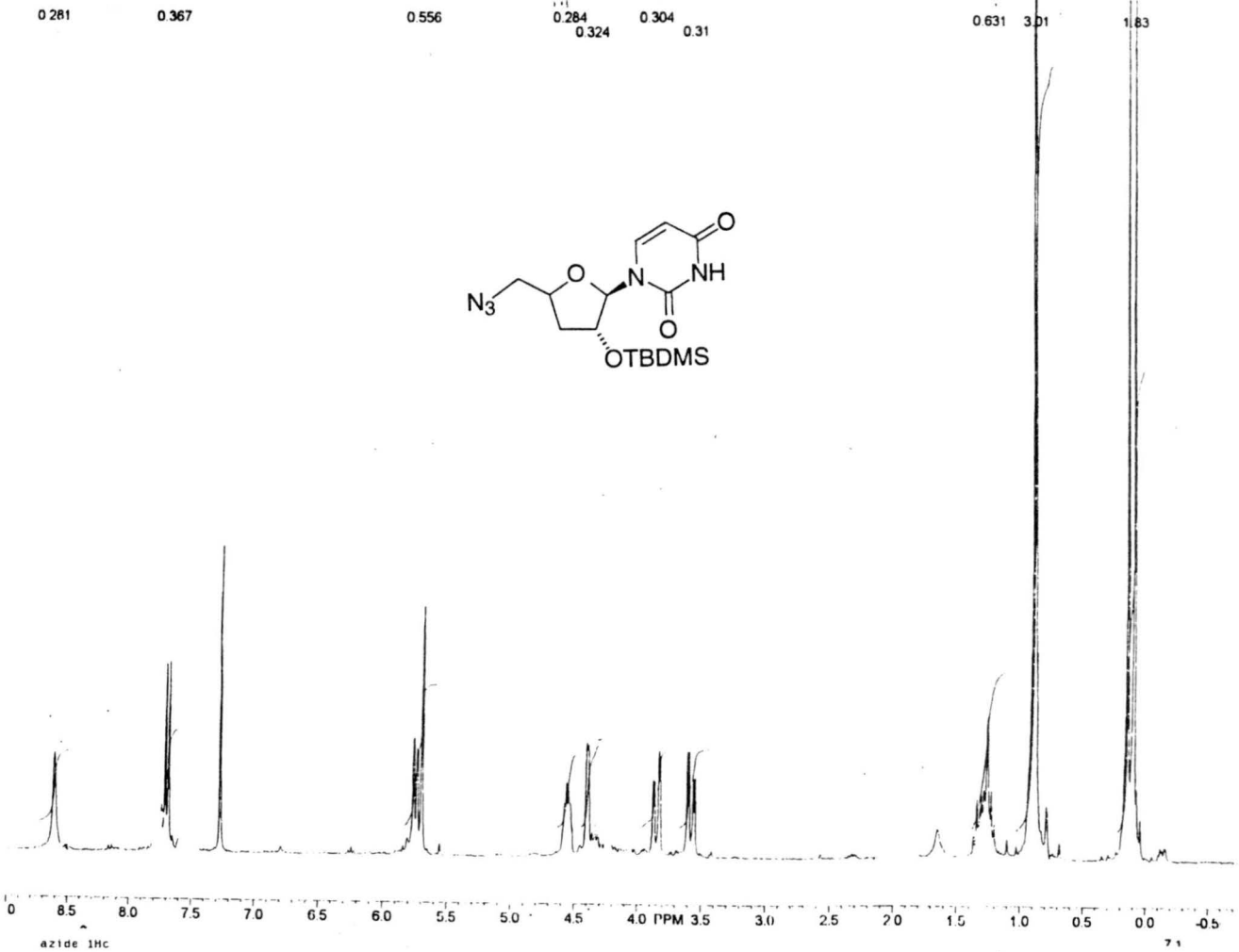
2'-O-(*tert*-butyldimethylsilyl)-3'-deoxyuridine (500 mg, 1.46 mmol) was dissolved in freshly distilled THF (10 mL) and stirred at room temperature uner argon. Triphenylphosphine (422 mg, 1.61 mmol) was added followed by HN_3 (1.52 mL of a 1.06 M sol. in C_6H_6, 1.61 mmol) and DEAD (254 µL, 1.61 mmol). The reaction was stirred at room temperature under argon for 24 h. The solvent was removed and the residue was chromatographed over silica gel (CH_2Cl_2,MeOH, 95:5, v/v) to yield 498 mg (93%) of 2'-O-(*tert*-butyldimethylsilyl)-3',5'-dideoxy-5'-azidouridine as a light yellow oil.

R_f=0.39 (CH_2Cl_2,MeOH, 95:5, v/v).

^{1}H NMR (300 MHz) ($CDCl_3$) δ 0.12 (6H, d), 0.88 (9H, s), 1.69–2.01 (2H, m) 3.52 - 3.61 (1H, dd, J=13.5, 3.9 Hz), 3.82–3.88 (1H, dd, J=13.5, 3.3 Hz), 4.39 (1H, m), 4.55 (1H, m), 5.70 (1H, s), 5.74 (1H, d, J=8.1 Hz), 7.69 (1H, d, J=8.1 Hz), 8.62 (1H, s, D_2O exchangeable).

^{13}C NMR (75.48 MHz) ($CDCl_3$) δ -5.22, -4.77, 14.37, 17.82, 25.56, 35.32, 52.95, 62.06, 78.68, 93.25, 101.74, 139.56, 150.13, 163.65.

IR (NaCl, CH_2Cl_2) 3211, 3056, 2983, 2954, 2930, 2856, 2102, 1713, 1590, 1521, 1462, 1437, 1379, 1340, 1259, 1223, 1181, 1119, 1097, 1068. cm -1.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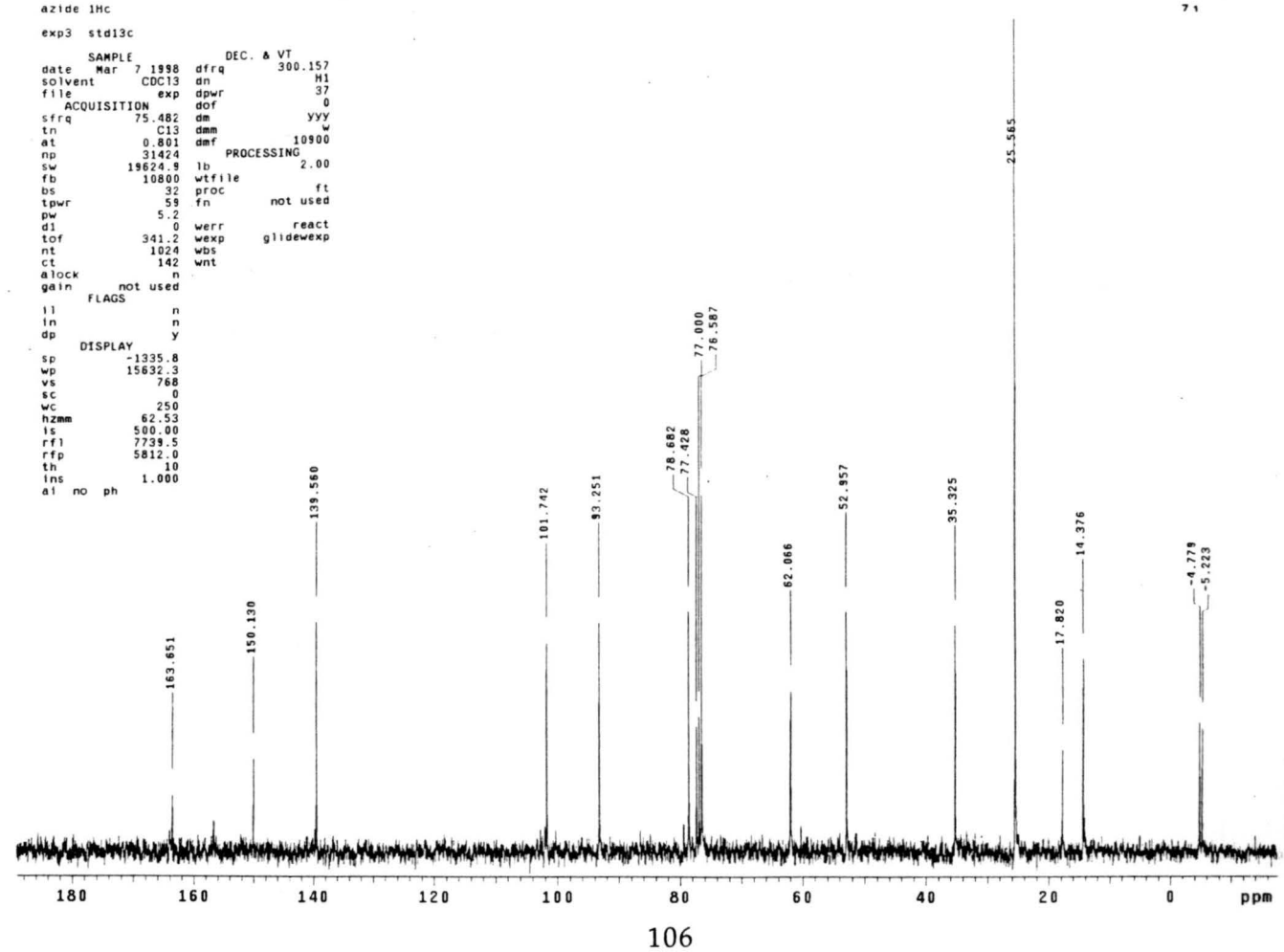
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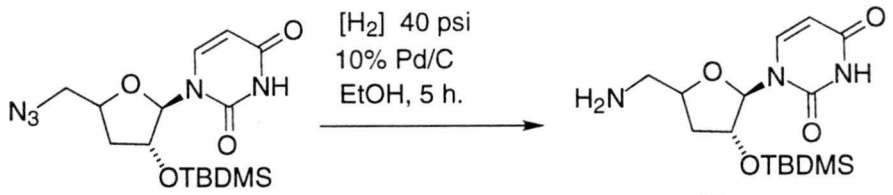


64 65

2'-O-(*tert*-butyldimethylsilyl)-3',5'-dideoxy-5'-aminouridine (65)

2'-O-(*tert*-butyldimethylsilyl)-3',5'-dideoxy-5'-azidouridine (3.00 g, 6.57 mmol) was combined with 10% Pd/C in absolute ethanol and hydrogenated under 40 psi of H_2 for 8 h. The reaction mixture was concentrated to a clear oil. The product crystallized upon the addition of ether. 200 mg (90%) of the title compound were isolated.

R_f=0.18 (CH_2Cl_2,MeOH, 8:2, v/v, stains ninhydrin).

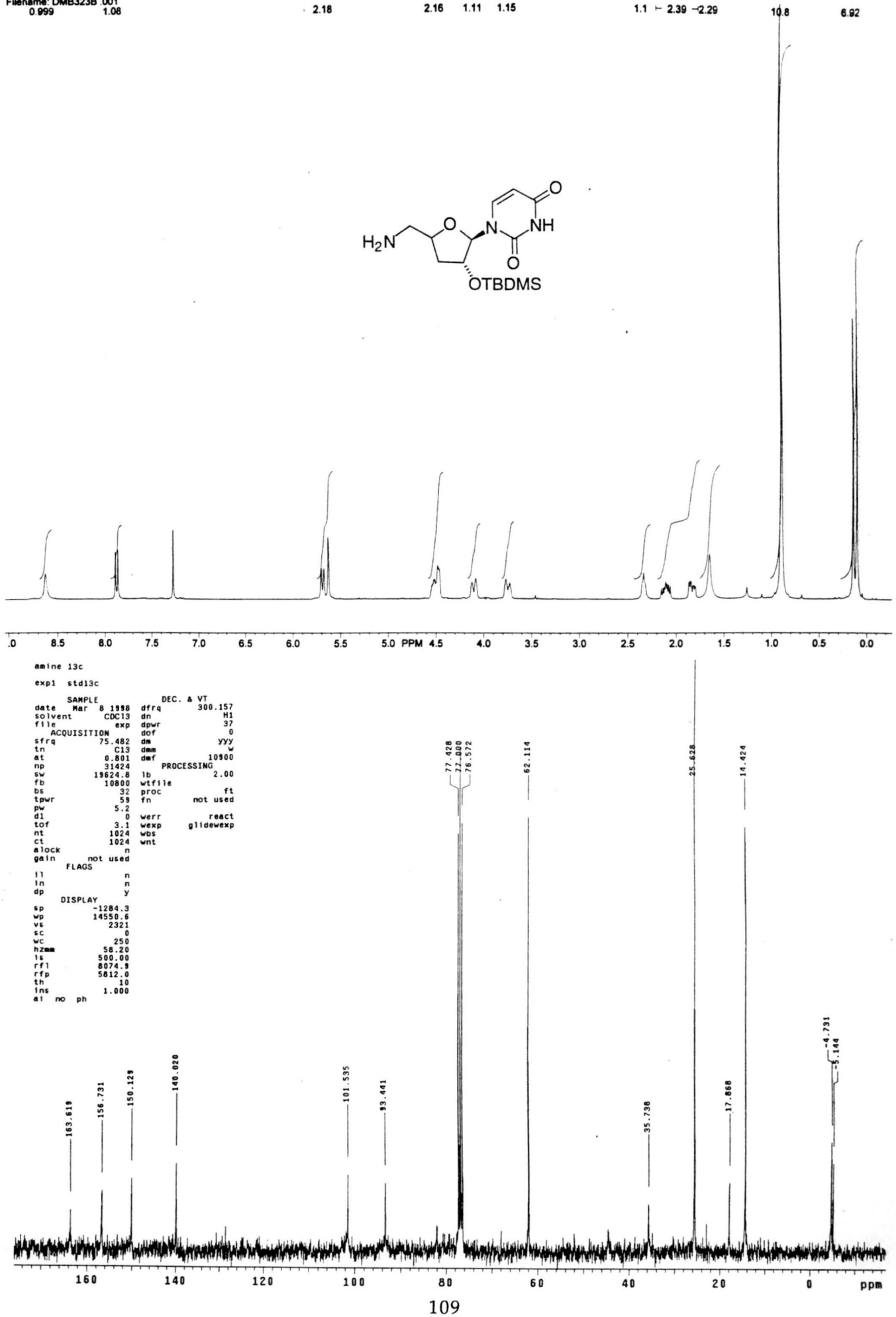
m.p. 89-91°C.

^{1}H NMR (300 MHz) ($CDCl_3$) δ 0.12 (6H, d), 0.89 (9H, s), 1.65 (2H, s, D_2O exchangeable), 1.81 (1H, dd, J=5.7, 2.4 Hz), 1.86 (1H, dd, J=5.7, 2.1 Hz), 2.06 - 2.15 (1H, m), 3.75 (1H, d, J=12.0 Hz), 4.10 (1H, d, J=11.7 Hz), 4.48 (2H, m), 5.63 (1H, d, J=J=0.9 Hz), 5.69 (1H, d, J=8.1 Hz) 7.87 (1H, d, J=8.1 Hz), 8.62 (1H, s, D_2O exchangeable).

^{13}C NMR (75.48 MHz) ($CDCl_3$) δ -5.14, -4.73, 14.42, 17.88, 25.63, 35.74, 62.11, 93.44, 101.53, 140.02, 150.13, 156.73, 163.62.

IR ($NaCl/CH_2Cl_2$) 3853, 3744, 3196, 2953, 2928, 2855, 1694, 1558, 1506, 1458, 1386, 1259, 1099 cm^{-1}.

HRMS (FAB+) m/e (M+H) 342.1853 ($C_{15}H_{27}N_3O_4Si$ + H requires 342.1849).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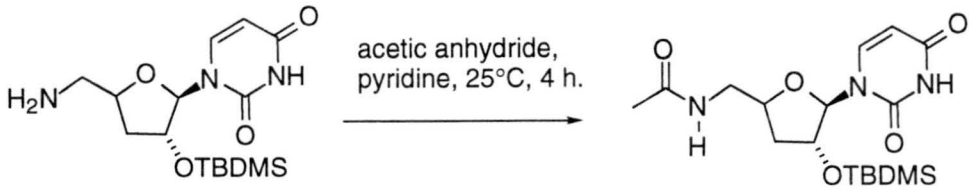


65 66

2'-O-(*tert*-butyldimethylsilyl)-3',5'-dideoxy-5'-N-acylaminouridine (66)

2'-O-(*tert*-butyldimethylsilyl)-3',5'-dideoxy-5'-aminouridine (100 mg, 0.293 mmol) was combined with acetic anhydride (83 µL, 0.88 mmol) in dry pyridine (1 mL) and stirred ar room temperature under argon for 24 h. The pyridine was removed *in vacuo* and the crude residue was purified by preparative TLC (eluted with CH_2Cl_2,MeOH, 9:1, v/v) to yield 96 mg (86%) of 2'-O-(*tert*-butyldimethylsilyl)-3',5'-dideoxy-5'-N-acylaminouridine as a white solid.

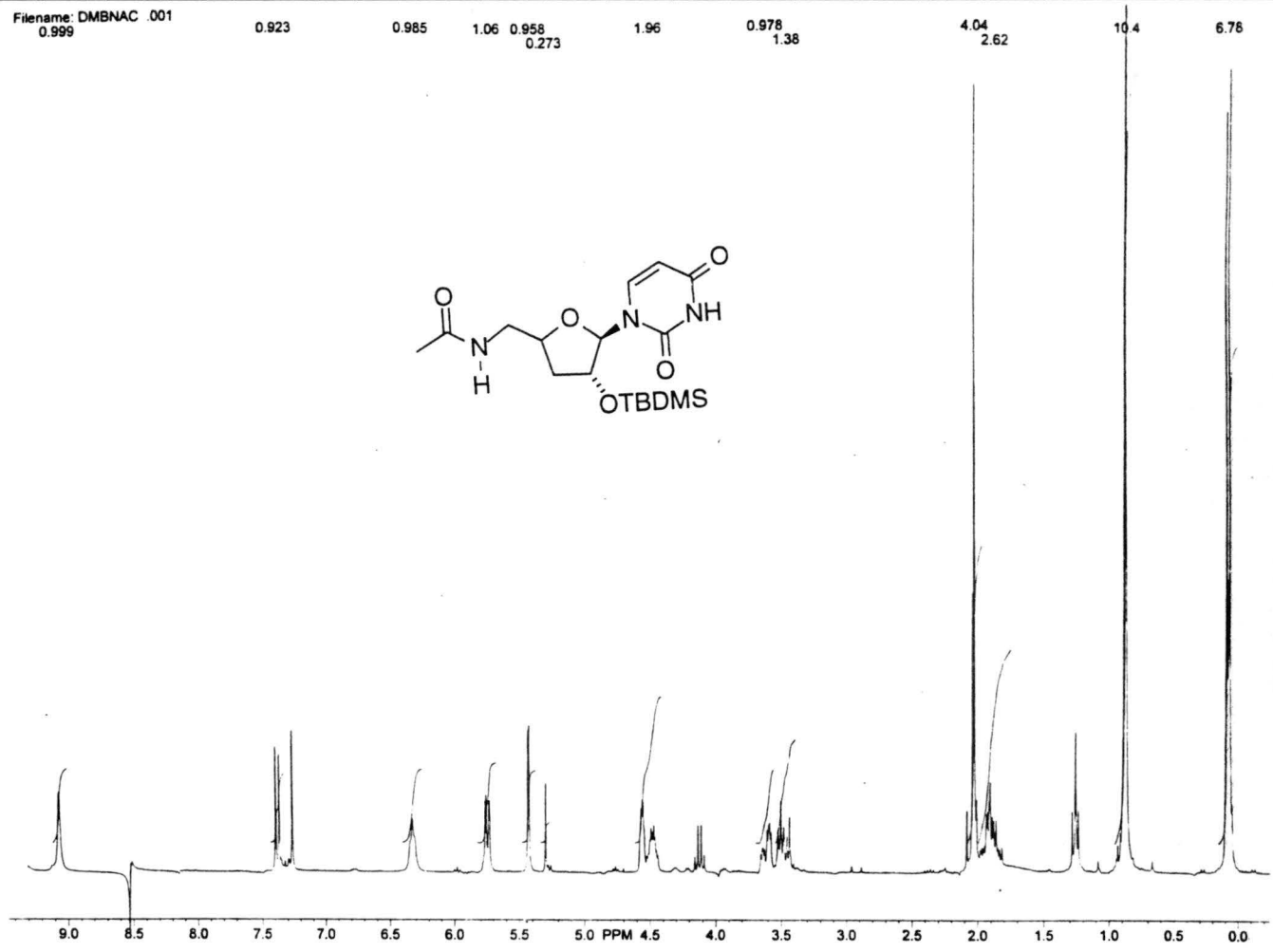
R_f=0.46 (CH_2Cl_2,MeOH, 9:1, v/v, stains ninhydrin).

^{1}H NMR (300 MHz) ($CDCl_3$) δ 0.09 (6H, d), 0.88 (9H, s), 1.78 - 1.94 (2H, m), 2.04 (3H, s), 3.44 - 3.51 (1H, m), 3.53 - 3.65 (1H, m), 4.45 (1H, m), 4.48 (2H, m), 4.56 (1H, m), 5.43 (1H, d, J=2.1 Hz) 5.75 (1H, dd, J=7.8, 1.8 Hz), 6.33 (1H, t, D_2O exchangeable), 7.38 (1H, d, J=8.1 Hz), 9.07 (1H, s, D_2O exchangeable).

^{13}C NMR (75.48 MHz) ($CDCl_3$) δ -5.08, -4.84, 17.83, 22.96, 25.58, 36.34, 42.57, 75.76, 79.42, 95.88, 101.86, 141.19, 150.16, 163.96, 171.03.

IR ($NaCl/CH_2Cl_2$) 3321, 3059, 2930, 2856, 1684, 1558, 1458, 1373, 1260, 1190, 1097, 1073 cm^{-1}.

LRMS (FAB+) m/e (M+H) 384.7 ($C_{17}H_{29}N_3O_5Si$ + H requires 384.5).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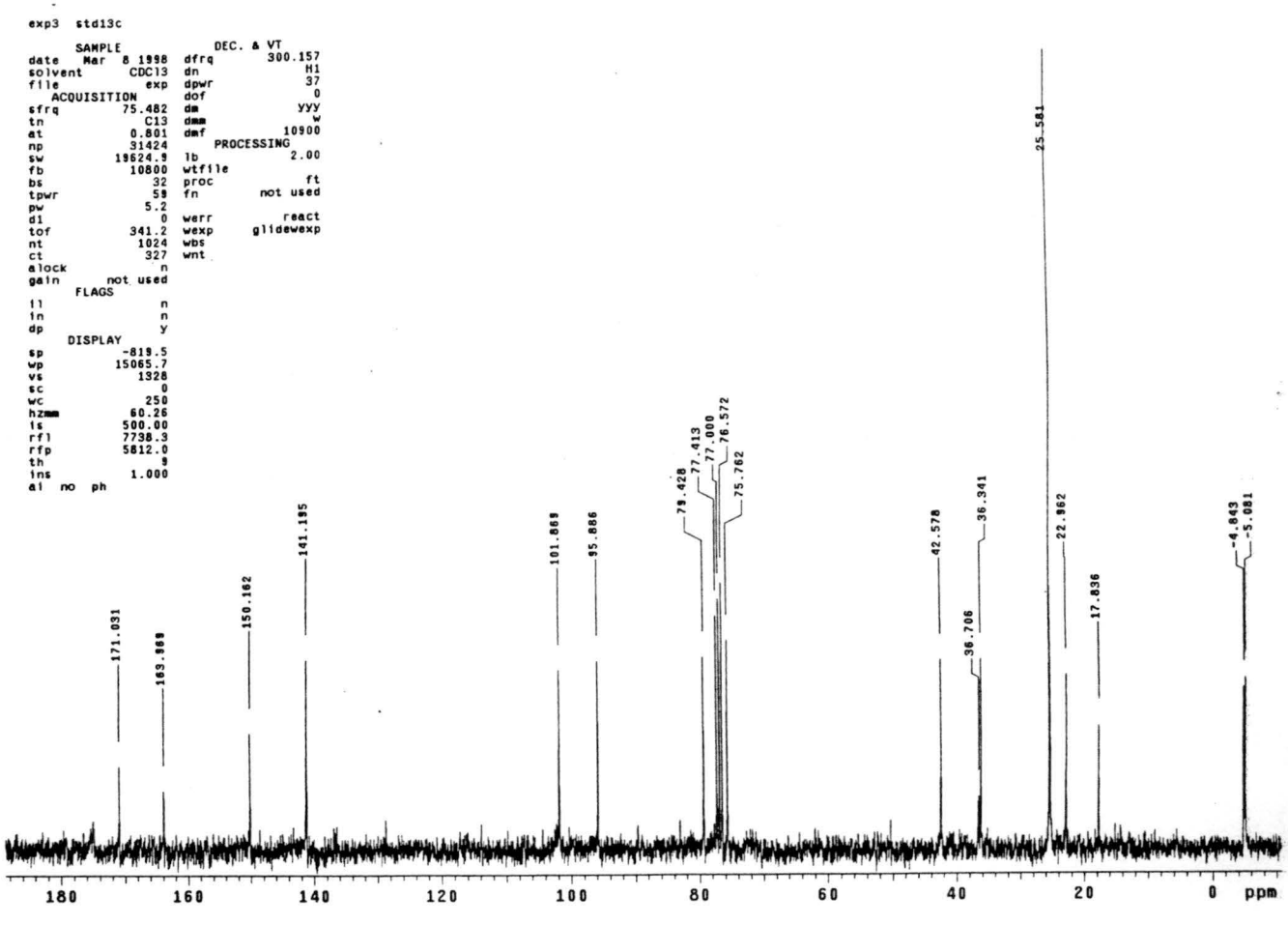
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SAMPLE
date Mar 8 1998
solvent CDCl3
file exp
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at 0.801
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bs 32
tpwr 58
pw 5.2
d1 0
tof 341.2
nt 1024
ct 327
alock n
gain not used
FLAGS
il n
in n
dp y
DISPLAY
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wp 15065.7
vs 1328
sc 0
wc 250
hzmm 60.26
is 500.00
rf1 7738.3
rfp 5812.0
th 9
ins 1.000
al no ph
DEC. & VT
dfrq 300.157
dn H1
dpwr 37
dof 0
dm yyy
dmm w
dmf 10900
PROCESSING
lb 2.00
wtfile
proc ft
fn not used
werr react
wexp glidewexp
wbs
wnt
171.031
169.868
150.162
141.195
101.868
85.886
79.428
77.413
77.000
76.572
75.762
42.578
36.706
36.341
25.581
22.962
17.836
-4.843
-5.081
180
160
140
120
100
80
60
40
20
0
ppm

O-(*tert*-butyldiphenylsilyl)-3-amino propanol

3-aminopropanol (2.0 g, 26.6 mmol) was dissolved in methylene chloride (50 mL) and stirred at 25°C. DMAP (0.33 g, 2.66 mmol) was added followed by *tert*-butyldiphenylsilyl chloride (10.4 mL, 39.9 mmol) and triethylamine (7.4 mL, 53.2 mmol) and the mixture was stirred overnight. The reaction was concentrated and the product was purified by Kugelrhor distillation (210°C at 0.1 mm Hg). The product was isolated as a clear oil in 57% yield.

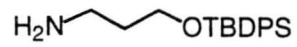
^{1}H NMR (300 MHz) ($CDCl_3$) δ 1.09 (9H, s), 1.22 (1H, quint), 1.98 (1H, s), 2.73 (1H, t), 3.73 (1H, t), 7.24-7.81 (10H, m).

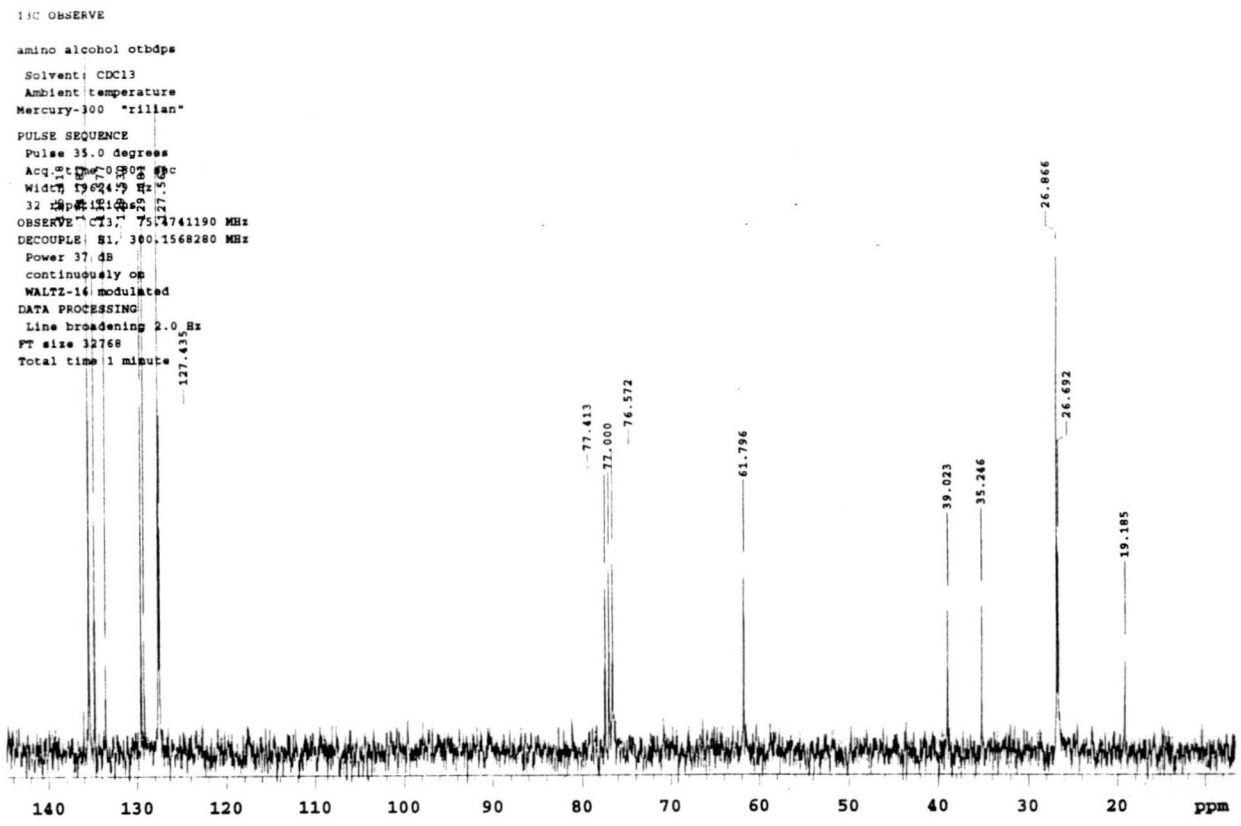
^{13}C NMR (75.48 MHz) ($CDCl_3$) δ 19.18, 26.69, 26.86, 35.24, 39.02, 61.79, 127.43, 127.56, 129.18, 129.53, 133.57, 134.78, 135.41.

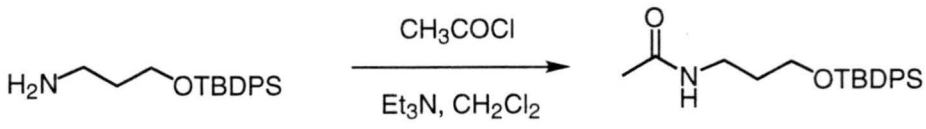
IR (NaCl/ CH_2Cl_2) 3363, 3133, 3069, 3048, 2997, 2930, 2856, 1773, 1601, 1524, 1471, 1445, 1427, 1389, 1306, 1246, 1228, 1187, 1110, 1029 cm^{-1}.

Filename DMB331DI 001
10.6

1.88 1.77 2.2 1.88 10







67

O-(*tert*-butyldiphenylsilyl)-3-N-acetylamino propanol (67)

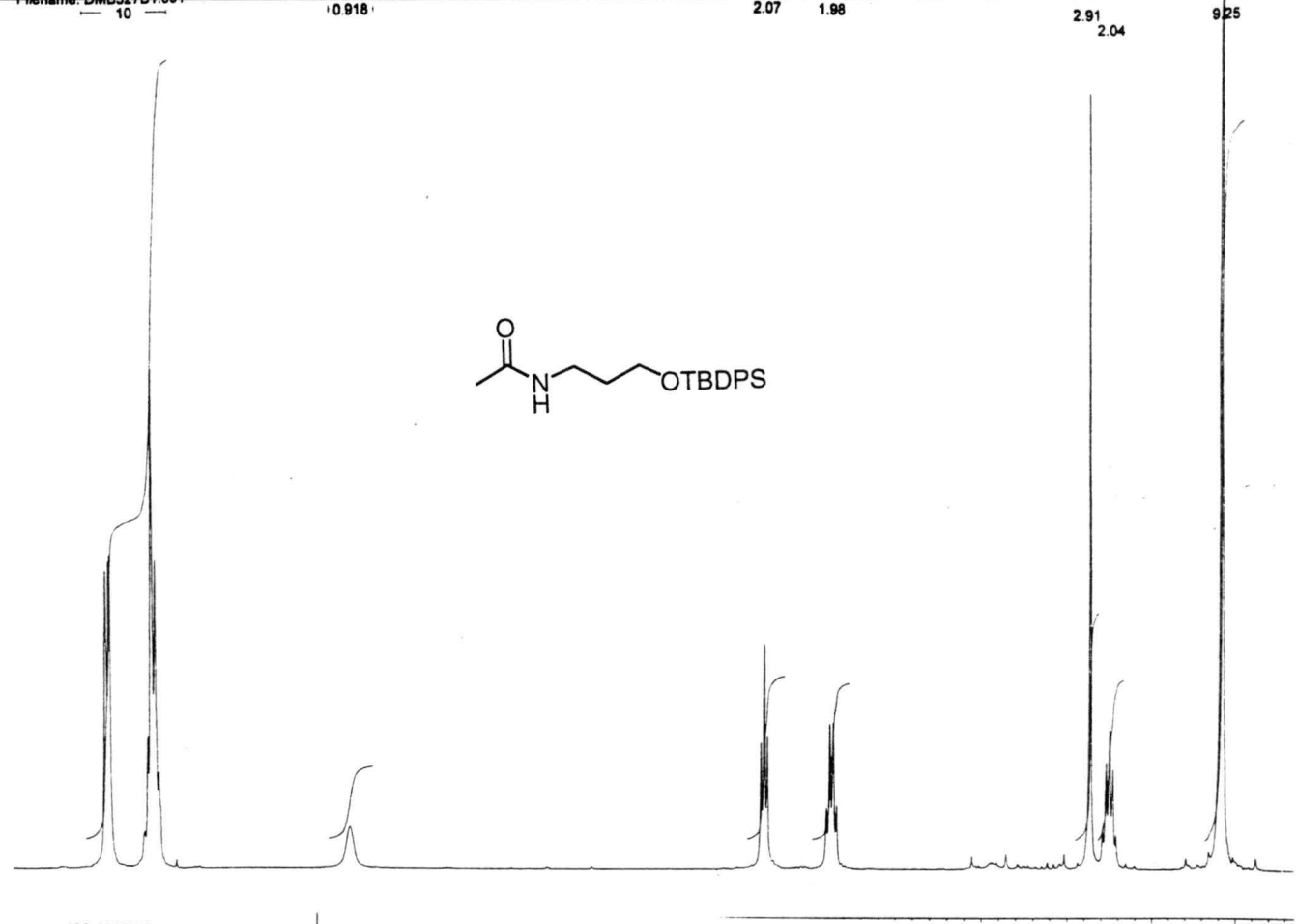
O-(*tert*-butyldiphenylsilyl)-3-amino propanol was dissolved in methylene chloride (50 mL). Acetyl chloride (2.08 mL, 29.26 mmol) and triethylamine (11.1 mL, 79.8 mmol) were added and the reaction was stirred overnight at 25°C under argon. The mixture was diluted with chloroform (50 mL) and extracted three times with saturated brine. The organics were combined, dried ($MgSO_4$) and concentrated to give a brown oil which was purified by chromatography ($MeOH/CH_2Cl_2$, 1:99, v/v then $MeOH/CH_2Cl_2$, 5:95, v/v) to give 3.5 g (74%) of **66** as a light yellow oil.

^1H NMR (300 MHz) ($CDCl_3$) δ 1.09 (9H, s), 1.72 (1H, quint), 1.86 (3H, s), 3.34 (1H, q), 3.73 (1H, t), 6.22 (1H, br s, D_2O exchangeable), 7.24-7.81 (10H, m).

^{13}C NMR (75.48 MHz) ($CDCl_3$) δ 18.97, 22.47, 26.54, 31.27, 37.54, 62,47, 127.18, 127.51, 128.89, 129.54, 133.05, 134.62, 135.21, 135.87, 169.79.

IR (NaCl/ CH_2Cl_2) 3292, 3070, 3049, 2997, 2956, 2930, 2856, 1771, 1739, 1652, 1588, 1558, 1472, 1427, 1389, 1369, 1292, 1218, 1193, 1111 cm^{-1}.

LRMS (ES+) (M+H) 356.2 (C21H29NO2Si + H requires 356.6)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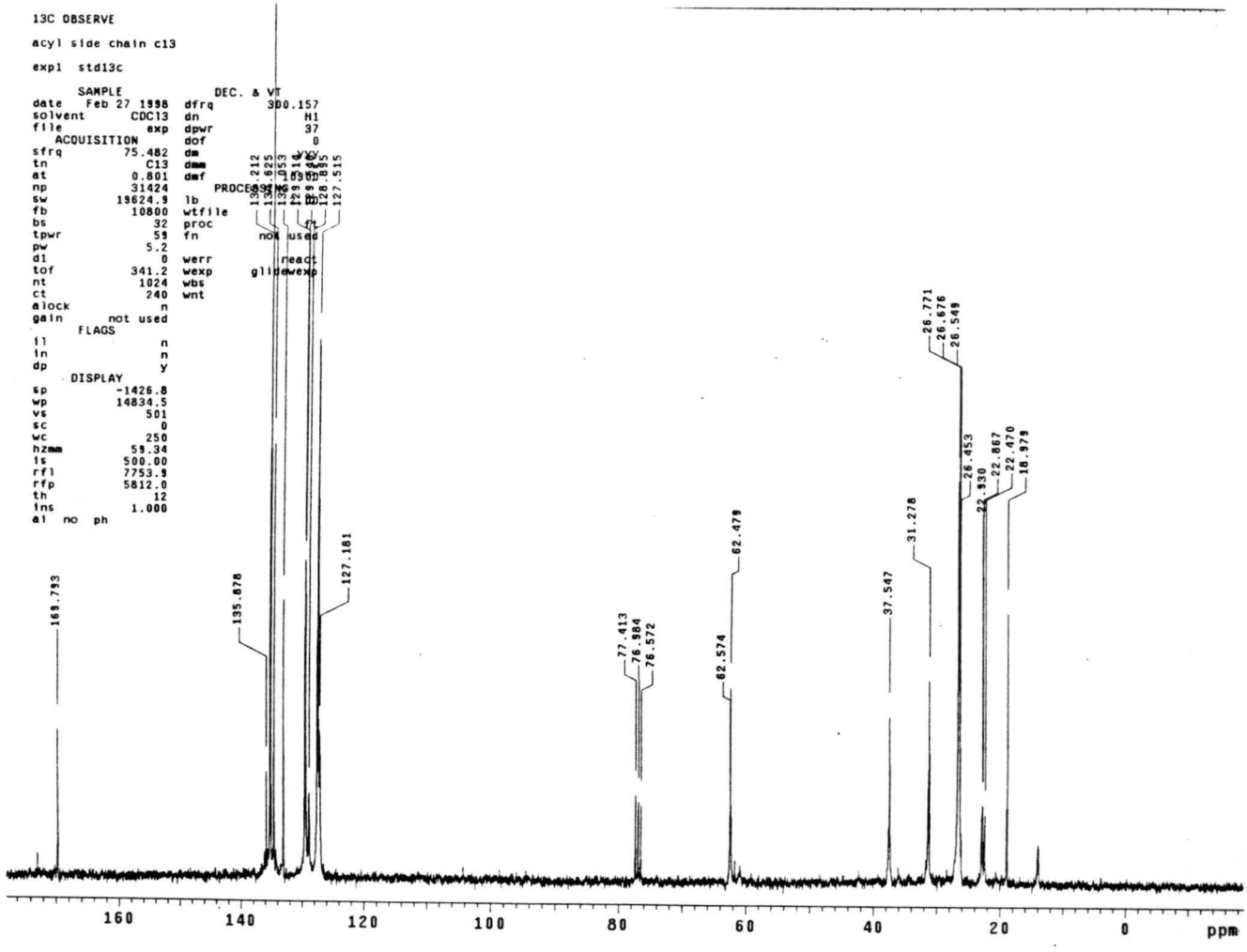
2.07
1.98
2.91
2.04
9.25
O
N
H
OTBDPS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exp1 std13c
SAMPLE
date Feb 27 1998
solvent CDCl3
file exp
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np 31424
sw 19624.9
fb 10800
bs 32
tpwr 59
pw 5.2
d1 0
tof 341.2
nt 1024
ct 240
alock n
gain not used
FLAGS
il n
in n
dp y
DISPLAY
sp -1426.8
wp 14834.5
vs 501
sc 0
wc 250
hzmm 59.34
is 500.00
rfl 7753.9
rfp 5812.0
th 12
ins 1.000
al no ph
DEC. & VT
dfrq 300.157
dn H1
dpwr 37
dof 0
dm
dmm
dmf
PROCESSING
lb
wtfile
proc
fn not used
werr react
wexp glidewexp
wbs
wnt
169.793
135.878
127.181
77.413
76.984
76.572
62.574
62.478
37.547
31.278
26.771
26.676
26.549
26.453
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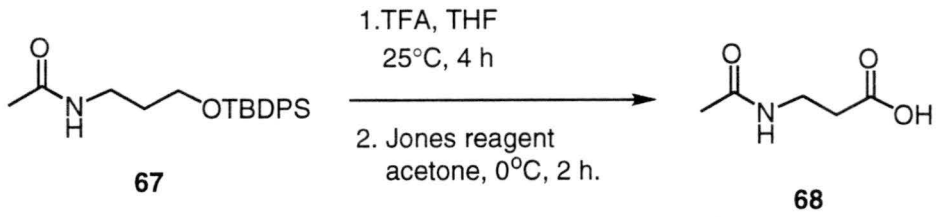
22.867
22.470
18.979
160
140
120
100
80
60
40
20
0
ppm



67 68

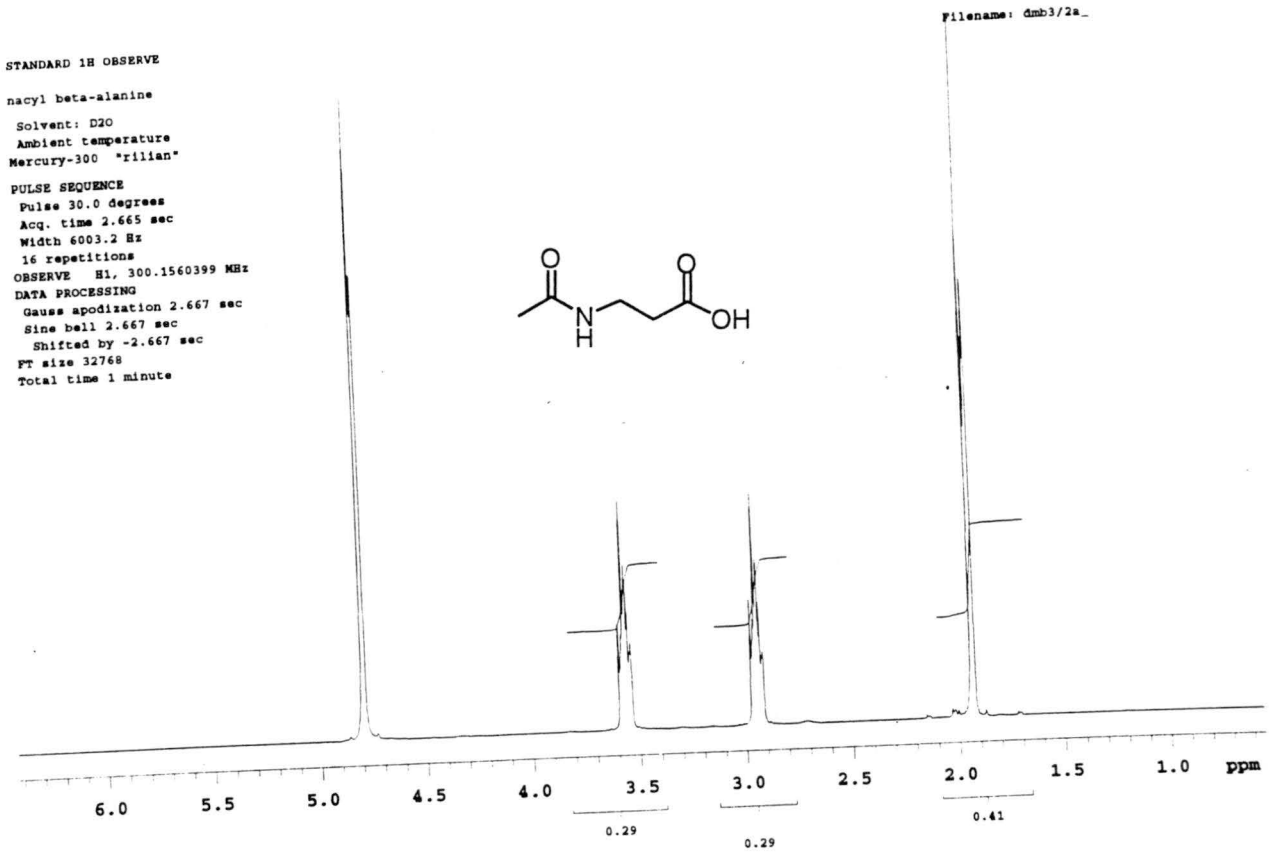
N-acetylamino propanoic acid (68)

Compound **66** was treated with trifluoroacetic acid in THF and stirred at 25°C for 4 h. The solvent was removed and the crude residue was immediately dissolved in acetone and treated with Jones reagent at 0°C. The reaction was stirred at 0° for 2 h. Water was added and the mixture turned a deep blue color. The aqueous phase was extracted three time with ethyl acetate. The organics were dried ($MgSO_4$) and concentrated to give **68** as a yellow oil.

^1H NMR (300 MHz) (D_2O) δ 1.91 (3H, s), 2.92 (1H, t), 3.56 (1H, t).

^{13}C NMR (75.48 MHz) (D_2O) δ 16.86, 22.32, 32.70, 170.45, 172.42.

IR (NaCl/ CH_2Cl_2) 3409, 2938, 2812, 1664, 1594, 1498, 1438, 1417, 1341, 1316, 1255, 1104, 1062 cm^{-1}.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Ambient temperature
Mercury-300 "rilian"
PULSE SEQUENCE
Pulse 30.0 degrees
Acq. time 2.665 sec
Width 6003.2 Hz
16 repetitions
OBSERVE H1, 300.1560399 MHz
DATA PROCESSING
Gauss apodization 2.667 sec
Sine bell 2.667 sec
Shifted by -2.667 sec
FT size 32768
Total time 1 minute
O
O
N
H
OH
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
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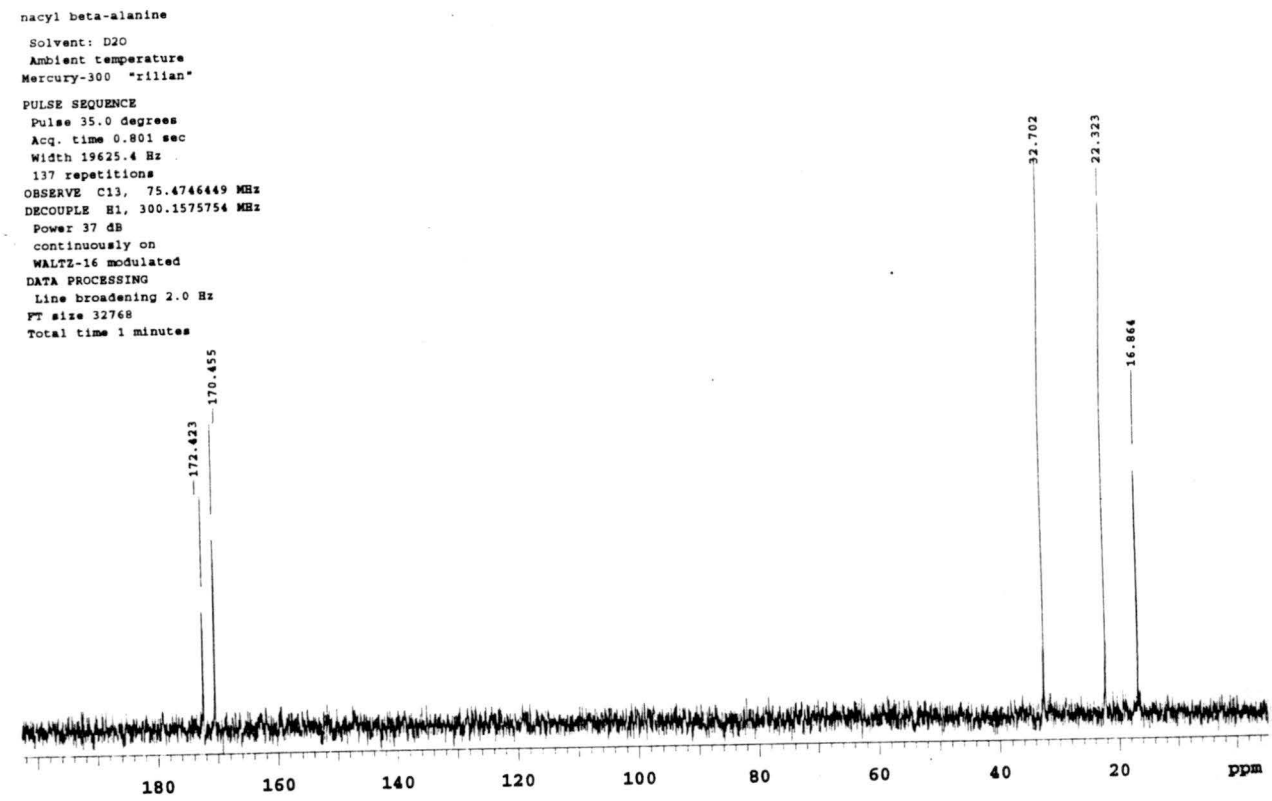
0.29
0.41


nacyl beta-alanine
Solvent: D2O
Ambient temperature
Mercury-300 "rilian"
PULSE SEQUENCE
Pulse 35.0 degrees
Acq. time 0.801 sec
Width 19625.4 Hz
137 repetitions
OBSERVE C13, 75.4746449 MHz
DECOUPLE H1, 300.1575754 MHz
Power 37 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 2.0 Hz
FT size 32768
Total time 1 minutes
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170.455
32.702
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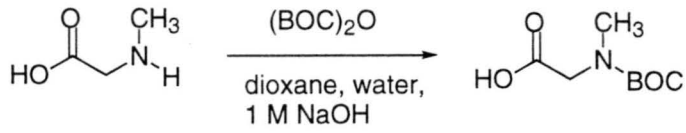
180
160
140
120
100
80
60
40
20
ppm



N-(*tert*butoxycarbonyl) sarcosine

Sarcosine (1.0 g, 11.2 mmol) was stirred in dioxane (20 mL), water (10 mL) and 1 M NaOH (11.2 mL) at 0°C. $(BOC)_2O$ was added and the reaction was stirred for 30 min. The mixture was concentrated to a volume of approximately 10 mL and cooled to 0°C. Ethyl acetate was added and the mixture was acidified to pH 2 with 10% $KHSO_4$. The layers were separated and the procedure was repeated twice more. The combined organic layers were washed with water, dried ($MgSO_4$) and concentrated to a white solid.

m.p. 49-51°C.

^1H NMR (300 MHz) ($CDCl_3$) δ 1.41 (9H, s), 2.91 (3H, s), 3.97 (2H, d), 10.15 (1H, s).

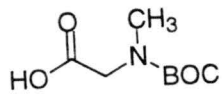
^{13}C NMR (75.48 MHz) ($CDCl_3$) δ 28.15, 35.53, 49.92, 80.50, 155.47, 174.19.

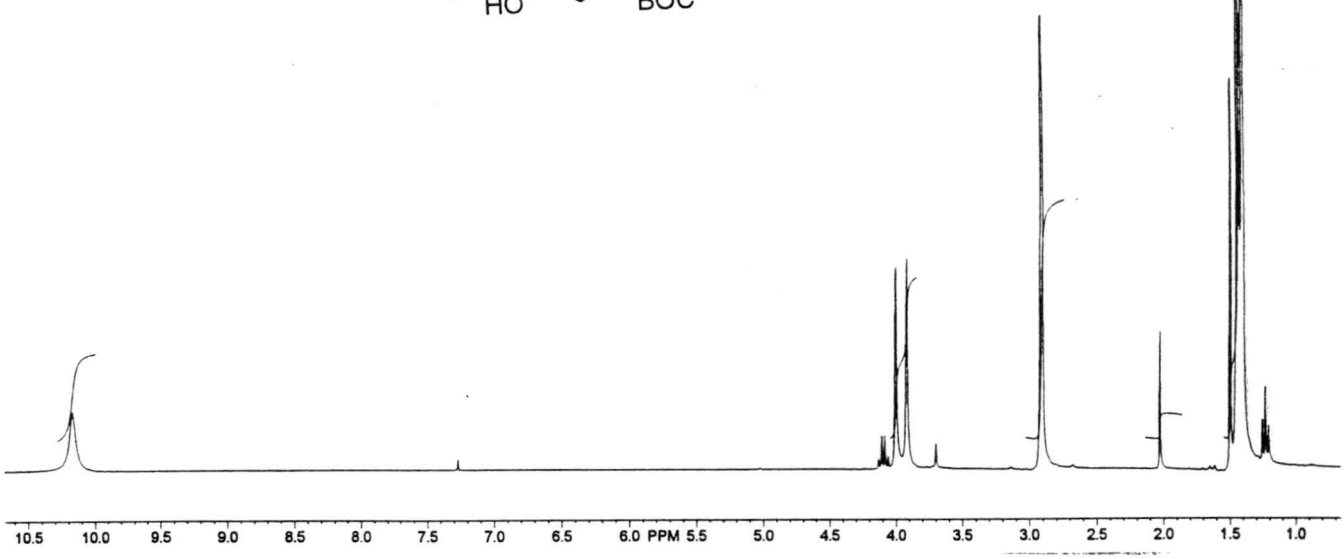
IR ($NaCl/CH_2Cl_2$) 3115, 2978, 2935, 2645, 1753, 1701, 1546, 1484, 1455, 1394, 1368, 1342, 1302, 1250, 1154, 1061, 1035 cm^{-1}.

Filename: DMB329A1.001
0.614

BOC-sarcosine

1.14 1.67 0.162 5.7





boc-sarc

expl std13c

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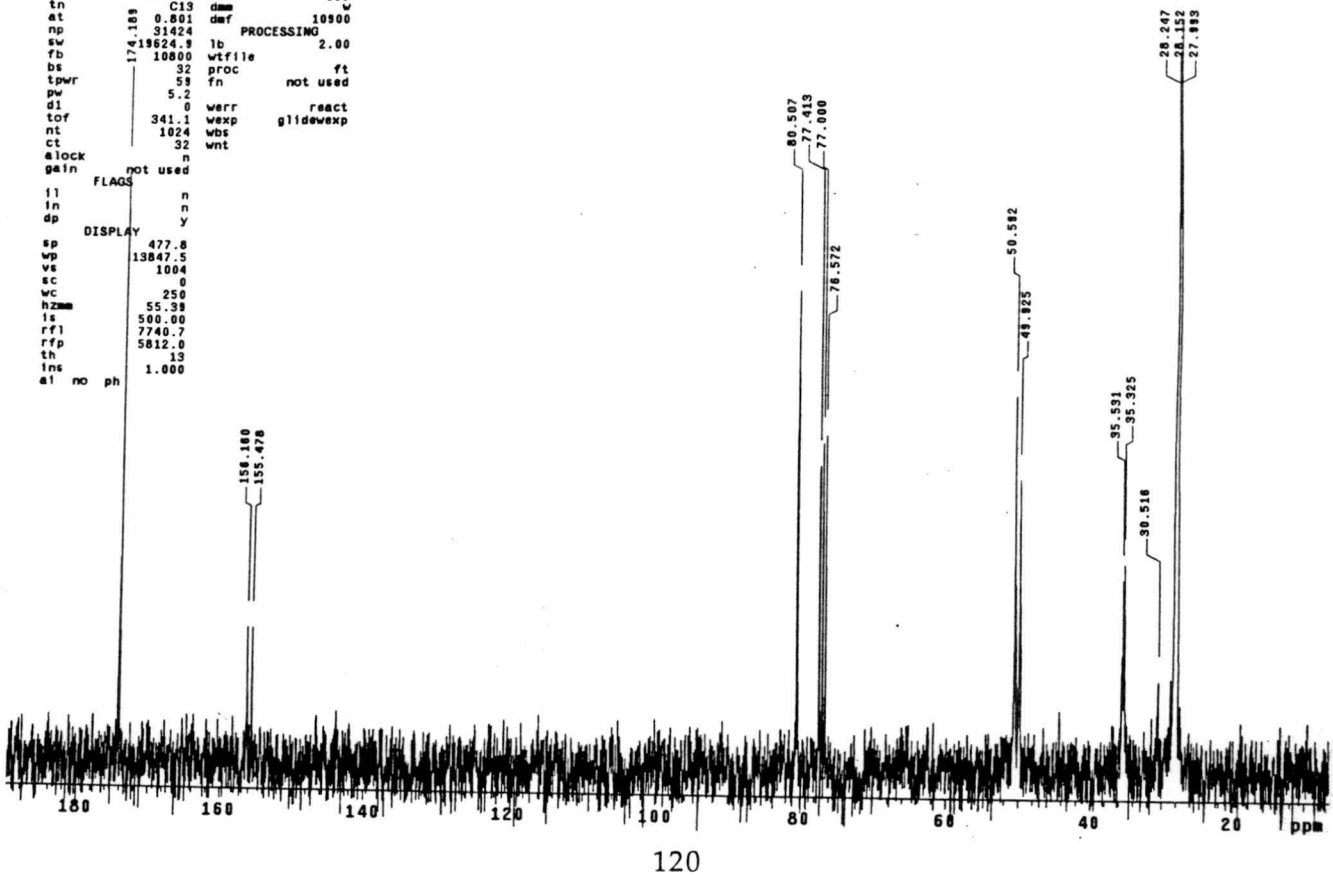
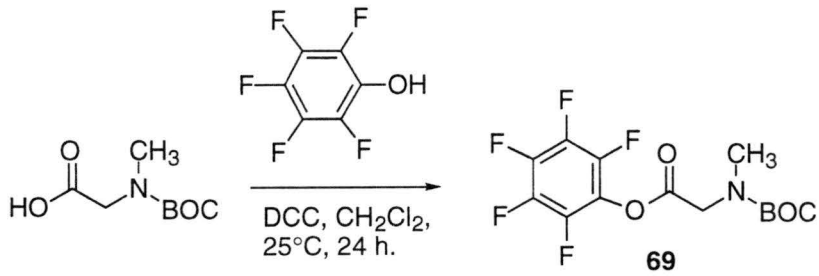
tn	C13	dmm	w
at	0.801	dmf	10900
np	31424	PROCESSING	
sw	19624.9	lb	2.00
fb	10800	wtfile	
bs	32	proc	ft
tpwr	59	fn	not used
pw	5.2		
d1	0	werr	react
tof	341.1	wexp	glidewexp
nt	1024	wbs	
ct	32	wnt	
alock	n		
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
DISPLAY			
sp	477.8		
wp	13847.5		
vs	1004		
sc	0		
wc	250		
hzmm	55.39		
is	500.00		
rfl	7740.7		
rfp	5812.0		
th	19		
ins	1.000		

al no ph





N-(*tert*butoxycarbonyl) sarcosine pentafluorophenyl ester (69)

BOC-sarcosine (0.20 g, 1.06 mmol) was dissolved in CH_2Cl_2 (3 mL). Dicyclohexylcarbodiimide (0.24 g, 1.16 mmol) and pentafluorophenol (0.21 g, 1.16 mmol) were added. A white precipitate formed immediately. The reaction was stirred at 25°C under argon overnight. The reaction mixture was concentrated and the crude residue was applied to a column of silica gel (hexanes/ethyl acetate, 2:1, v/v) to yield 0.35 g (92%) of a yellow oil.

R_f=0.69 (hexanes/ethyl acetate, 2:1, v/v)

^{1}H NMR (300 MHz) ($CDCl_3$) δ 1.48 (9H, s), 3.07 (3H, s), 4.26 (2H, d).

^{13}C NMR (75.48 MHz) ($CDCl_3$) δ 28.15, 35.48, 49.71, 50.33, 80.68, 136.11, 139.37, 142.70, 154.85, 166.11.

IR (NaCl/CH_2Cl_2) 3089, 2987, 1724, 1218, 1187 cm^{-1}.

STANDARD 1H OBSERVE

c6f5 1H

exp3 std1h

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tn	H1	dmm	c
at	2.665	dmf	10900
np	32000	PROCESSING	
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fb	3400	sbs	-2.667
bs	16	gf	2.667
ss	2	gfs	not used
tpwr	56	wtfile	
pw	3.7	proc	ft
d1	0	fn	not used
tof	0.0		
nt	16	werr	react
ct	16	wexp	glidewexp
alock	y	wbs	
gain	4	wnt	
FLAGS			
il	n		
in	n		
dp	y		
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vs	240		
sc	0		
wc	250		
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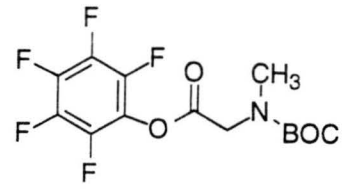
ins	1.000		

al cdc ph



13C OBSERVE

c6f5 13c

exp3 std13c

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file	exp	dpwr	37
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sfrq	75.482	dm	yyy
tn	C13	dmm	w
at	0.801	dmf	10900
np	31424	PROCESSING	
sw	19624.8	lb	2.00
fb	10800	wtfile	
bs	32	proc	ft
tpwr	59	fn	not used
pw	5.2		
d1	0	werr	react
tof	341.2	wexp	glidewexp
nt	1024	wbs	
ct	224	wnt	
alock	n		
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
DISPLAY			
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wp	12156.1		
vs	1866		
sc	0		
wc	250		
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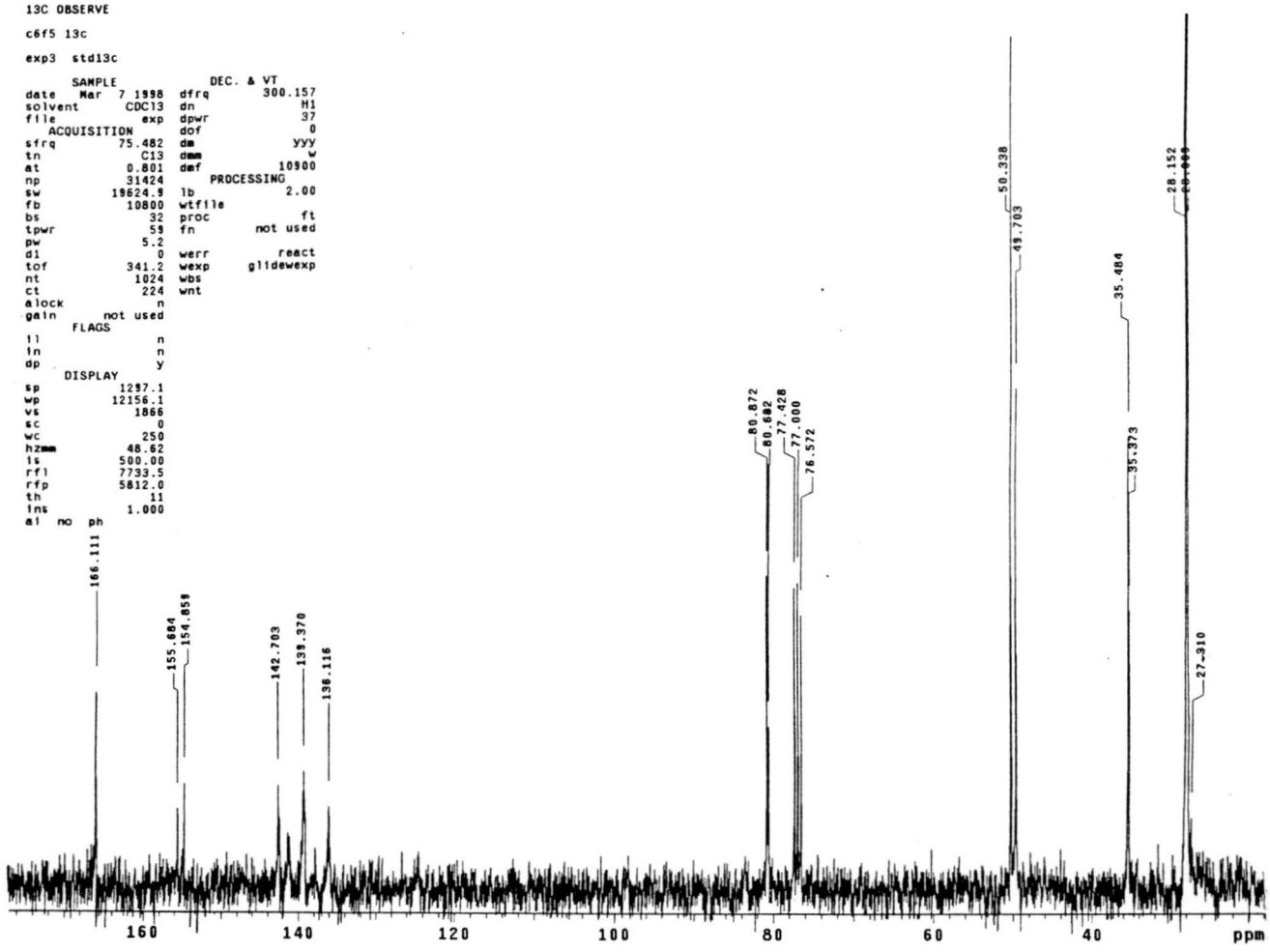
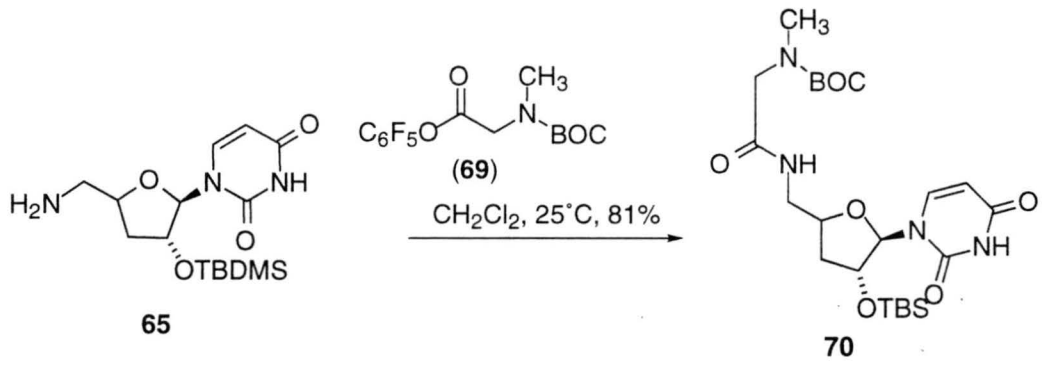
is	500.00		
rfl	7733.5		
rfp	5812.0		
th	11		
ins	1.000		

al no ph





Protected Coupled Product

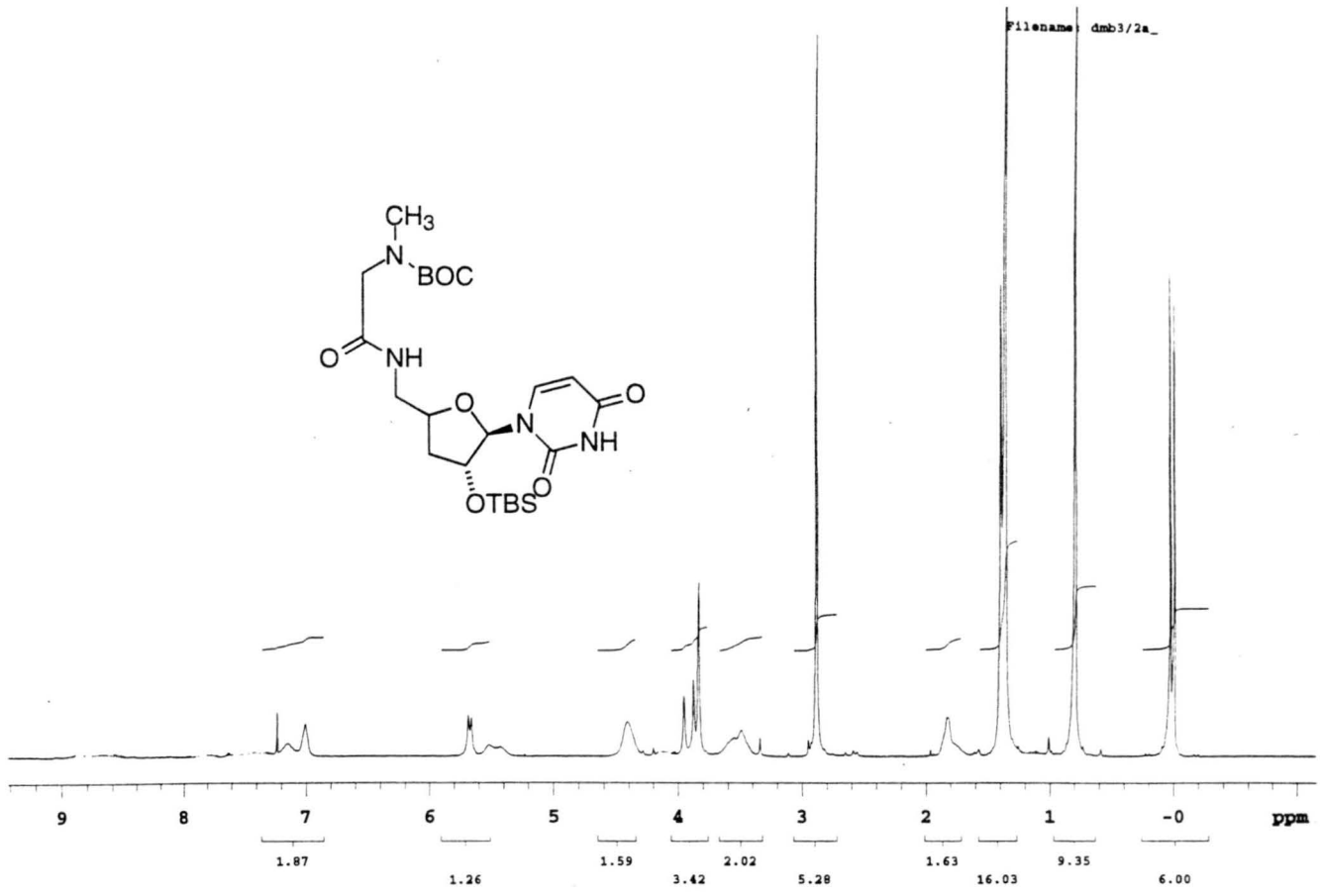
2'-O-(*tert*-butyldimethylsilyl)-3',5'-dideoxy-5'- aminouridine (**18**) (0.10 g, 0.29 mmol) was combined with protected sarcosine **69** in pyridine (2 mL) and stirred at 25 °C under argon overnight. The solvent was removed and the crude residue was applied to a column of silica gel ($MeOH/CH_2Cl_2$, 95:5, v/v) to give 0.12 g (81%) of the desired compound as a white solid.

R_f=0.51 (CH_2Cl_2/MeOH, 95:5, v/v)

^1H NMR (300 MHz) ($CDCl_3$) δ 0.07 (6H, d), 0.87 (9H, s), 1.41 (9H, m), 1.81-1.96 (2H, m), 2.95 (3H,s), 3.46-3.63 (2H, m), 3.96 (2H, m), 4.41 (2H, m), 5.50 (1H, d, J=8.1 Hz), 5.69 (1H, d, J=1.9 Hz), 7.01 (1H, s, D_2O exchangeable), 7.11 (1H, d, J=8.1 Hz).

^{13}C NMR (75.48 MHz) ($CDCl_3$) δ -5.17, -4.89, 14.32, 17.75, 25.50, 28.15, 36.46, 50.26, 50.97, 52.89, 61.90, 76.42, 79.34, 101.95, 125.02, 138.47, 146.92, 150.05, 163.51, 172.51.

IR ($NaCl/CH_2Cl_2$) 3311, 2930, 2856, 1691, 1541, 1458, 1390, 1367, 1259, 1152, 1099 cm^{-1}.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BOC
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NH
O
N
NH
O
O
OTBS
9
8
7
6
5
4
3
2
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-0
ppm
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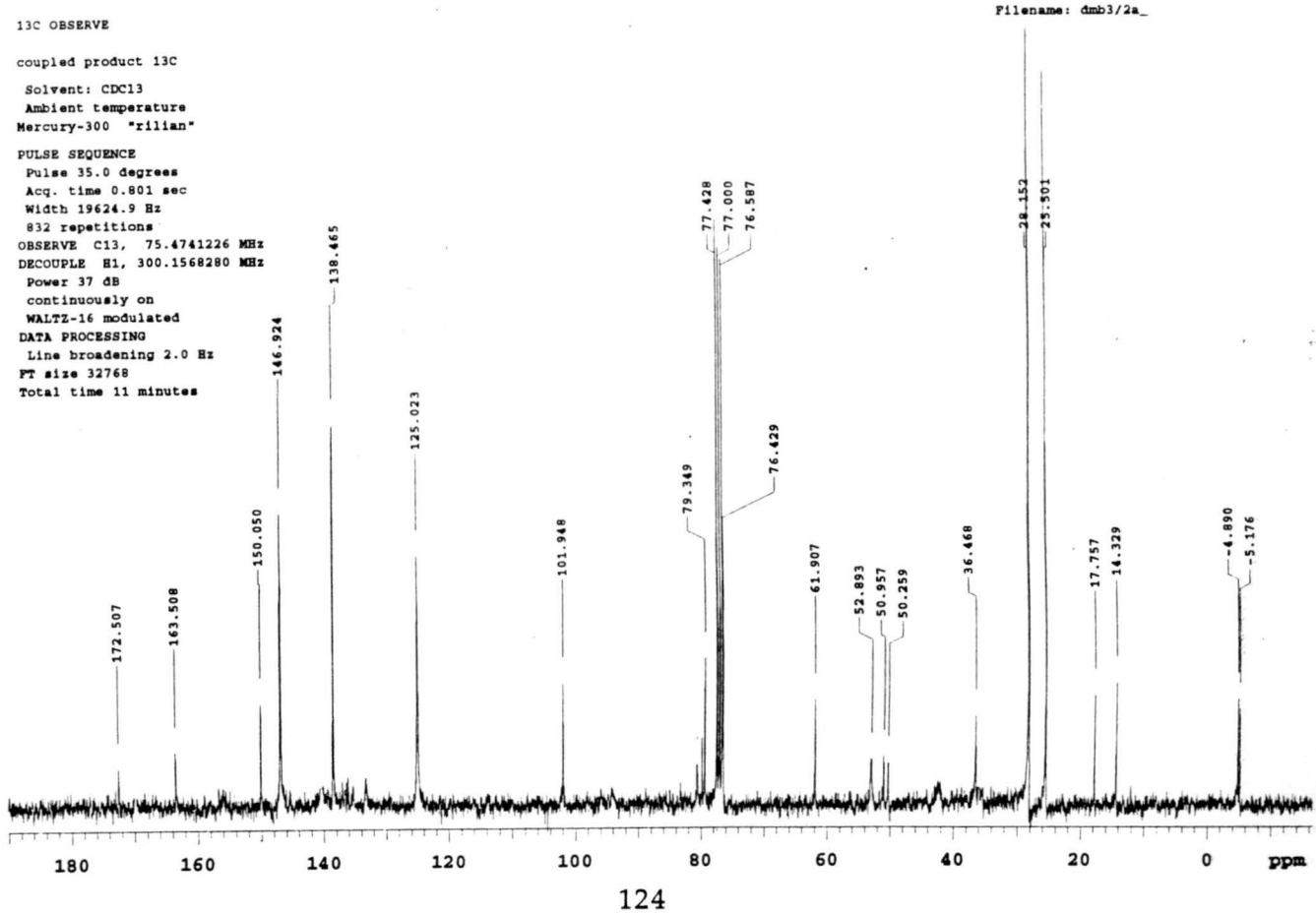
3.42
2.02
5.28
1.63
16.03
9.35
6.00


13C OBSERVE
coupled product 13C
Solvent: CDCl3
Ambient temperature
Mercury-300 "rilian"
PULSE SEQUENCE
Pulse 35.0 degrees
Acq. time 0.801 sec
Width 19624.9 Hz
832 repetitions
OBSERVE C13, 75.4741226 MHz
DECOUPLE H1, 300.1568280 MHz
Power 37 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 2.0 Hz
FT size 32768
Total time 11 minutes
Filename: dmb3/2a_
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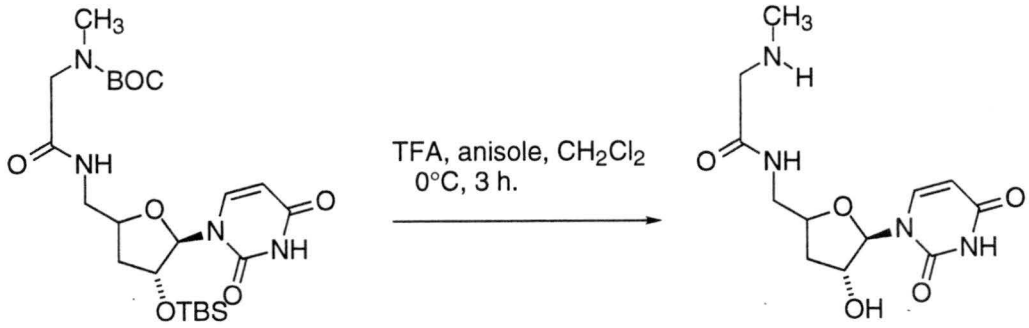
163.508
150.050
146.924
138.465
125.023
101.948
79.349
77.428
77.000
76.587
76.429
61.907
52.893
50.957
50.259
36.468
28.152
25.501
17.757
14.329
-4.890
-5.176
180
160
140
120
100
80
60
40
20
0
ppm



69

Coupled Product

The coupled peptidylnucleoside (0.10 g, 0.19 mmol) was dissolved in CH_2Cl_2 (4 mL) and cooled to 0°C while stirring under argon. Anisole (2 mL) was added followed by TFA (0.46 mL, 3.90 mmol) and the reaction was stirred at 0°C for 3 h. The solvent was removed and the residue was chromatographed (MeOH/CH_2Cl_2, 95:5, v/v) to give 0.053 g, (91%) of the desired compound.

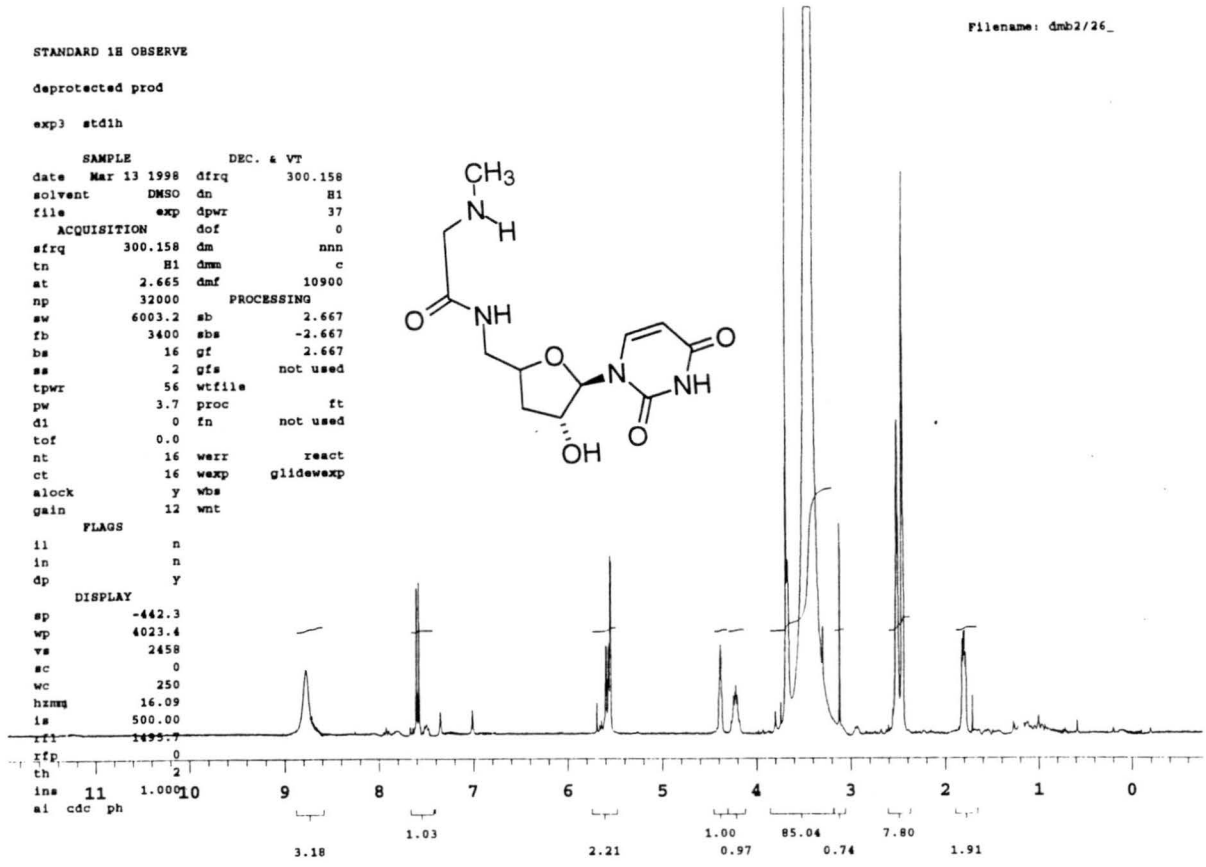
R_f=0.12 (MeOH/CH_2Cl_2, 1:9, v/v)

^{1}H NMR (300 MHz) (DMSO-d_6) 1.81 (2H, m), 2.46 (3H,s), 3.61 (2H, m), 4.21 (1H, m), 4.38 (1H, m), 5.50 (1H, s), 5.60 (1H, d, J=8.1 Hz), 7.61 (1H, d, J=8.1 Hz), 8.98 (1H, s, D_2O exchangeable).

^{13}C NMR (75.48 MHz) ($CDCl_3$) δ 17.69, 25.61, 32.72, 49.02, 54.92, 75.80, 101.54, 113.82, 140.45, 150.34, 163.13, 165.32.

IR (NaCl/CH_2Cl_2) 3418, 3093, 2987, 2856, 1689, 1642, 1451, 1383, 1334, 1272, 1254, 1119, 1089, 1021 cm^{-1}.

LRMS (FAB+) m/e (M+H) 299.6 ($C_{12}H_{18}N_4O_5$ + H requires 299.3)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gain 12
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N
NH
O
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11
10
9
8
7
6
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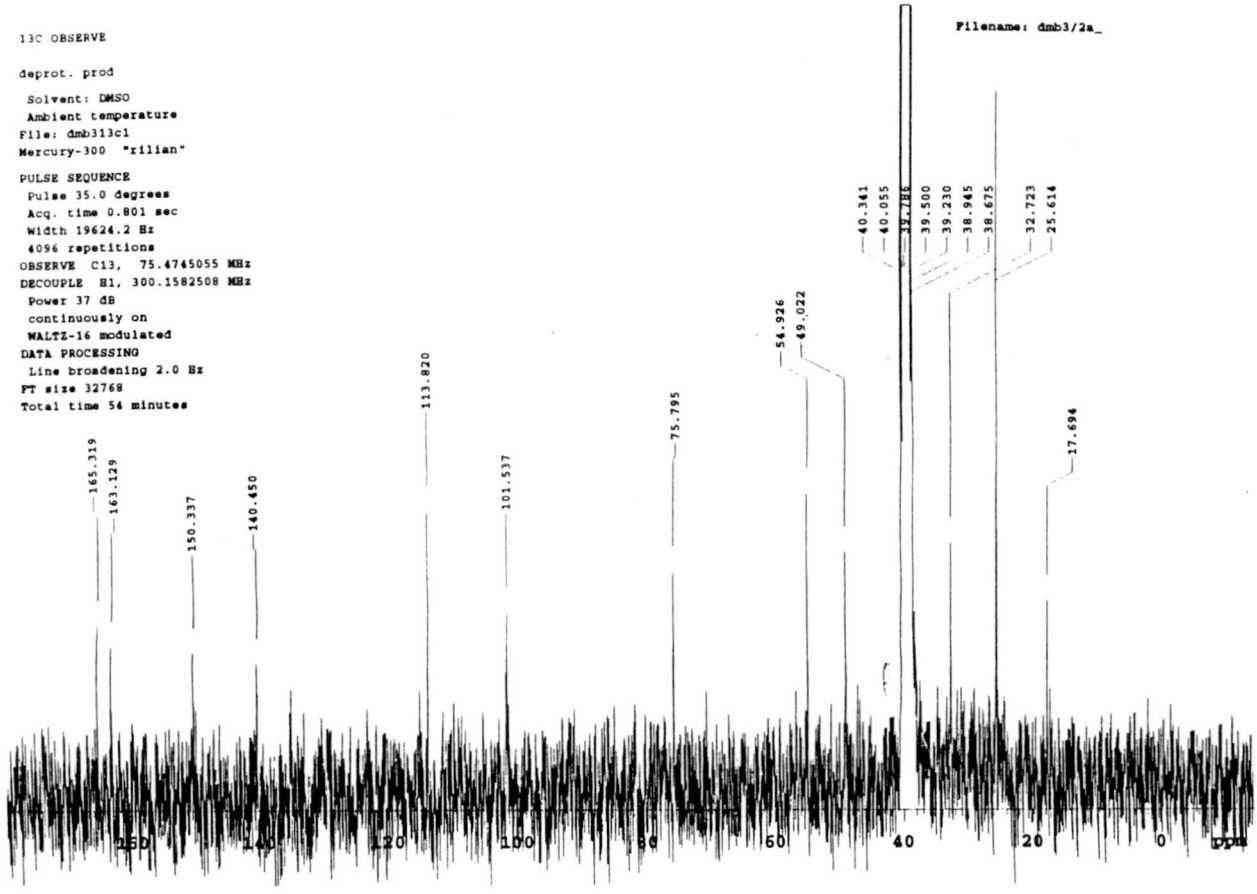
1.91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WALTZ-16 modulated
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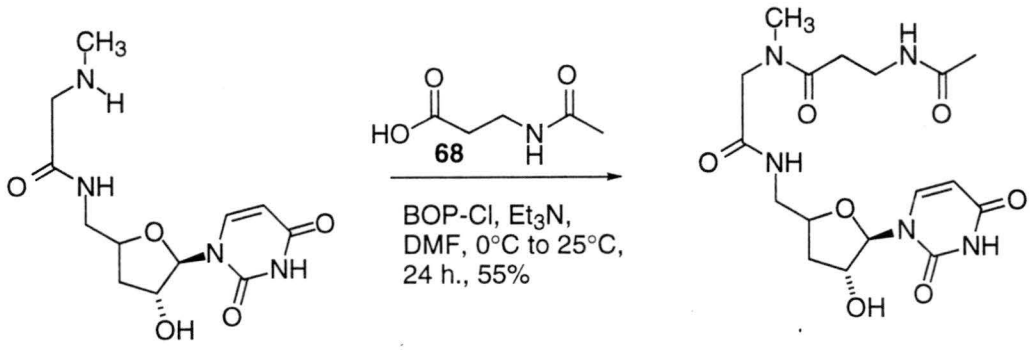


Mureidomycin Analog DMB-I-418B

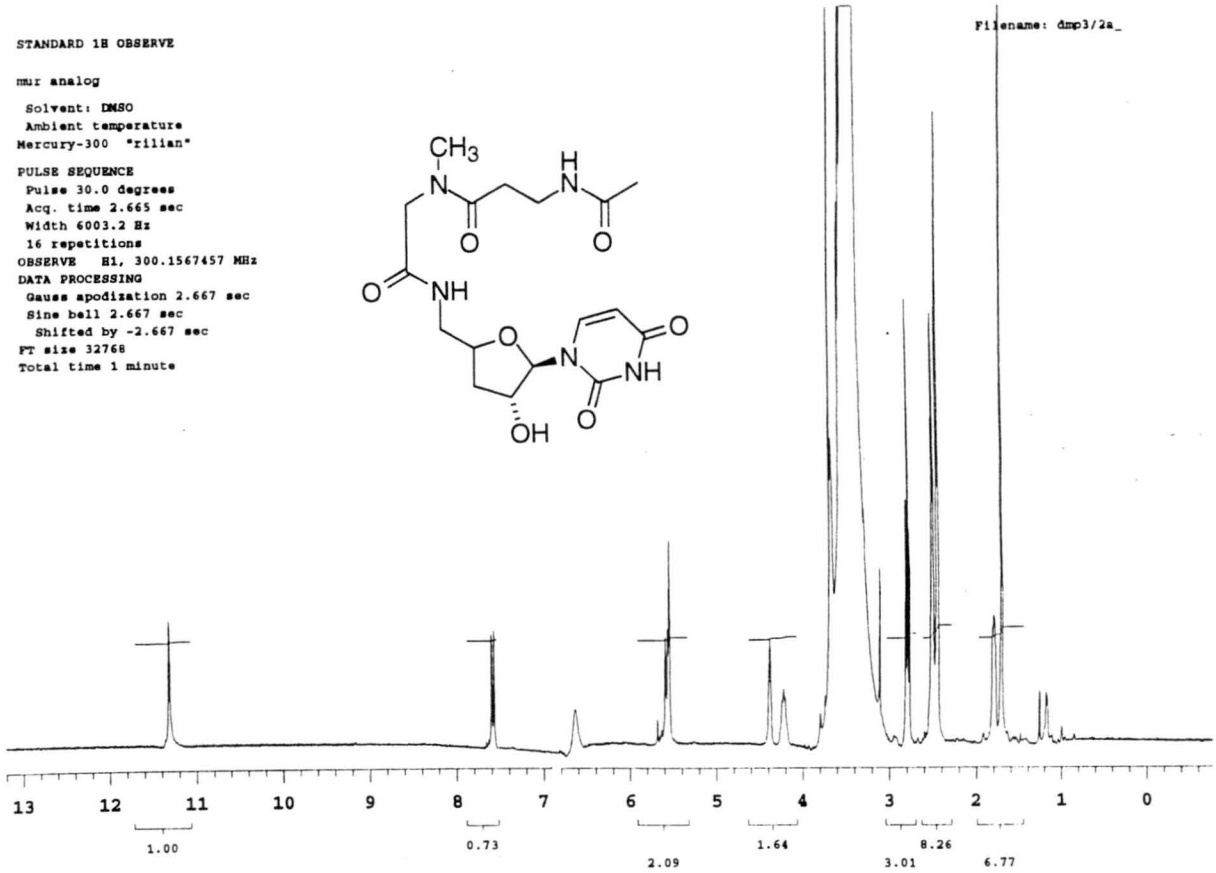
N-acetyl-beta-alanine (**68**) (0.010g, 0.074 mmol) was dissolved in DMF (1 mL) and cooled to 0°C. Triethylamine (10μL, 0.074 mmol) was added followed by BOP-Cl (0.019 g, 0.074 mmol) and the reaction was stirred at 0°C for 10 min. **70** was added and the reaction was stirred at 25°C under argon overnight. Solvent was removed *in vacuo* and the residue was chromatographed ($MeOH/CH_2Cl_2$, 1:9, v/v) to give **58** as a white solid.

^1H NMR (300 MHz) (DMSO-d_6) 1.88(3H, s), 1.91 (2H, m), 2.45 (3H,s), 2.79 (2H, m),3.65 (2H, m), 4.09 (1H, m), 4.38 (1H, m), 5.51 (1H, s), 5.58 (1H, d, J=8.1 Hz), 6.60 (1H, s), 7.61 (1H, d, J=8.1 Hz), 11.24 (1H, s, D_2O exchangeable).

^{13}C NMR (75.48 MHz) ($CDCl_3$) δ 17.70, 22.52, 25.64, 28.44, 32.75, 49.05, 54.93, 75.82, 92.02, 101.56, 113.84, 140.48, 150.35, 153.20, 165.34, 171.52, 171.65.

IR ($NaCl/CH_2Cl_2$) 3418, 3093, 2987, 2856, 1689, 1642, 1451, 1383, 1334, 1272, 1254, 1119, 1089, 1021 cm^{-1}.

LRMS (FAB+) m/e (M+H) 412.4 ($C_{17}H_{25}N_5O_7$ + H requires 412.2)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Shifted by -2.667 sec
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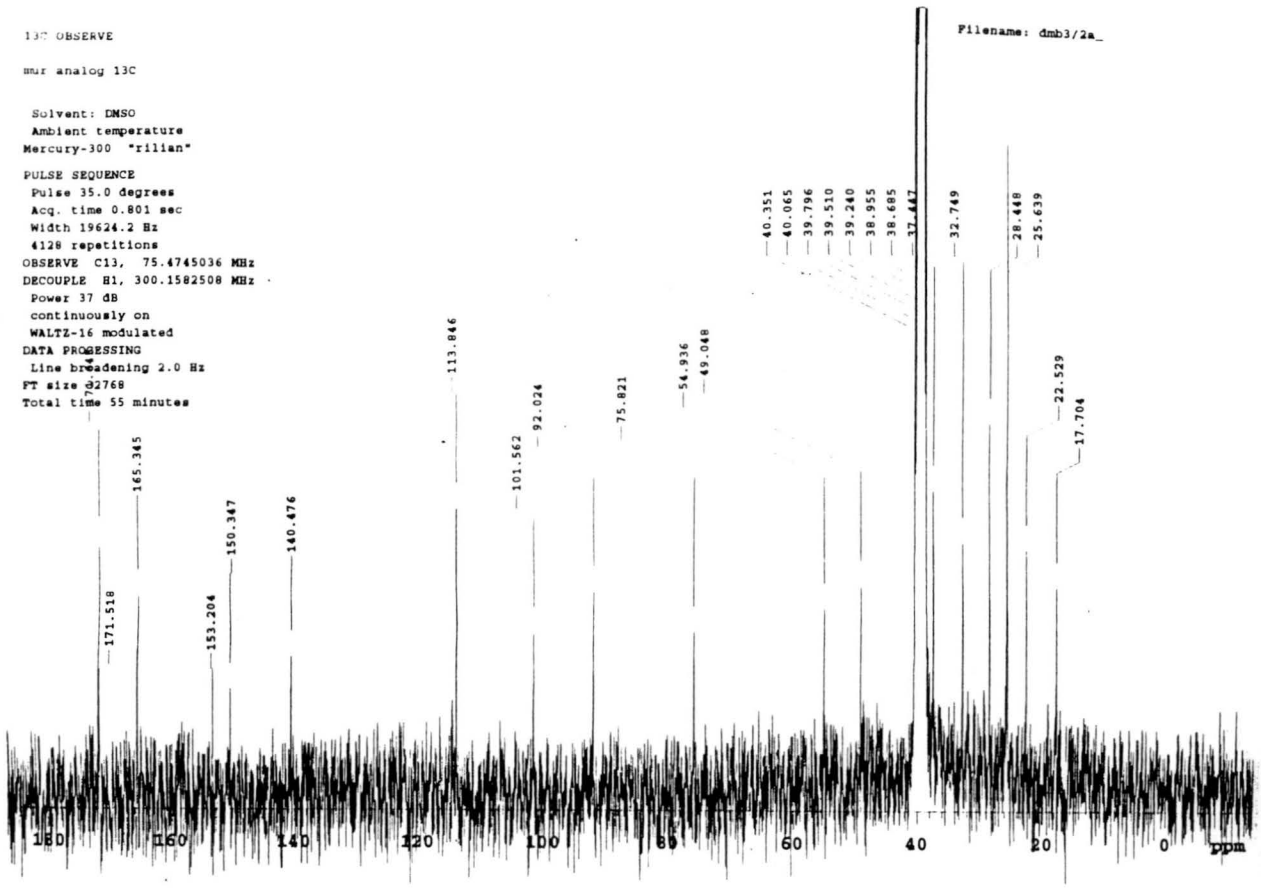
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Mercury-300 "rilian"
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OBSERVE C13, 75.4745036 MHz
DECOUPLE H1, 300.1582508 MHz
Power 37 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 2.0 Hz
FT size 32768
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39.796
39.510
39.240
38.955
38.685
32.749
28.448
25.639
22.529
17.704
180 160 140 120 100 80 60 40 20 0 ppm

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Appendix A

Publications

An Efficient Synthesis of (*S*)-*m*-Tyrosine

David M. Bender and Robert M. Williams

Department of Chemistry, Colorado State University,
Fort Collins, Colorado 80523

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An Efficient Synthesis of (*S*)-*m*-Tyrosine

David M. Bender and Robert M. Williams*

Department of Chemistry, Colorado State University Fort Collins, Colorado 80523

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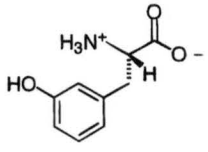
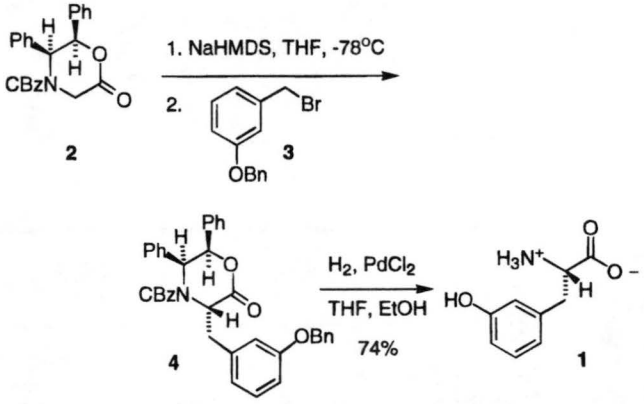
The amino acid (*S*)-*m*-tyrosine[1] (**1**) has found wide use in the area of medicinal chemistry since its discovery. This amino acid has been utilized extensively in the study of the metabolic pathways of the central nervous system.[2] The biological effects of this molecule have been shown to be identical to that of L-Dopa (3,4-dihydroxyphenylalanine), which has been used in the treatment of Parkinson's disease.[3] More recently, this unnatural amino acid has been found in a new class of peptidylnucleoside antibiotics, the mureidomycins[4] and the pacidamycins.[5] In addition, (*S*)-*m*-tyrosine has been used in the synthesis of several aminodiol HIV protease inhibitors.[6] Despite the simplicity of this amino acid, there exist very few methods reported in the literature[7,8] for its synthesis in optically pure form. The method most frequently used to obtain this amino acid appears to be resolution of *d,l*-*m*-tyrosine.[1a,c] We report here a very simple and convenient procedure that can be utilized to unambiguously prepare either (*S*)- or (*R*)-*m*-tyrosine in high optical purity.



1, *meta*-Tyrosine

Optically active (>98% ee) oxazinone **2**[9,10] was condensed with *m*-(benzyloxy)benzyl bromide (**3**) via formation of the sodium enolate (NaHMDS, THF, HMPA, −78 °C). The alkylation product (**4**) was obtained in 87% yield with a diastereomeric excess of >95%. This substance was conveniently converted into *m*-tyrosine (**1**) by catalytic hydrogenation (74% overall from **4**) (Scheme 1). Mosher amide analysis[11] of this material by NMR and GC revealed that the product was obtained in an enantiomeric excess of >96%.

Scheme 1



The current methodology provides a mild and efficient means to prepare *m*-tyrosine in optically active form of high enantiomeric purity. Since both antipodes of **2** are commercially available,[9] this procedure permits the stereochemically unambiguous synthesis of either (*R*)- or (*S*)-*m*-tyrosine in a rapid and convenient manner.

Experimental Section[12]

Preparation of *m*-(Benzyloxy)benzyl Bromide. Commercially available 3-benzyloxy benzyl alcohol (Aldrich) (5.0 g, 23.4 mmol) was converted to the benzyl bromide derivative **3** by reaction with Ph_3P (6.74 g, 25.7 mmol) and CBr_4 (8.50 g, 25.7 mmol) in THF (100 mL) at 25 °C for 1 h. Solid material was removed by filtration, and the crude product was purified by flash chromatography (hexanes) to yield **3** (5.89 g, 91%) as a white solid (recryst hexanes), mp 37−39 °C (dec) ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.39 (2H, s), 4.98 (2H, s), 6.83−6.95 (3H, m), 7.17−7.39 (6H, m). ^{13}C NMR (300 MHz, $CDCl_3$): δ 33.6, 70.2, 115.1, 115.6, 121.7, 127.7, 128.2, 128.8, 130.0, 136.9, 139.4, 159.1. IR ($NaCl/CH_2Cl_2$): 3013, 2985 cm^{-1} HRMS (ES^+) calcd for $C_{13}H_{13}OBr$ 276.0150, found 276.0145.

(3S,5S,6R)-4-[(Benzyloxy)carbonyl)]-5,6-diphenyl-3-{[3′-(benzyloxy)phenyl]methyl}-2,3,5,6-tetrahydro-4*H*-1,4-oxazin-2-one (4). NaHMDS (12.3 mL, 12.3 mmol, 1 M solution in THF) was added dropwise to a solution of oxazinone **2**[9] (3.17 g, 8.20 mmol) (Aldrich) and *m*-(benzyloxy)benzyl bromide (**3**) (2.50 g, 9.02 mmol) in THF (160 mL) and HMPA (16 mL) at −78 °C. After 3 h, the reaction mixture was poured into ethyl acetate and extracted with brine and H_2O. The organic extracts were dried ($MgSO_4$) and concentrated to a yellow oil which was purified by flash chromatography (CH_2Cl_2/MeOH, 99:1) to give **4** (4.15 g, 87%) as a white solid (recryst CH_2Cl_2/hexanes), mp 146−148 °C (dec). ^{1}H NMR (300 MHz, DMSO-d_6, 393 K): δ 3.37 (1H, dd, J = 13.8, 3.9 Hz), 3.49 (1H, dd, J = 13.5, 8.1 Hz), 5.04 (2H, s), 5.09 (2H, s), 5.14 (2H, s), 5.47 (1H, s (br)), 6.59 (2H, d, J = 7.5 Hz), 6.83−7.42 (22H, m). ^{13}C NMR (300 MHz, DMSO-d_6): δ 39.3, 59.9, 67.7, 69.9, 78.4, 114.8, 122.7−138.6 (unresolved), 154.6, 159.6, 168.6. IR (KBr): 1698, 1750, 2950, 3030 cm^{-1} $[a]^{25}_D$ = +52.45° (*c* 2.0, $CHCl_3$). Anal. Calcd for $C_{38}H_{33}NO_5$: C, 78.19; H, 5.69; N, 2.39. Found: C, 78.18; H, 5.52; N, 2.19.

Synthesis of (*S*)-*m*-Tyrosine Hydrochloride. To a solution of compound **4** (0.5 g, 0.857 mmol) in ethanol (5 mL) and

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(9) Both antipodes of **3** are commercially available from Aldrich Chemical Co.: **3**, catalog #33,187-2; antipode of **3**, catalog #33-185-6.

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THF (5 mL) was added $PdCl_2$ (0.045 g, 0.254 mmol). The reaction mixture was hydrogenated at 50 psi for 18 h. The mixture was purged with nitrogen and filtered through Celite to remove the catalyst. Removal of the solvents *in vacuo*, followed by trituration with Et_2O, produced 0.154 g (99%) of *m*-tyrosine (**1**). This compound was dissolved in 1 N HCl and concentrated, followed by trituration with Et_2O, to give (*S*)-*m*-tyrosine hydrochloride. $[a]^{25}_D$ −7.4° (*c* 2.0, 1 N HCl) (lit.[1b] $[a]^{25}_D$ (*S*)-*m*-tyrosine hydrochloride −7.9° (*c* 2.0, 1 N HCl)) . ^1H NMR (300 MHz, D_2O vs HOD): δ 3.11 (1H, dd, J = 14.7, 7.5 Hz), 3.24 (1H, dd, J = 14.4, 5.4 Hz), 4.29 (1H, dd, J = 7.5, 5.7 Hz), 6.71−6.85 (3H, m), 7.26 (1H, t).

Determination of Optical Purity. Oxalyl chloride (48.0 mL, 0.550 mmol) was added dropwise to a solution of the amino acid **1** in ethanol (1 mL) at 0 °C, followed by refluxing for 2 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. The crude amino ester hydrochloride salt was combined with (+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (24.6 mL, 0.132 mmol) and propylene oxide (40 mL, 0.571 mmol) in THF (1 mL) and heated at 50 °C for 2 h. Optical purity was measured by examination of the ^1H NMR spectrum of the resulting Mosher amide and glc analysis (Alltech AT-1, nonpolar polymethylsiloxane) (>96% ee).

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