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BOUVARDIN ANALOGS

The University of Arizona M.S. 1986

BOUVARDIN ANALOGS

by

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A Thesis Submitted to the Faculty of the

DEPARTMENT OF CHEMISTRY

In Partial Fulfillment of the Requirements
For the Degree of

MASTER OF SCIENCE

In the Graduate College

THE UNIVERSITY OF ARIZONA

1 9 8 6

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ACKNOWLEDGMENTS

I wish to thank Dr. Robert B. Bates for his guidance and patience. I also want to acknowledge Vladmir Bowlin and Maria Bishop for their help.

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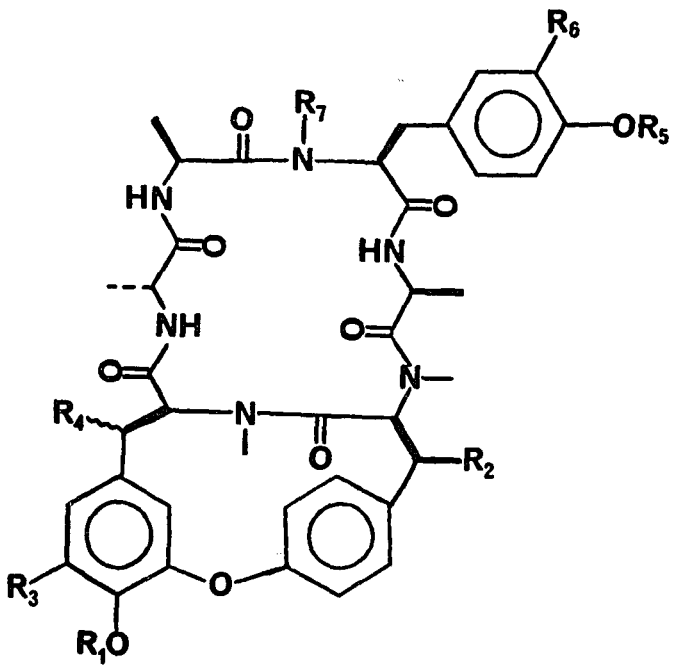
ABSTRACT

The three parts of this thesis relate to the preparation of bouvardin analogs which may be useful in discovering the mechanism of the antitumor action of the bouvardin group. Iododeoxybouvardin was prepared for use in studying the binding parameters of deoxybouvardin to the eukaryotic ribosome. A tetrapeptide which may be used in the synthesis of 3-N-desmethylbouvardin was prepared for use in finding out which configuration of bouvardin is active. Finally, the synthesis of a dipeptide with a 14-membered ring useful in making deoxybouvardin was continued but not completed.

INTRODUCTION

Bouvardin (1) and two of its derivatives (Fig. 1), deoxybouvardin (2) and 6-O-methylbouvardin[1] (3), were isolated from the methanol extract of Bouvardia ternifolia (Rubiaceae) and found to be the substances responsible for the plant's antitumor activity.[2] Two other active derivatives, 4 and 5 (Fig. 1), were later isolated from plants of the same family.[3,4] These compounds show high activity toward P388 lymphocytic leukemia and marginal activity toward B16 melanoma.[5] The activity is caused by bouvardin's ability to inhibit protein synthesis without significantly inhibiting DNA and RNA biosynthesis.[6] Studies aimed at defining the mechanism of this inhibition may lead to a better understanding of the nature of protein synthesis in mammalian cells.[7] Thus, the preparation of bouvardin analogs should be useful for biological probes as well as helpful in cancer research.

Determining the structure of bouvardin (1) and its analogs involved several techniques. The structure of bouvardin itself was resolved using x-ray and hydrolytic studies.[2] The structures of 2 and 3 were then determined by comparing their NMR spectra with the NMR of bouvardin.



	R_1	R_2	R_3	R_4	R_5	R_6	R_7
1	H	OH	H	H	Me	H	Me
2	H	H	H	H	Me	H	Me
3	Me	OH	H	H	Me	H	Me
4	Me	H	H	H	Me	H	Me
5	Me	H	H	OH	Me	H	Me
6	H	OH	H	H	H	H	Me
7	H	OH	H	H	H	OH	Me
8	H	H	I	H	Me	H	Me
9	H	OH	I	H	Me	H	Me
1Ø	Me	H	H	H	Me	H	H

Fig. 1. Bouvardin Analogs

The actual NMR assignments were not finished until five years after the x-ray study because of the complexity of their spectra.[1]

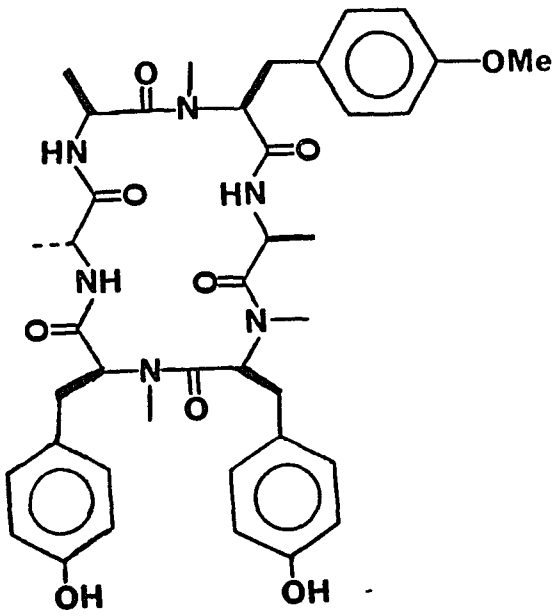
This complexity is partially because when bouvardin is in chloroform solution at room temperature, two slowly interconverting isomers exist; this was discovered while interpreting HPLC[8] and NMR[1] data. The minor stereoisomer (~15%) was found to be separated from the major stereoisomer by an energy barrier of 20.6 kcal/mol. This value was obtained from data collected using low temperature NMR. It is believed that this isomer is due to a rotation about the trans-tyr-3 and/or the trans-tyr-5 bond of the N-methyltyrosines. The major form was shown to have the same configurations, all amides trans except for the one in the 14-membered ring, as the crystalline form. It is not known which of these two is the active form of bouvardin.

Several analogs of bouvardin (1) have been prepared which give some general indications about the part of the bouvardin molecule responsible for its activity. One study involved the conversion of bouvardin (1) to O-desmethylbouvardin (6) and bouvardin catechol (7; Fig. 1) using microbiological modification.[9] These two compounds were not active which indicates that the top portion of bouvardin (1) contains the active site. Another derivative, which was prepared synthetically, is

cyclo-N-methyl-L-tyr-N-methyl-L-tyr-D-ala-L-ala-O,N-dimethyl-L-tyr-L-ala (11; Fig. 2).[10] This compound differs from deoxybouvardin (2) by only two hydrogen atoms, yet it shows no antitumor activity. This is probably because the 14-membered ring in 2 changes the conformation of the top portion to give it activity. Analog 11 was originally made in hopes of converting it to deoxybouvardin (2); however, all attempts to oxidize 11 to 2 failed.[11]

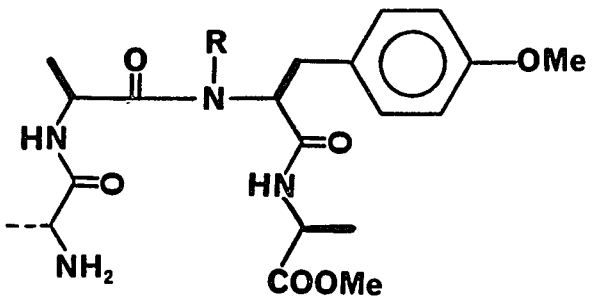
Progress toward the preparation of three analogs of bouvardin (1) is the topic of this thesis. One analog, which when prepared in radioactive form should be a useful biological probe, is iododeoxybouvardin (8; Fig. 1). This analog, if active as an antitumor agent, would be useful in studying the binding parameters of deoxybouvardin to the eukaryotic ribosome.[12] An attempt to make iodobouvardin (9) from bouvardin (1) failed, probably because of the sensitive secondary alcoholic group in 1.[13] This problem was avoided by using deoxybouvardin (2).

Another analog, 10 (Fig. 1), which may be useful in determining the active form of bouvardin (1), differs from 4 by the replacement of the tyr-3-N-methyl group by a hydrogen atom. This involved preparing tetrapeptide 12, similar to 13 (Fig. 3), which was prepared for the synthesis of 11.[10] The synthesis of a dipeptide with a 14-membered ring such as 16 (Fig. 4) needed to complete a



11

Fig. 2. Deoxybouvardin Analog



	R
12	H
13	Me

Boc-L-tyr → 13

1) NaH, MeI
2) L-ala Me ester, DCC
3) F_3CCOOH
4) Boc-L-ala, DCC
5) F_3CCOOH
6) Boc-D-ala, DCC
7) F_3CCOOH

Fig. 3. Preparation of 13

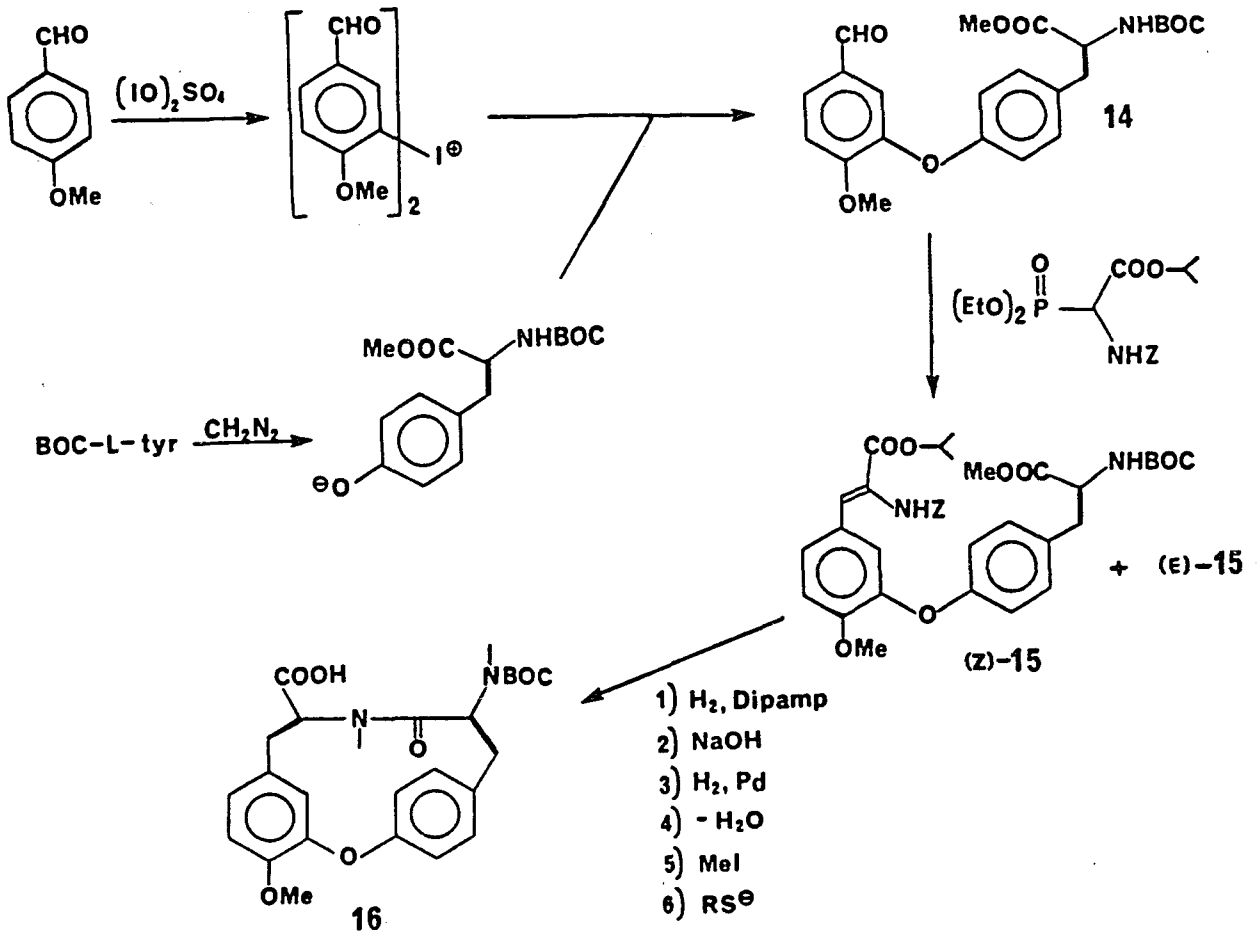


Fig. 4. Synthetic Route to 16

synthesis of 10 has been under way for several years (Fig. 4). 14[14] and 15[15] have been prepared and the synthesis of 16 is in progress. Once it is prepared, 12 can be joined to 16 to make 10 in the same manner as 13 was joined to 17 (Fig. 5) to make 11.

The synthetic preparation of a third analog, 4, would be useful because it is costly to isolate from the plant for study. 4 can be synthesized in the same manner as 10 using 13 as the tetrapeptide (Fig. 5).

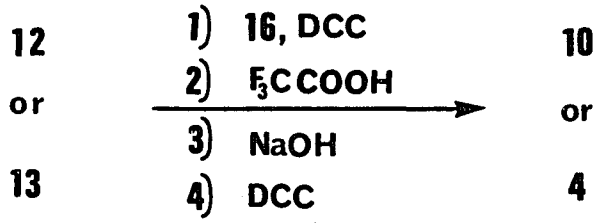


Fig. 5. Preparation of 10 and 4

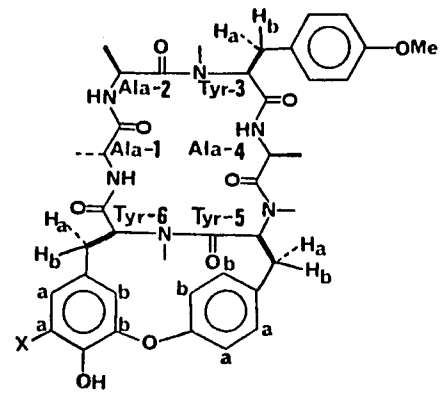
RESULTS AND DISCUSSION

Iododeoxybouvardin

Deoxybouvardin (2) was iodinated in a similar manner as tyrosine,[14,16] giving iododeoxybouvardin (8) in 88% yield. If more than an equimolar amount of iodine was added to the reaction, a mixture of products which were not characterized was obtained.

Iododeoxybouvardin (8) was characterized by comparing its ^1H NMR spectrum to that of deoxybouvardin (2) (Table 1). The most obvious difference comes from adding the iodine ortho to the phenolic group which eliminates the ^1H NMR adsorption from the tyr-6ϵ_a proton. Elimination of this proton also changes the proton adsorption of tyr-6δ_a from a doublet of doublets to a doublet; the iodine shifts its adsorption downfield from δ6.51 to 7.02. The tyr-6OH is also shifted downfield from δ5.58 to 6.05. The three NH's (ala-4NH, ala-2NH, and ala-1NH) are all shifted upfield which is due to a change in the concentration of the NMR sample. Finally, the tyr-6δ_b proton is shielded even more than in 2, shifting upfield from δ4.35 to 4.27.

Table 1. ^1H NMR ($CDCl_3$) Data for 2 and 8



	X
2	H
8	I

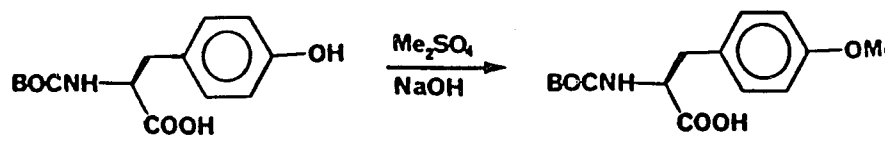
^1H	2[1,a]	8[b]
Ala-4β	1.09 d (6.7)	1.11 d (7.5)
Ala-1β	1.31 d (7.3)	1.30 d (6.8)
Ala-2β	1.36 d (7.3)	1.36 d (7.2)
Tyr-5β_a	2.62 dd (11.4, 2.4)	2.64 dd (11.3, 2.9)
Tyr-6N-Me	2.70 s	2.68 s
Tyr-3N-Me	2.87 s	2.86 s
Tyr-6β_a	2.91 dd (20.2, 4.6)	2.92 dd (19.0, 11.7)
Tyr-6β_b	3.00 dd (20.2, 12.4)	3.02 dd (19.0, 4.4)
Tyr-5N-Me	3.11 s	3.12 s
Tyr-3β	3.33 m	3.36 m
Tyr-3α	3.61 dd (9.8, 6.1)	3.58 dd (10.1, 5.7)
Tyr-5β_b	3.65 dd (11.4, 8.6)	3.70 t (11.3)
Tyr-3O-Me	3.80 s	3.80 s
Tyr-6δ_b	4.35 bs	4.27 d (1.9)
Ala-1α	4.42 ~p (7)	4.35 p (6.8)
Tyr-6α	4.56 dd (12.4, 4.6)	4.54 dd (11.7, 4.4)
Ala-4α	4.79 ~p (7)	4.75 p (7.2)
Ala-2α	4.81 ~p (7)	4.84 p (7.5)
Tyr-5α	5.42 dd (11.4[c], 2.4)	5.39 dd (11.3, 2.9)
Ala-1NH	6.48 d (7)	6.18 d (7.5)
Tyr-6δ_a	6.51 dd (8.5, 1.8)	7.02 d (1.9)
Ala-2NH	6.52 d (7.3)	6.42 d (6.8)
tyr-6OH	5.58 s[c]	6.05 s
Ala-4NH	6.76 d (7.9)	6.68 d (7.2)
Tyr-6ϵ_a	6.81 d (8.5)	
Tyr-3ϵ	6.83 ~d (8.5)	6.83 d (8.6)
Tyr-5ϵ_b	6.83 dd (8.5, 2.4)	6.84 dd (8.4, 2.4)
Tyr-3δ	7.05 ~d (8.5)	7.05 d (8.6)
Tyr-5ϵ_a	7.20 dd (8.5, 2.4)	7.21 dd (8.4, 2.4)
Tyr-5δ_b	7.27 dd (8.5, 1.8	7.28 dd (8.5, 2.1)
Tyr-5δ_a	7.43 dd (8.5, 1.8)	7.42 dd (8.5, 2.1)

[a] Corrected from values in reference by correspondance with G. S. Linz
[b] Rotamer peaks appear at δ3.78, 7.10
[c] Corrected from values in reference 1

Tetrapeptide

O-Methyl-N-Boc-L-tyr (18) was synthesized by preparing the disodium salt of Boc-L-tyr and reacting it with dimethyl sulfate (Fig. 6).[17] This method gave a much better yield than adding the dimethyl sulfate to the water solution used to prepare the salt.[18] 18 was used in the preparation of tetrapeptide 25 (Fig. 7). When 16 is prepared 12 will be prepared by removing the Boc group from 25 and the two will be combined (Fig. 5) to prepare 1Ø.

1Ø may be a useful tool in determining the active form of deoxybouvardin (2). If this compound is active it can be assumed that trans-tyr-3 is the active rotamer. Also if the NMR only shows one form it would prove that the major and minor rotomers arise from a rotation about the tyr-3 amide bond. These assumptions can be made because the trans form of 1Ø is much more stable than the cis form and would be present virtually all of the time. If the NMR still shows the presence of two rotamers, then the rotamers arise from rotation about the tyr-5 amide bond. No assumptions can be made if 1Ø is not active because the tyr-3-N-methyl group may be necessary for activity.



18

Fig. 6. Preparation of 18

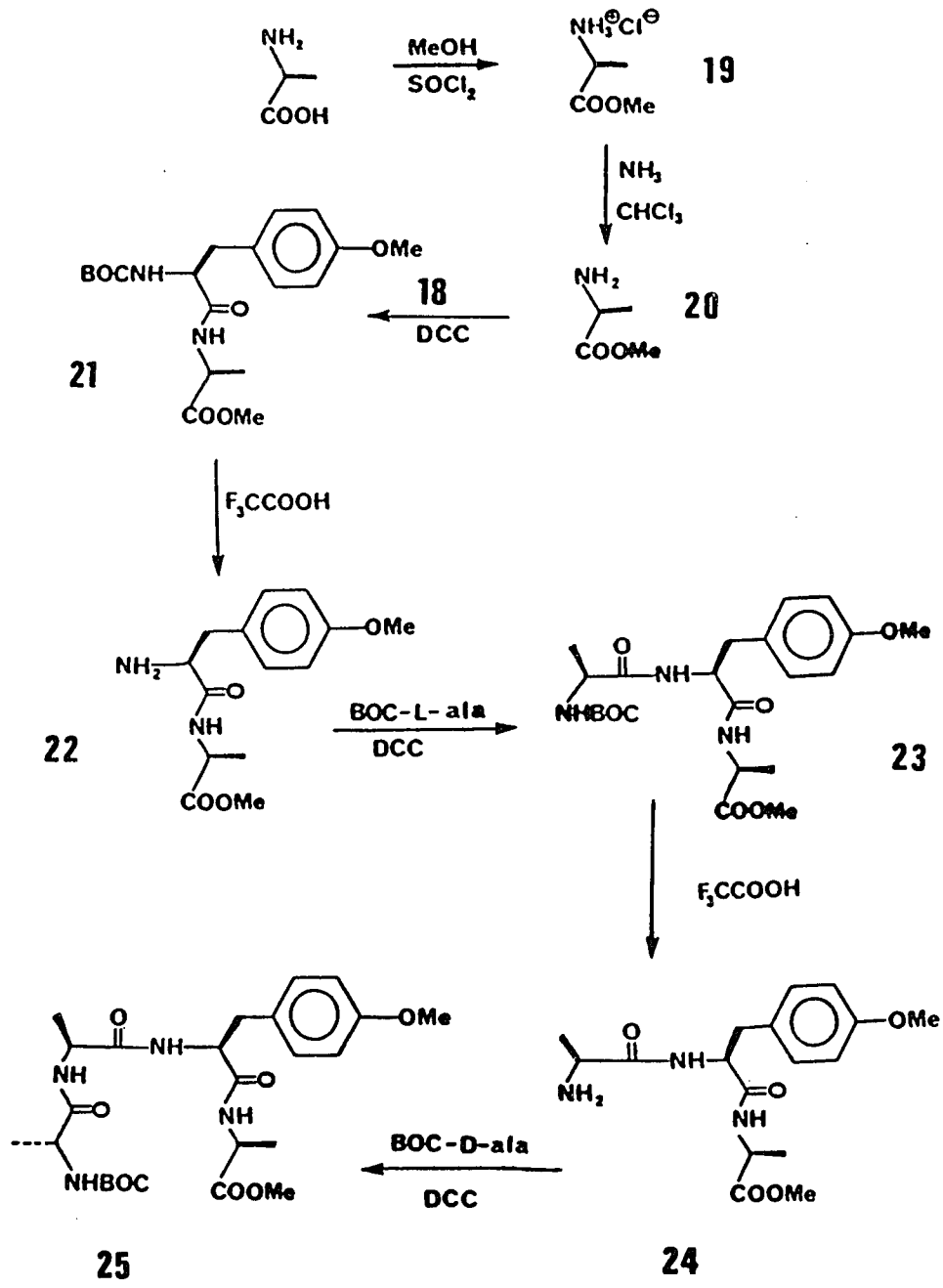


Fig. 7. Preparation of 25

14-Membered Ring

A mixture of 30% **15** and 70% **27**[19] (Fig. 8) was purified by analytical HPLC using a 10 1 silica column and chloroform for use in the preparation of **16**. The second fraction was identified as **27** by ^{1}H NMR ($CDCl_3$): δ1.12d (6.2), 1.21d (6.2), 1.42s, 3.00m, 4.53m, 4.53m, 4.94m, 5.08s, 5.23d (8.1), 6.74s, 6.82d (8.6), 6.88s, 7.01d (8.5), 7.34s; (Fig. 9). Not much of this compound was availible (3 mg) because of the long purification process so another hydrogenation was carried out (Fig. 8). More catalyst (**26**) and a higher temperature were used in hopes of obtaining a higher yield so that purification would not be so difficult. This hydrogenation not only reduced the double bond, but some of the Z protecting group was cleaved as well (**28**), giving an even greater mixture of products (Fig. 8).

The next step in preparing **16** involved the cleavage of the methyl ester to give **29** or **30** depending on which starting material was used (Fig. 10). One way of cleaving methyl esters is by using strong solutions of sodium hydroxide. The problem with this method is that the presence of a strong base could cause racemization at the two chiral centers. A study using a model compound (Boc-L-tyr methyl ester) was carried out to determine the extent of racemization. The methyl ester was cleaved using sodium hydroxide and then replaced with diazomethane.

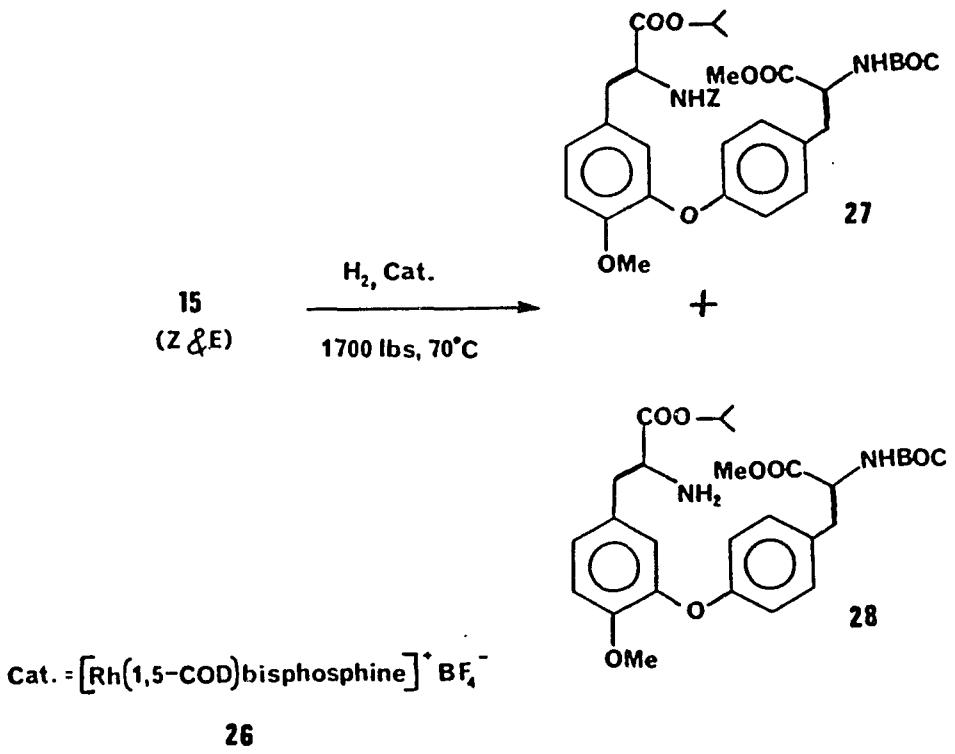


Fig. 8. Hydrogenation of 15

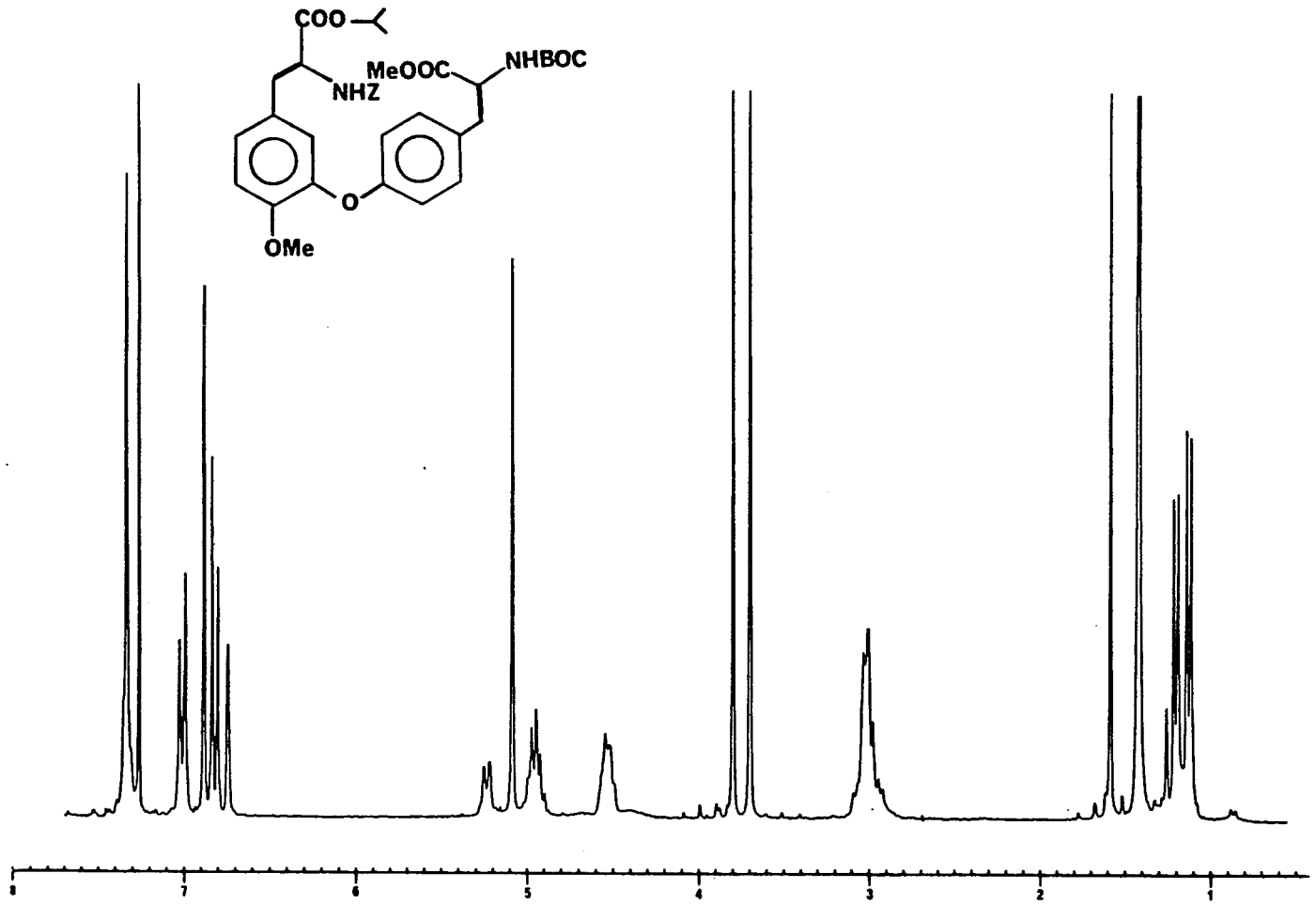


Fig. 9. ^{1}H NMR Spectrum ($CDCl_3$) of 27

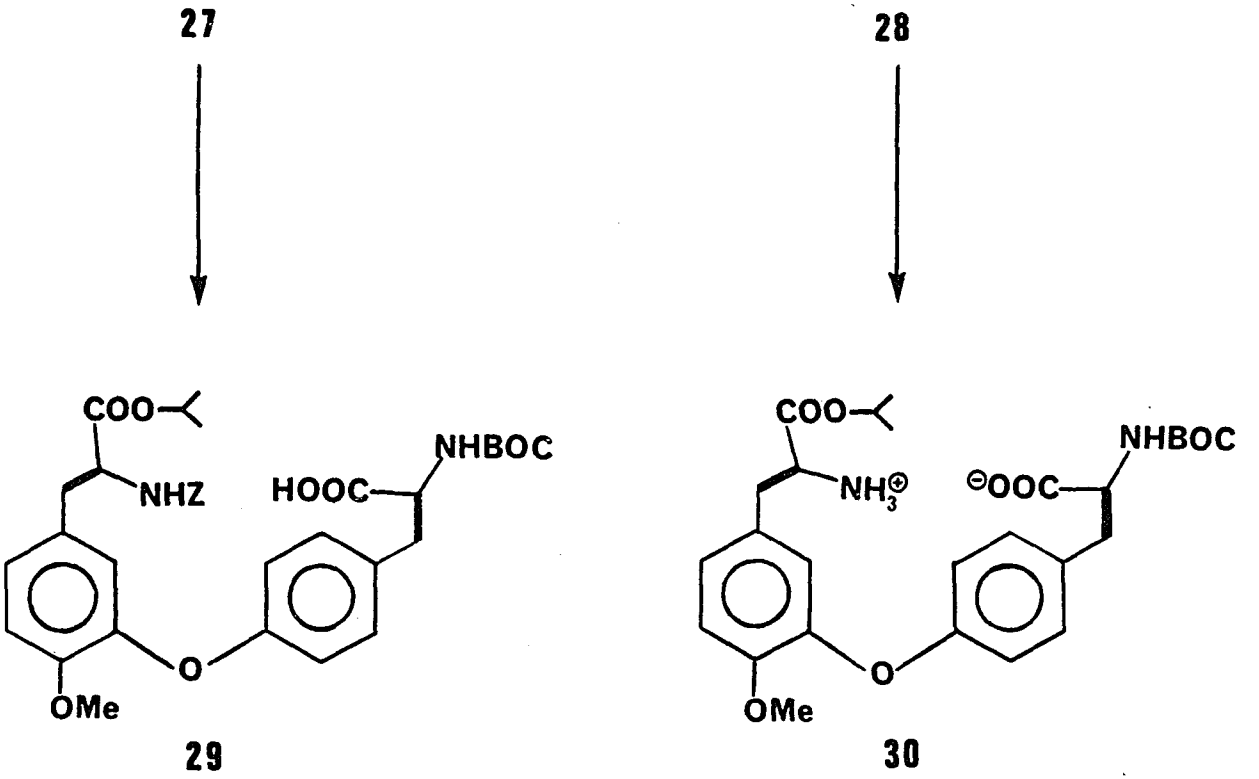


Fig. 1Ø. Preparation of 29 and 3Ø

The optical rotation of the original Boc-L-tyr methyl ester (9.0°) was compared to the rotation of the freshly prepared Boc-L-tyr methyl ester (6.1°); 32% racemization had occurred.

Since significant racemization had occurred, other methods of cleaving the methyl ester of 27 and 28 were attempted. The first method invloved using an esterase enzyme. This enzyme cleaved the methyl ester of Boc-L-tyr methyl ester (31) but not of 27 or 28; many conditions were attempted but cleavage never occurred (Table 2) because of solubility problems. Another method that was tried involved using dilute, equimolar sodium hydroxide. This reaction did not work either; perhaps the solution was too dilute.

At this point more concentrated sodium hydroxide was used although partial racemization would occur. This reaction worked but because the starting material 27 contained 15 a mixture of products was produced. Purification of this reaction mixture failed.

Other methods of preparing 16 which do not involve the cleavage of the methyl ester include heating 28 in DMF or converting 28 into a reactive aluminum reagent[20,21] to form the peptide bond (Fig. 11). 28 was prepared in good yield from 27 using hydrogen and palladium. These reactions are still being conducted.

Table 2. Enzyme Reactions

Rxn	Solv	mL	pH of Buffer	mL	E[a] Type	E µL	Cpd	Cpd mg	time h
1	EtOAc	Ø.25			1	8	31	45.Ø	Ø.Ø8
2	EtOAc	Ø.Ø1			1	1Ø	31	5.Ø	21
3					1	1Ø	31	41.Ø	19
4[b]					1	43	B[c]	Ø.Ø1mL	18
5[b]	EtOH	Ø.2	8.Ø	1.Ø	1	1Ø	31	4.2	22
6	EtOH	Ø.Ø5	8.Ø	Ø.25	1	5	27	1.4	23
7	EtOH	Ø.2	8.Ø	1.Ø	1	1Ø	27	1.4	24
8	EtOH	Ø.4	8.Ø	2.Ø	1	2Ø	27	6.6	26
9	EtOH	Ø.4	8.Ø	2.Ø	1	7Ø	27	6.6	24
1Ø	EtOH	Ø.2	8.Ø	1.Ø	1	7Ø	27	6.3	21
11[d]	EtOH	2.Ø	8.Ø	2.Ø	1	3Ø	27	6.6	25
12	EtOH	1.4	8.Ø	7.Ø	1	7Ø	27	6.3	28
13	EtOH	Ø.2	8.Ø	1.5	1	2Ø	28	3.Ø	24
14[e]	EtOH	2.Ø	8.Ø	2.Ø	1	3Ø	27	6.3	25
15	DMSO	3.Ø	8.Ø	3.Ø	1	5Ø	27	1Ø.Ø	24
16	$CDCl_3$	Ø.5	8.Ø	Ø.2	1	5Ø	27	1Ø.Ø	24
17	EtOH	Ø.4	6.Ø	3.Ø	2	6Ø	28	3.Ø	48
18			6.Ø	Ø.2	2	5Ø	28	1.Ø	48
19[f]	EtOH	Ø.2	8.Ø	1.Ø	2	5Ø	27	3.1	22
2Ø[f]	D[g]	Ø.2	8.7	Ø.2	1	2ØØ	27	2.8	21
21[h]	E[i]	Ø.5	8.7	1.Ø	1	17	27	2.8	28
22[j]	E[i]	Ø.3	8.7	1.Ø	2	17	27	2.8	24

[a] Esterase from porcine liver; Type 1 and Type 2
[b] Reaction worked
[c] Butyric acid
[d] pH of reaction was 6
[e] used NH_4OH to adjust pH to 8
[f] used sonic bath for 4 h
[g] Dioxane
[h] An additional Ø.2 mL of ethyl ether was added gradually Ø.1 mL/h
[i] Ethyl ether
[j] 1Ø µL of Triton X 1ØØ detergent

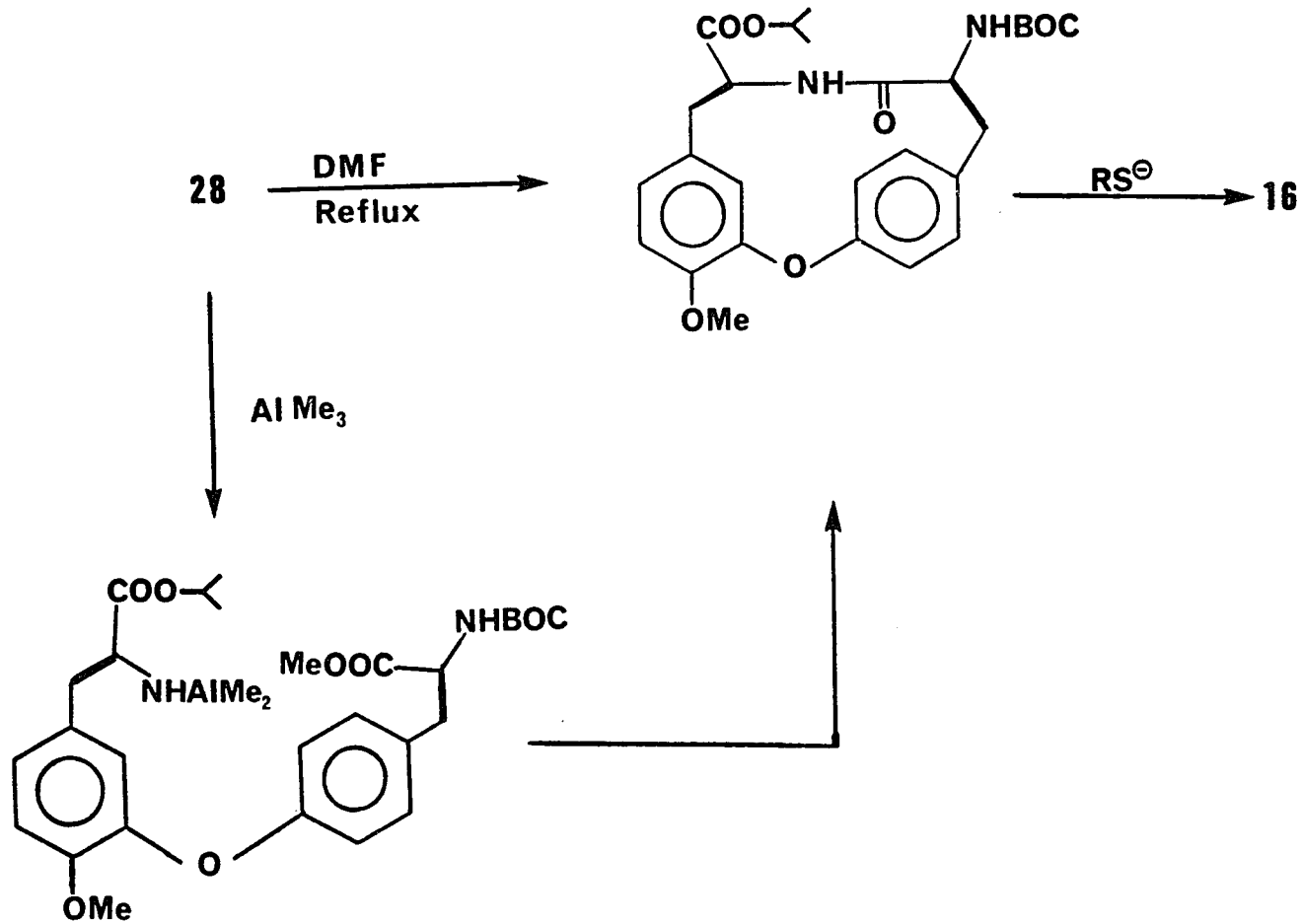


Fig. 11. Preparation of 16

EXPERIMENTAL

Iododeoxybouvardin (8)

Deoxybouvardin (2, 3.8 mg, 4.3 μmol) was partially dissolved in ammonium hydroxide (Ø.2 mL) and 2 N sodium hydroxide (3 μL, 6 mol). The mixture was degassed with argon and 1 mL of potassium iodide and iodine solution (5 mL water, 21 mg potassium iodide and 5 mg of iodine) was slowly added. After stirring for 24 h under argon, the solution was acidified with citric acid (pH = 3), extracted with ethyl acetate (3 x 3 mL), washed with water (3 x 5 mL), dried over magnesium sulfate, filtered, evaporated using rotary evaporation, and kept under vacuum (1 mm) overnight. The ^{1}H NMR spectrum showed an 88% yield. The iododeoxybouvardin (8) was separated from unreacted deoxybouvardin (2) using preparative TLC on silica gel, developing with dichlormethane-hexane-methanol (1Ø8:8Ø:12). The spots were detected using UV light (R_{f8} = Ø.39, R_{f2} = Ø.31). The product was identified by ^{1}H NMR (Table 1, Fig. 12).

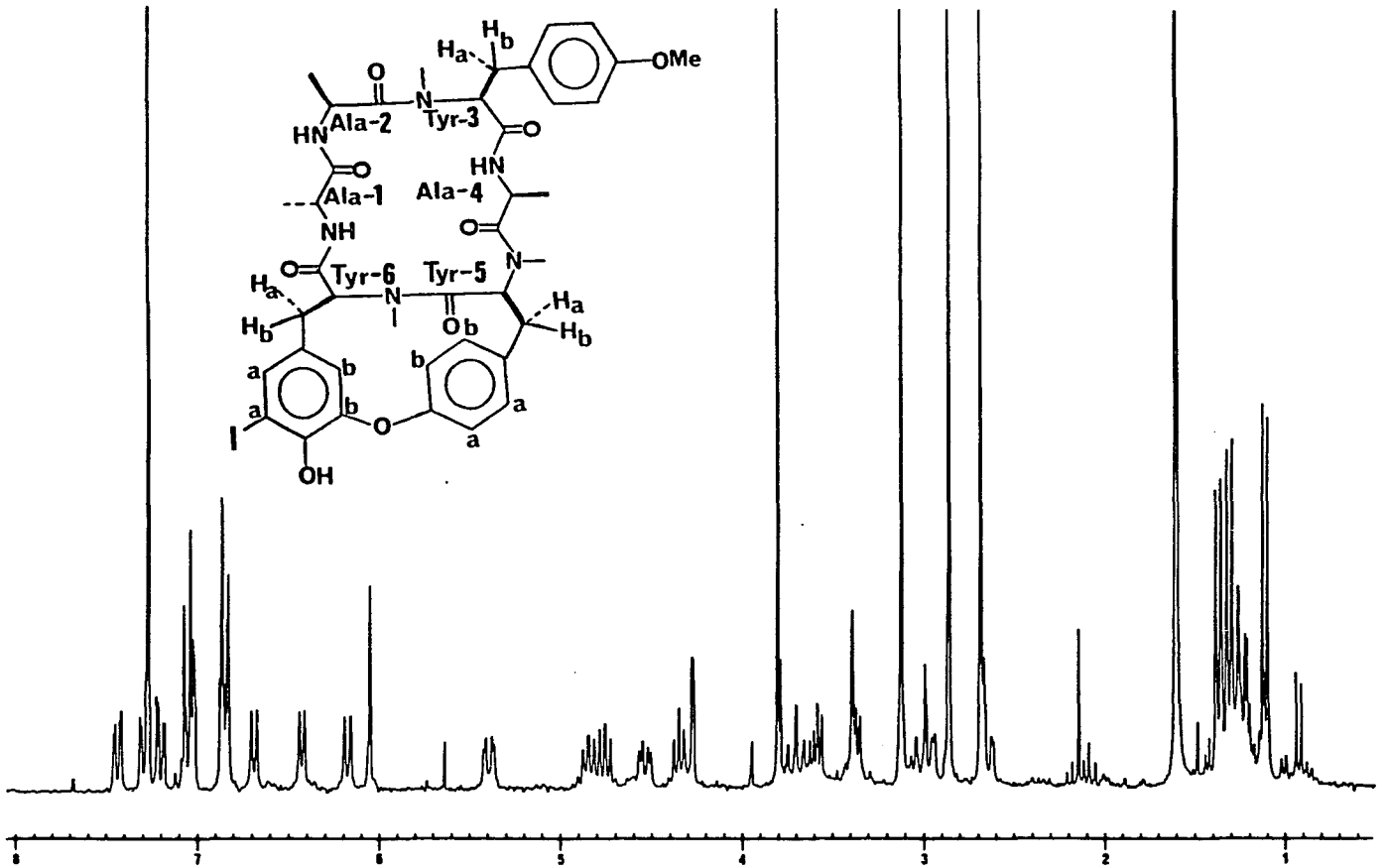


Fig. 12. ^{1}H NMR Spectrum ($CDCl_3$) of 8

Tetrapeptide (25)

O-Methyl-N-Boc-L-tyrosine (18)

Sodium hydroxide (2.37 g, 53.5 mmol) was dissolved in the least amount of methanol, added to t-Boc-L-tyr (5.01 g, 17.8 mmol) in 50 mL of methanol and stirred for 24 h at 25°C. The methanol was then removed by rotary evaporation, leaving the white sodium salt. Tetrahydrofuran (100 mL) was added to the solid and the solution was placed in a -10°C rock salt-ethanol-ice bath. When the solution had cooled, dimethyl sulfate (3.8 mL, 39.5 mmol) was added dropwise for one h to the stirring solution. After stirring for 24 h at 25°C a thick gelatinous precipitate remained. This precipitate dissolved when 2 N sodium hydroxide was added to destroy any excess dimethyl sulfate. The tetrahydrofuran was removed from the basic solution by rotary evaporation leaving a yellow solution which was washed with ethyl ether (3 x 25 mL). After the solution was acidified to pH 3 with 5 N hydrochloric acid, the product was extracted with ethyl acetate (5 x 100 mL) and dried over magnesium sulfate. The ethyl acetate was removed by rotary evaporation leaving a thick yellow oil which formed star-shaped crystals upon standing. ^{1}H NMR showed 100% conversion to the desired product and the yield by weight was 93%. The crystals were further purified

using a silica gel liquid chromatography column. The column, monitored by TLC, was eluted with 600 mL of 9:1 hexane:ethyl acetate and 1100 mL of 6:4 hexane:ethyl acetate. After evaporation, a thick oil was obtained and again after standing, star-shaped crystals (mp 105°C, α_D= +41.1°, 6.4 mg/mL of methanol) formed in the 6:4 hexane:ethyl acetate fractions. The crystals were identified by ^{1}H NMR (Figs. 13 and 14, Table 3).

L-Alanine Methyl Ester Hydrochloride (19)[22]

L-alanine (6.00 g, 67.4 mmol) was dissolved in 25 mL methanol and added slowly through a dropping funnel to 240 mL of thionyl chloride stirring under argon in a 3-necked 500 mL round bottom flask immersed in an ice bath. Even at 0°C the reaction was vigorous so a condenser was used. After the total addition of the thionyl chloride, the solution was refluxed for 0.5 h; the color changed from light yellow to dark yellow. The solution was then cooled to 0°C and methanol was added through the dropping funnel until a vigorous reaction no longer occurred. After refluxing for 0.5 h and stirring 18 h at 25°C under argon, the methanol and excess thionyl chloride were evaporated in the hood by stirring the solution in an

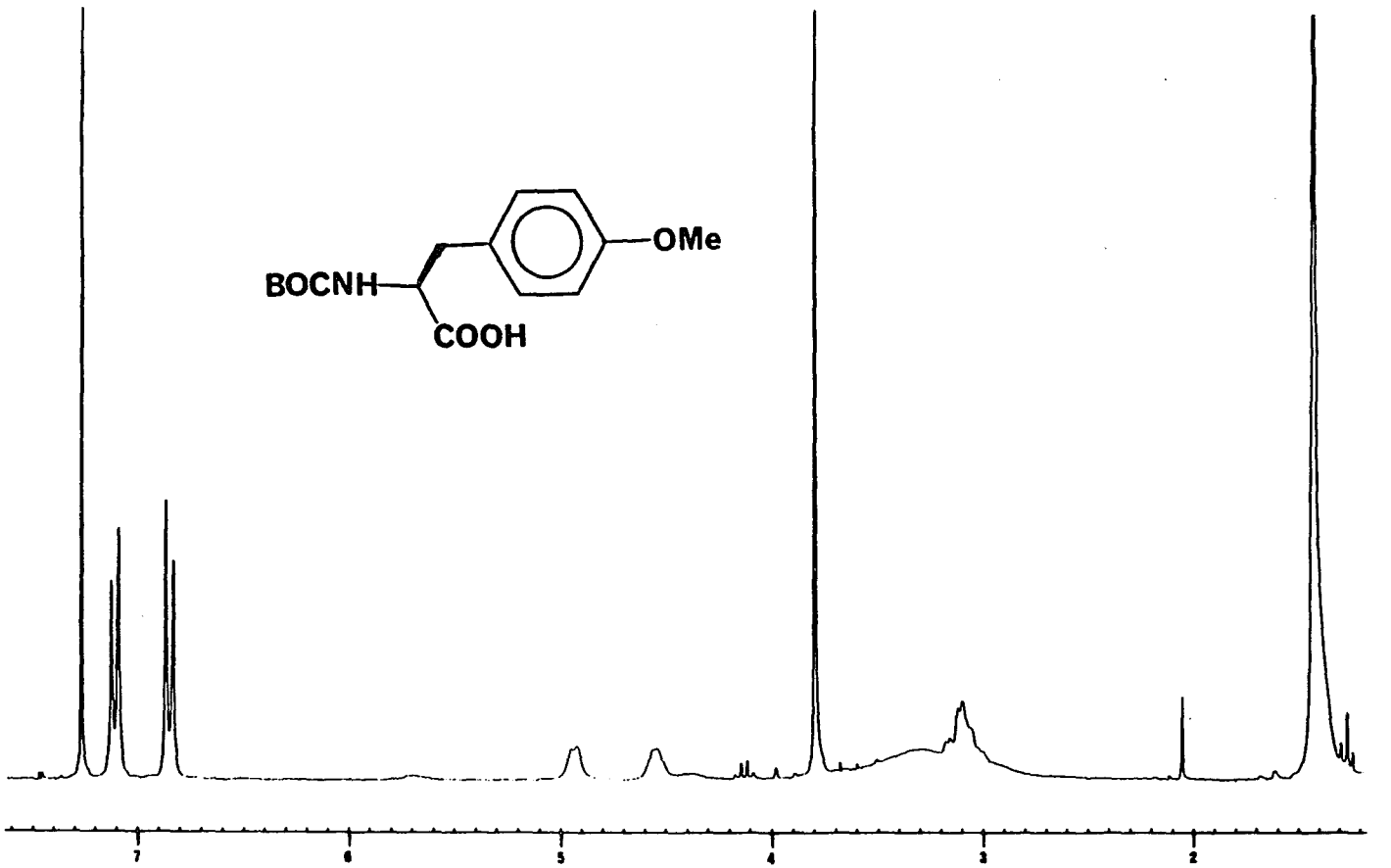


Fig. 13. ^{1}H NMR Spectrum ($CDCl_3$) of 18

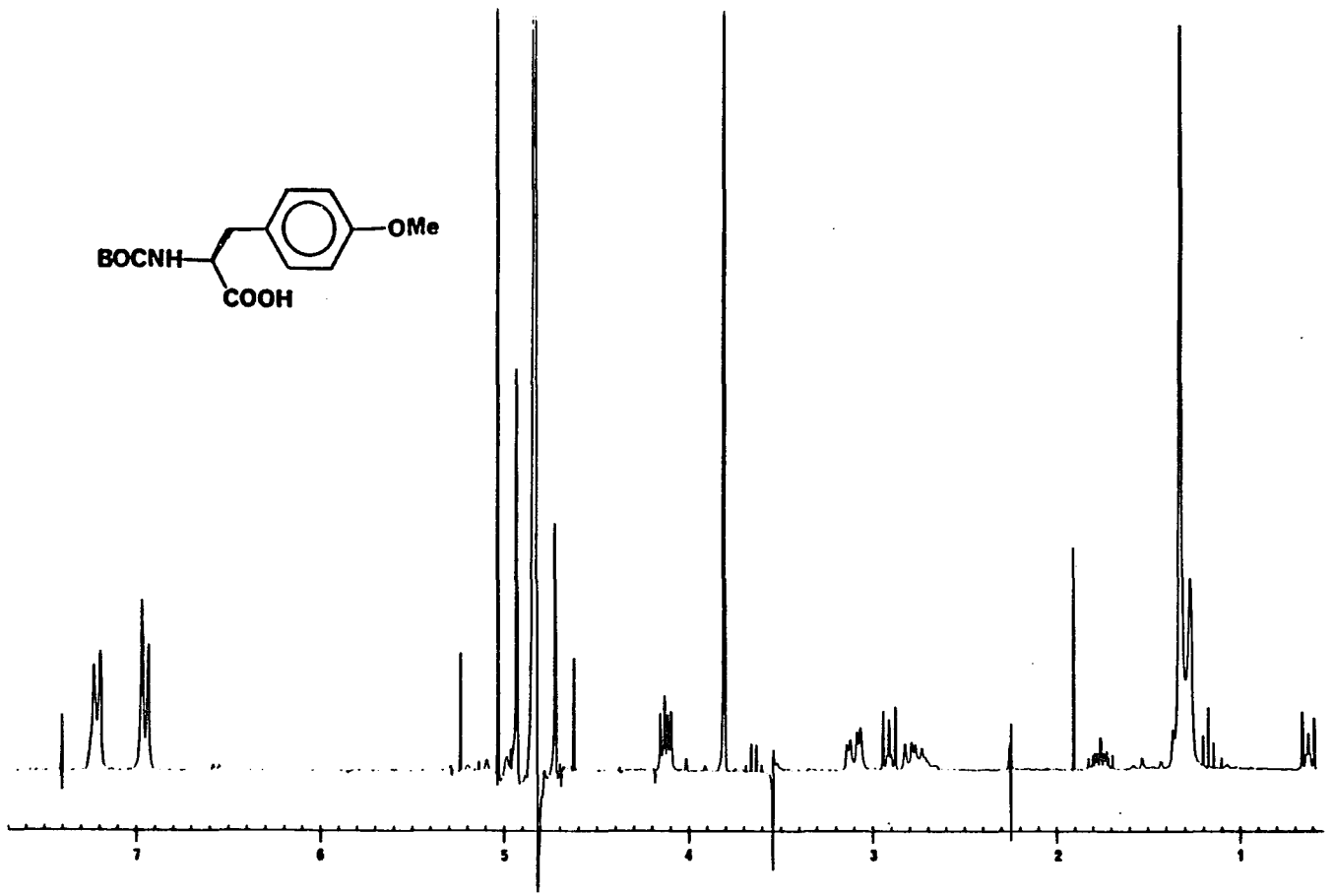
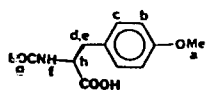
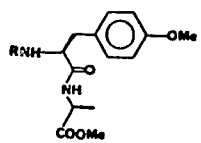
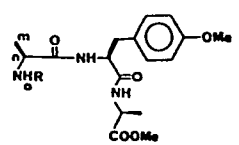


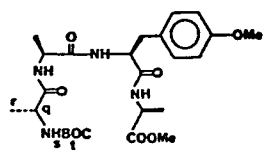
Fig. 14. ^{1}H NMR Spectrum (D_2O) of **18**

Table 3. ^1H NMR Assignments for the Synthesis of 25









R

19 $NH_3^+Cl^-$

20 NH_2

R

21 BOC (g)

22 H

R

23 BOC (p)

24 H

	18	18[b]	19[b]	20[b]	21	22	23	24	25
a	3.79s	3.81s			3.79s	3.79s	3.78s	3.78s	3.78s
b	6.84d(8.5)	6.94d(8.5)			6.83d(8.6)	6.85d(8.6)	6.82d(8.6)	6.82d(8.7)	6.82d(8.7)
c	7.10d(8.5)	7.21d(8.5			7.12d(8.6)	7.13d(8.6)	7.12d(8.6)	7.14d(8.7)	7.14d(8.7)
d	3.08m	2.79dd(14.0,9.0)			2.98dd(14.0,6.8)	2.68dd(13.8,9.1)	2.98dd(13.9,6.9)	3.02dd(14.0,7.2)	2.98dd(14.2,8.5)
e	3.12m	3.09dd(14.0,4.6)			3.05dd(14.0,6.4)	3.15dd(13.8,4.0)	3.11dd(13.9,5.8)	3.06dd(14.0,6.8)	3.17dd(14.2,5.2)
f	4.91bs				5.00bs	1.50bs	6.56bs	7.78d(7.9)	6.43d(6.3)
g	1.42s	1.32s[c]			1.42s				
h	4.54m	4.12dd(9.0,4.6)			4.30-q(6.9)	3.59dd(9.1,4.0)	4.63-q(7.2)	4.57-q(7.6)	4.64-q(7.1)
i			4.24q(7.0)	4.21q(6.8)	6.39d(7.2)	7.75d(7.3)	6.63d(7.2)	6.57d(7.2)	6.78bs
j			1.58d(7.0)	1.56d(6.8)	4.52p(7.2)	4.59p(7.3)	4.49p(7.2)	4.48p(7.2)	4.47p(7.2)
k			3.85s	3.84s	1.35d(7.2)	1.38d(7.3)	1.34d(7.2)	1.36d(7.2)	1.38d(7.2)
l					3.72s	3.74s	3.71s	3.72s	3.72s
m							1.32d(7.0)	1.22d(7.0)	1.38d(7.0)
n							4.10p(7.0)	3.58q(7.0)	4.36p(7.0)
o							4.91bs	1.71bs	6.86bs
p							1.40s		
q									4.84p(6.8)
r									1.33d(6.8)
s									4.97bs
t									1.45s

[a] Spectra run in $CDCl_3$ unless indicated otherwise
[b] Spectra run in D_2O
[c] A minor rotamer with 1.26s was also observed

open beaker. A brownish-green solid was obtained (100% conversion indicated by ^1H NMR, Fig. 15, Table 3).

L-Alanine Methyl Ester (20)

19 (61.1 mg, 0.44 mmol) was added to an ammonia-chloroform solution (prepared by bubbling ammonia into chloroform for 1.5 hrs) and stirred for 0.5 h. The solution was filtered to remove ammonium chloride and evaporated using rotary evaporation to remove the chloroform. The yield (100%) was established by ^1H NMR (Fig. 16, Table 3). 20 was immediately reacted with 18 to prevent the formation of diketopiperazine.

Methyl O-Methyl-N-Boc-tyr-L-alaninate (21)

18 (3.35 g, 11.3 mmol) was added to freshly prepared 20 (2.40 g, 23.3 mmol) dissolved in 100 mL of dichloromethane. The solution was protected with a drying tube and cooled to 0°C using an ice bath. DCC (7.20 g, 35.0 mmol) was added and the solution was stirred for 1.0 h at 0°C and 25 h at 25°C. The reaction mixture was then diluted to 250 mL with dichloromethane and washed with the following: 5% acetic acid (3 x 100 mL), water (1 x 50 mL), saturated sodium bicarbonate (2 x 100 mL), and again with

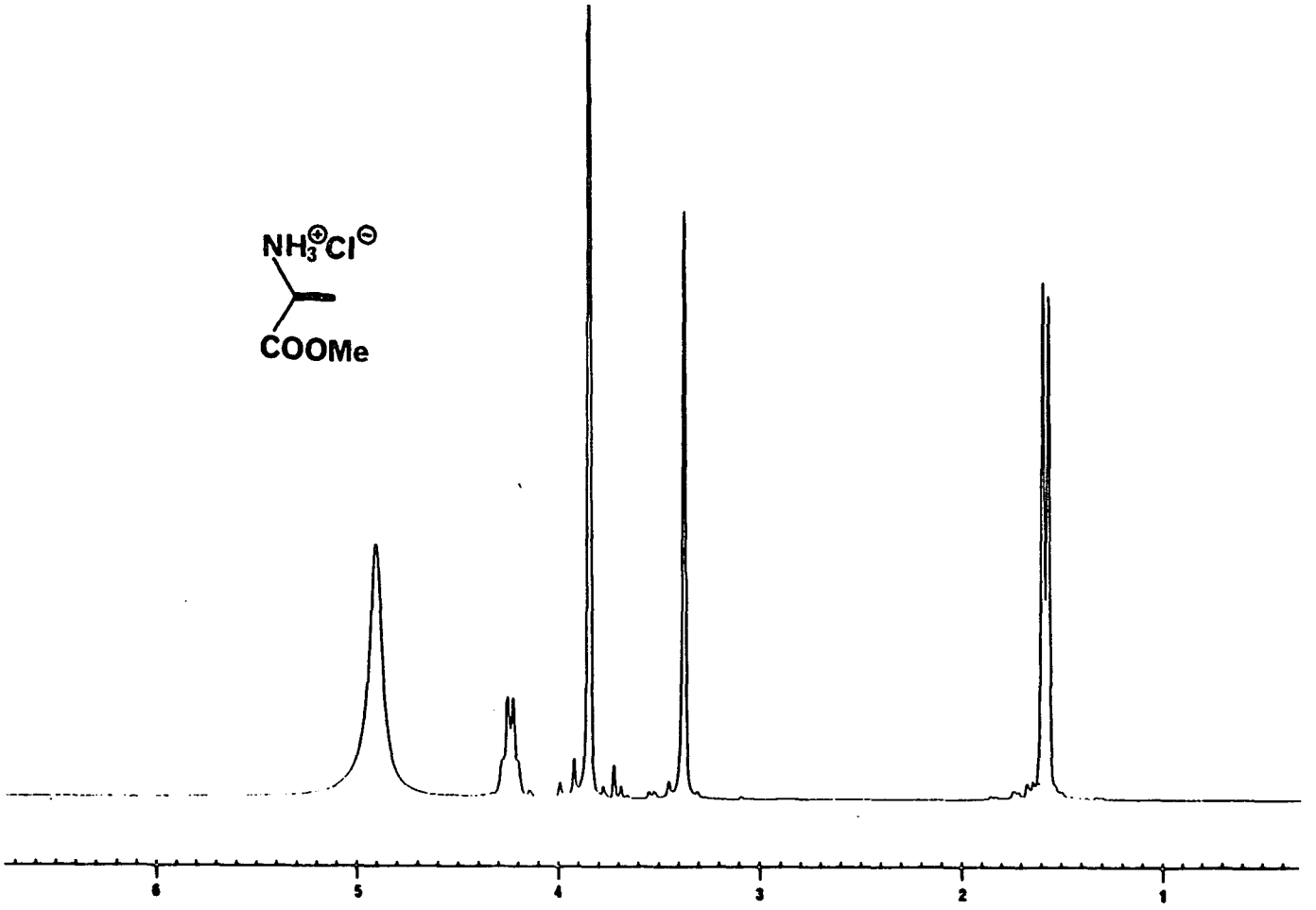


Fig. 15. ^{1}H NMR Spectrum (D_2O) of 19

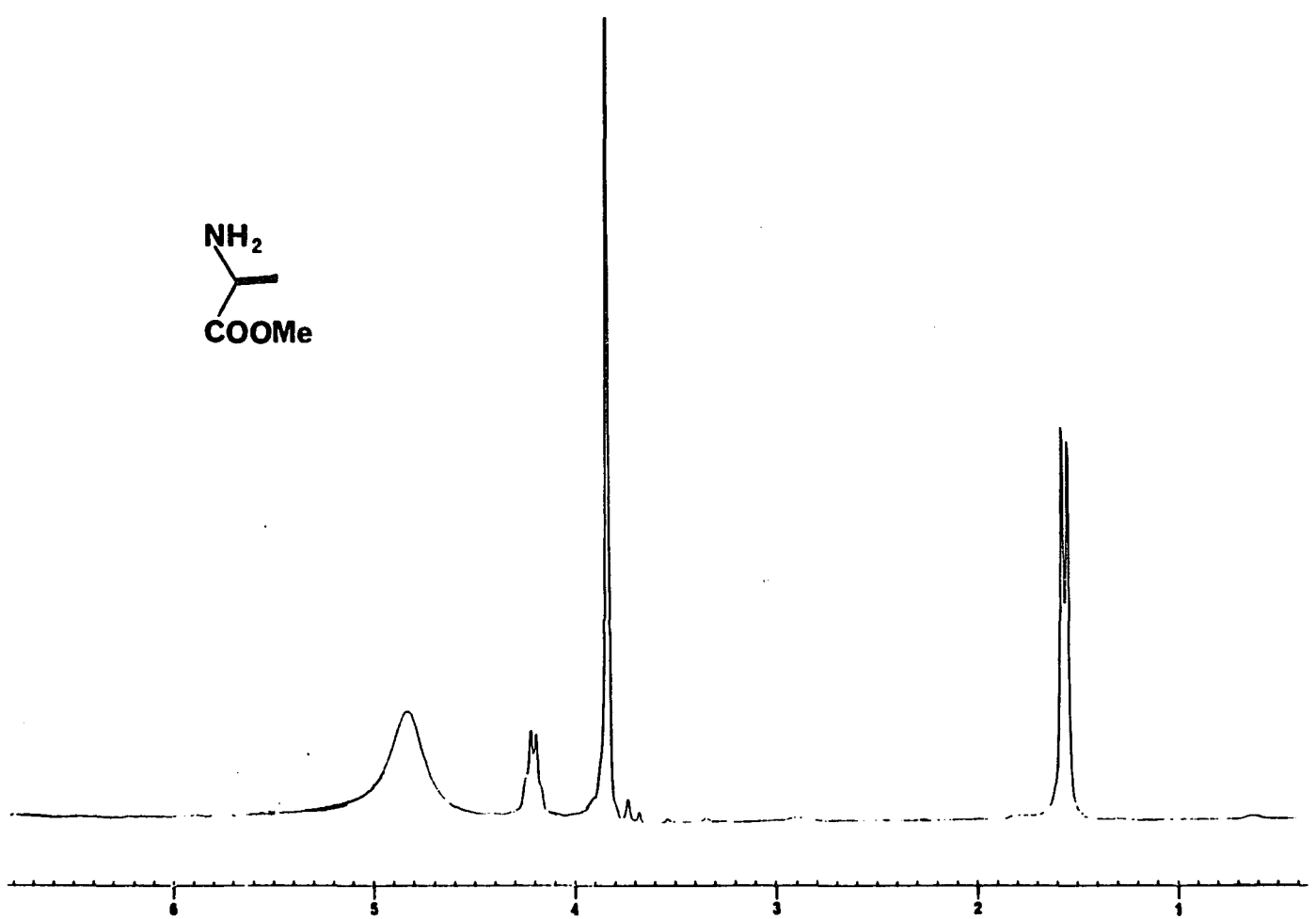


Fig. 16. ^{1}H NMR Spectrum (D_2O) of 2[illegible]

water (1 x 50 mL). The dichloromethane solution was then dried over magnesium sulfate, filtered, and evaporated using rotary evaporation. The product (17.61 g) contained urea so a sample was purified using column chromatography. The silica gel column was eluted with the following ethyl acetate:hexane mixtures: 3:7 (100 mL); 5:5 (100 mL); 7:3 (100 mL); and 8:2 (200 mL). The product (mp 102-103°C, α_D = -9.5°, 2.8 mg/mL of methanol) was characterized by ^1H NMR (Fig. 17, Table 3) and elemental analysis (Anal. Calcd for $C_{19}H_{28}O_6N_2$: C, 59.97; H, 7.43; N, 7.36. Found: C, 59.62; H, 7.41; N, 7.12).

Methyl O-Methyl-L-tyr-L-alaninate (22)

Dichloromethane (100 mL) was added to a mixture of 21 and urea (17.61 g). The urea was filtered off and the dichloromethane was removed using rotary evaporation. Trifluoroacetic acid (10 mL) was added to the residue and the mixture was stirred for 30 min at 25°C. The trifluoroacetic acid was evaporated using rotary evaporation. The oil which remained was dissolved in dichloromethane (20 mL), extracted twice with 0.1 N hydrochloric acid (1 x 170 mL and 1 x 35 mL), washed with dichloromethane (2 x 20 mL), and made basic with solid potassium bicarbonate. The basic solution was then saturated with sodium chloride, extracted

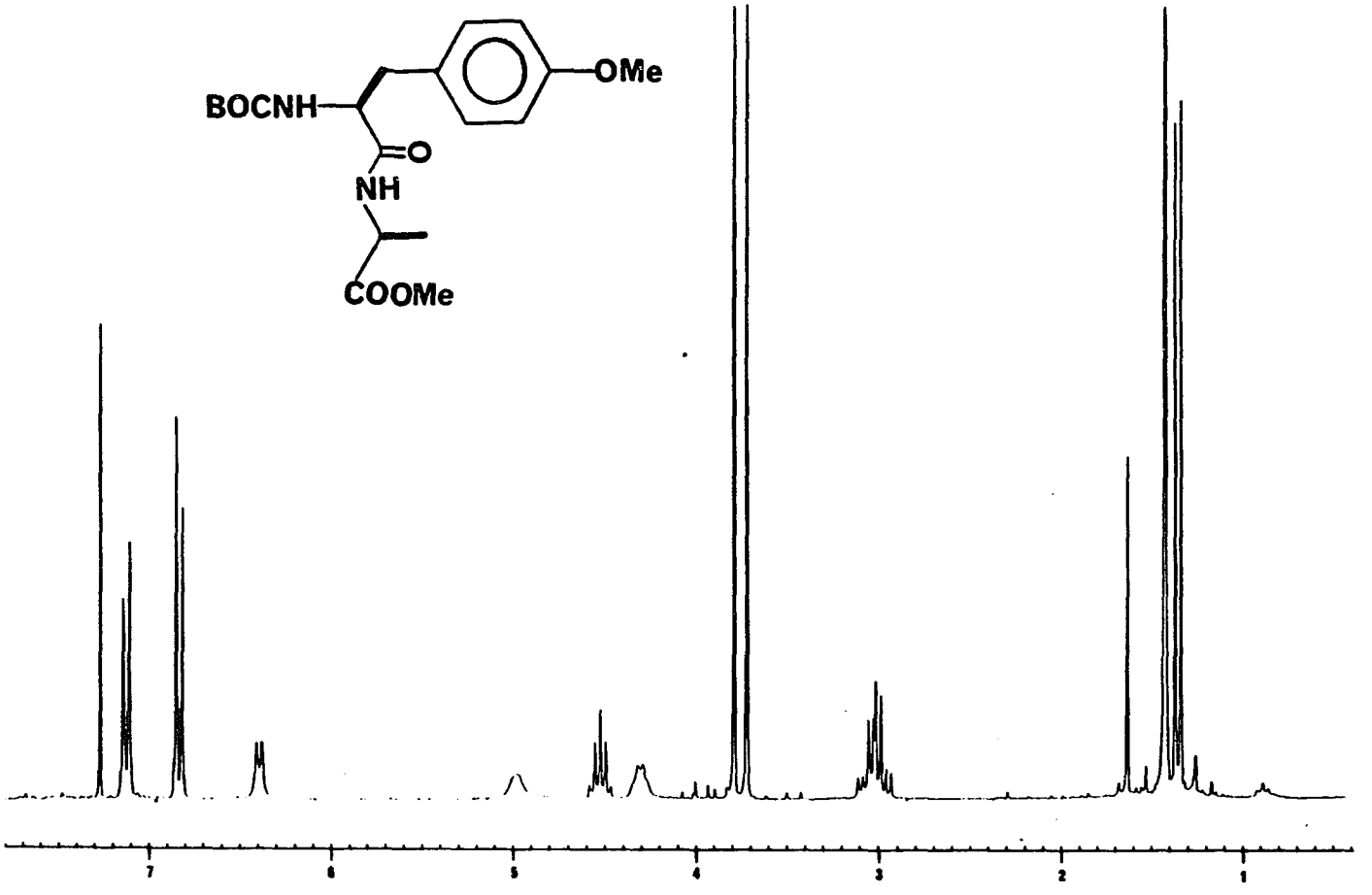


Fig. 17. ^{1}H NMR Spectrum ($CDCl_3$) of 21

with dichloromethane (3 x 100 mL), dried over magnesium sulfate, filtered, and evaporated using rotary evaporation to give the product (2.25 g) in 71% yield over two steps. **22** (mp 270°C) was identified by ^{1}H NMR (Fig 18, Table 3). The product was immediately reacted with Boc-L-alanine to prevent diketopiperazine formation.

Methyl N-Boc-L-ala-O-methyl-L-tyr-L-alaninate (23)

Boc-L-ala (4.0 g, 21.2 mmol) and **22** (3.72 g, 13.3 mmol) were dissolved in 100 mL of dichloromethane and cooled to 0°C with an ice bath. After DCC (6.97 g, 26.8 mmol) was added the solution was stirred for 1 h at 0°C and 23 h at 25°C. The reaction was then diluted with 150 mL of dichloromethane and filtered to remove undissolved urea. The remaining solution was washed with saturated potassium bicarbonate (3 x 100 mL) and with water (1 x 50 mL). The dichloromethane layer was dried over magnesium sulfate, filtered and evaporated, using rotary evaporation, leaving an oil (21.4 g) containing **23** and urea. **23** was isolated using silica gel liquid chromatography with a solvent system involving the following ratios of ethyl acetate-hexane: 5:5 (200 mL); 6:4 (200 mL); 7:3 (200 mL); 8:2 (100 mL); 9:1 (200 mL); and 10:0 (200 mL). **23** (mp 142-144°C,

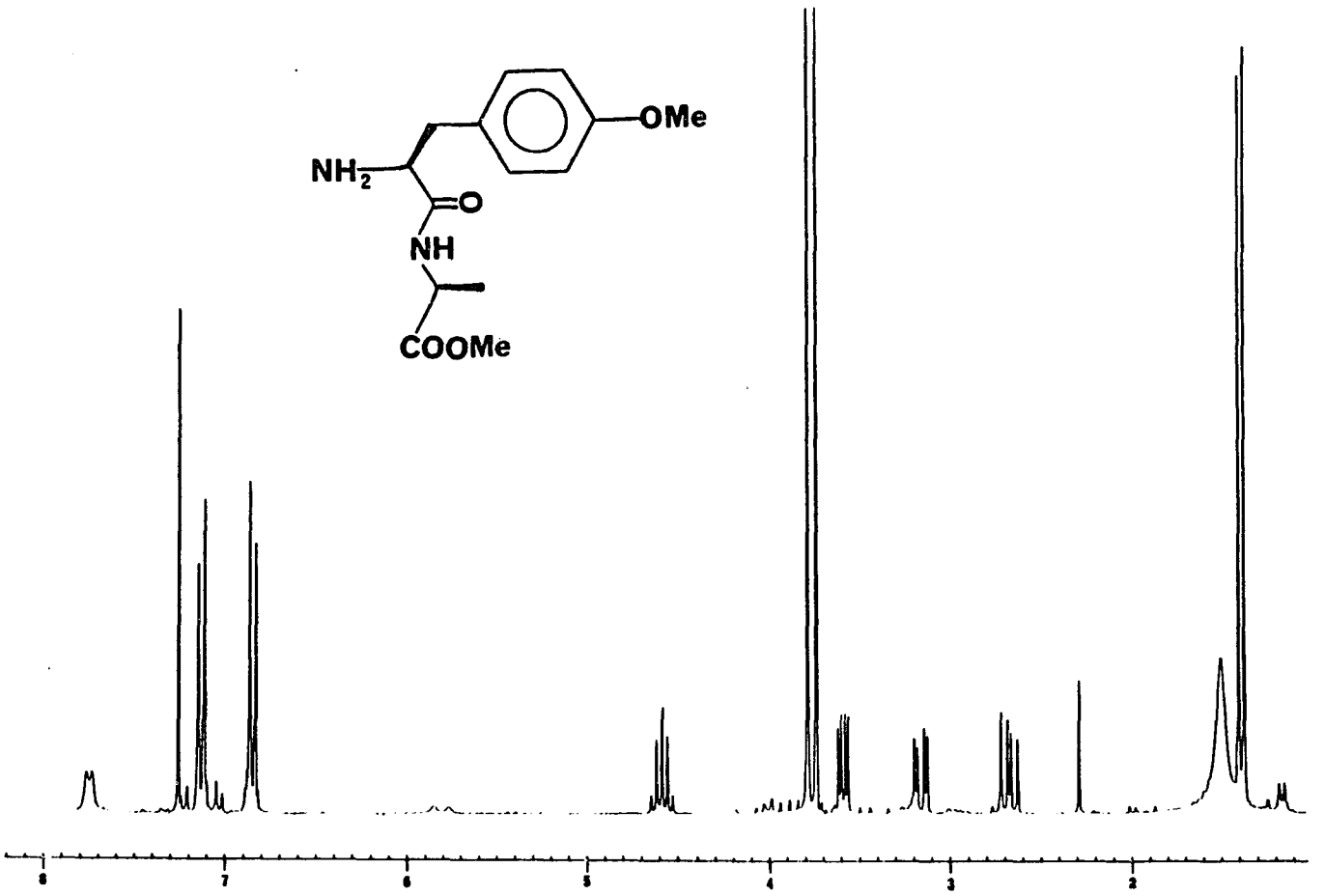


Fig. 18. ^{1}H NMR Spectrum ($CDCl_3$) of 22

α_D = -42.6°, 15.6 mg/mL of methanol) was characterized using ^1H NMR (Fig. 19, Table 3) and elemental analysis (Anal. Calcd for $C_{22}H_{33}N_3O_7$ H_2O: C, 57.40; H, 7.45; N, 9.12. Found: C, 57.55; H, 7.63; N, 8.86).

Methyl L-Ala-O-methyl-L-tyr-L-alaninate (24)

A mixture of 23 and urea (2.0355 g) was dissolved in 8 mL of dichloromethane. Trifluoroacetic acid (10 mL) was added, the solution was stirred for 0.5 h and the trifluoroacetic acid was removed by rotary evaporation. The residue was extracted with 0.1 N HCl (25 mL), washed with dichloromethane (2 x 20 mL), made basic with solid potassium bicarbonate, saturated with sodium chloride, extracted with dichloromethane (3 x 20 mL), dried over magnesium sulfate and rotary evaporated. The product (293 mg, 78% yield over two reactions) was characterized by ^1H NMR (Fig. 20, Table 3). 24 was reacted immediately with Boc-D-ala to prevent decomposition.

Methyl N-Boc-D-ala-L-ala-O-methyl-L-tyr-L-alaninate (25)

24 (0.3 g, 0.85 mmol) and Boc-D-ala (245 mg, 1.3 mmol) were dissolved in 5 mL of dichloromethane and cooled to 0°C in an ice bath. DCC (528 mg, 2.6 mmol) was added

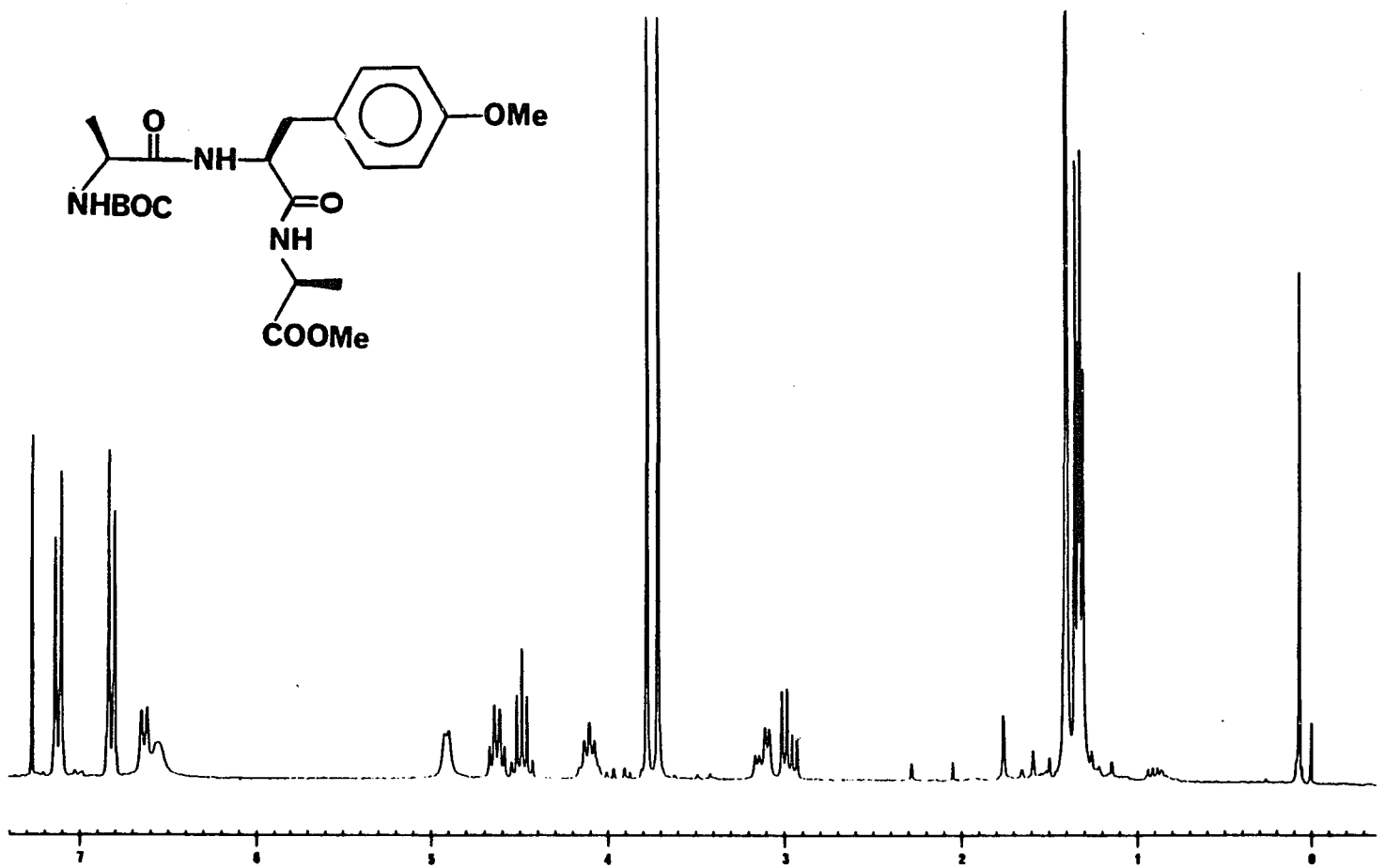


Fig. 19. ^{1}H NMR Spectrum ($CDCl_3$) of 23

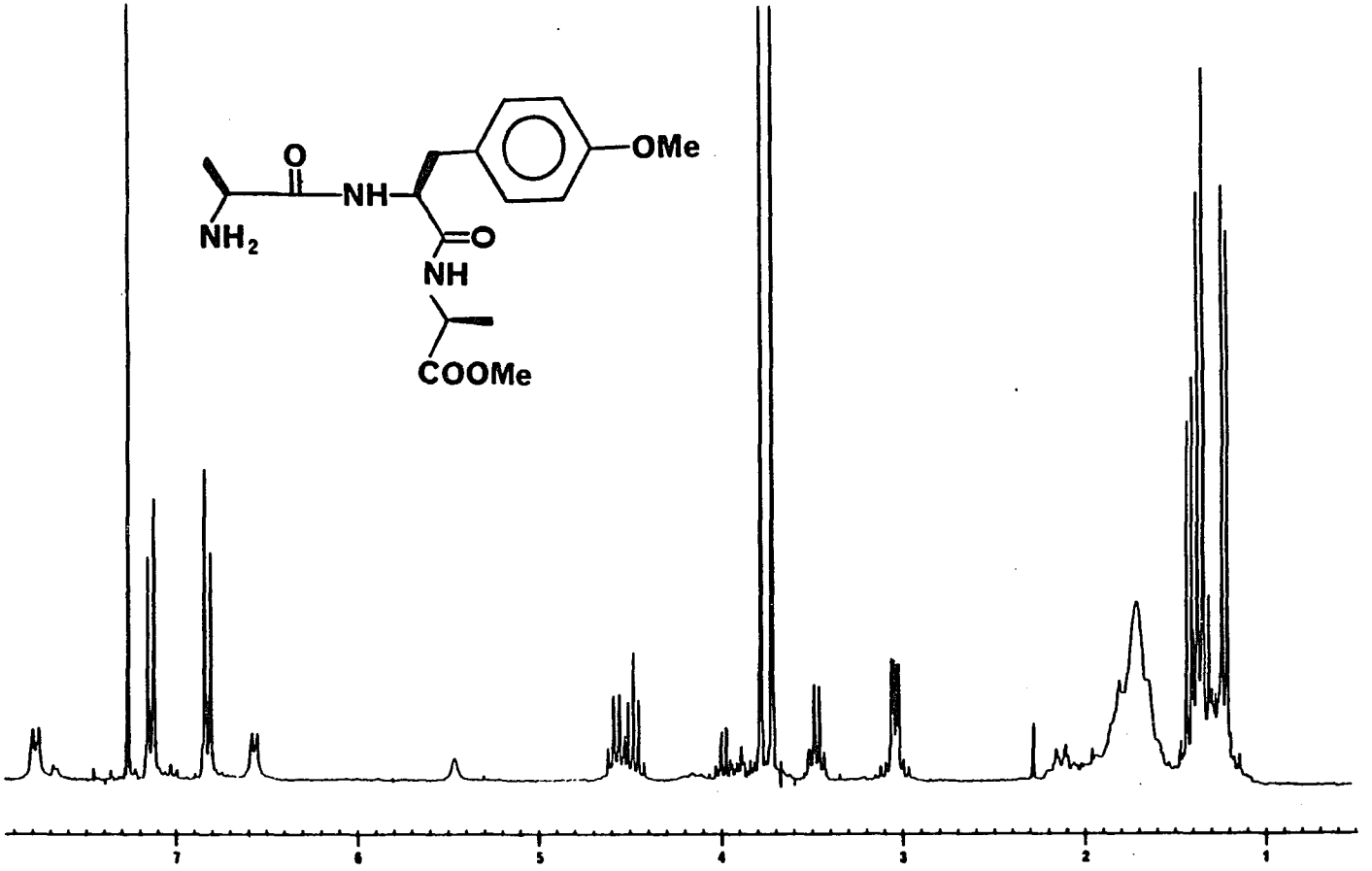


Fig. 20. ^{1}H NMR Spectrum ($CDCl_3$) of **24**

and the solution was stirred for 1.Ø h at ØºC and 3Ø h at 25ºC. The solution was then diluted with dichloromethane (25 mL) and water (1 x 25 mL), dried over magnesium sulfate, filtered, and rotary evaporated to dryness (673 mg). This product was purified by silica gel liquid chromatography, eluting with the following ratios of ethyl acetate-hexane: 7:34 (2ØØ mL); 8:2 (1ØØ mL); 9:1 (1ØØ mL); and 1:Ø (2ØØ mL). A pink product (356 mg, 8Ø% yield) was obtained after evaporation. A white solid (mp 148.5-149ºC, α_D = -21.6º, 5.Ø mg/mL of methanol) was obtained after recrystallization from ethyl acetate-hexane. 25 was identified using ^1H NMR (Fig. 21, Table 3) and elemental analysis (Anal. Calcd for $C_{25}H_{38}N_4O_8$: C, 57.64; H, 7.33; N, 1Ø.72. Found: C, 57.18; H, 7.36; N, 1Ø.56).

14-Membered Ring

26^{23}

(R,R)-1,2-Ethanediylbis-[(O-methoxyphenyl)phenyl-phosphine] (218 mg, Ø.58 mmol; kindly provided by W. S. Knowles of Monsanto Corp.) was added to 1 mL of 9Ø% methanol and placed under argon. Bis(cyclooctadiene-1,5) dichlorodirhodium, $[Rh(COD)Cl]_2$ (145 mg, Ø.29 mol), was added to the white slurry and it became yellow. After

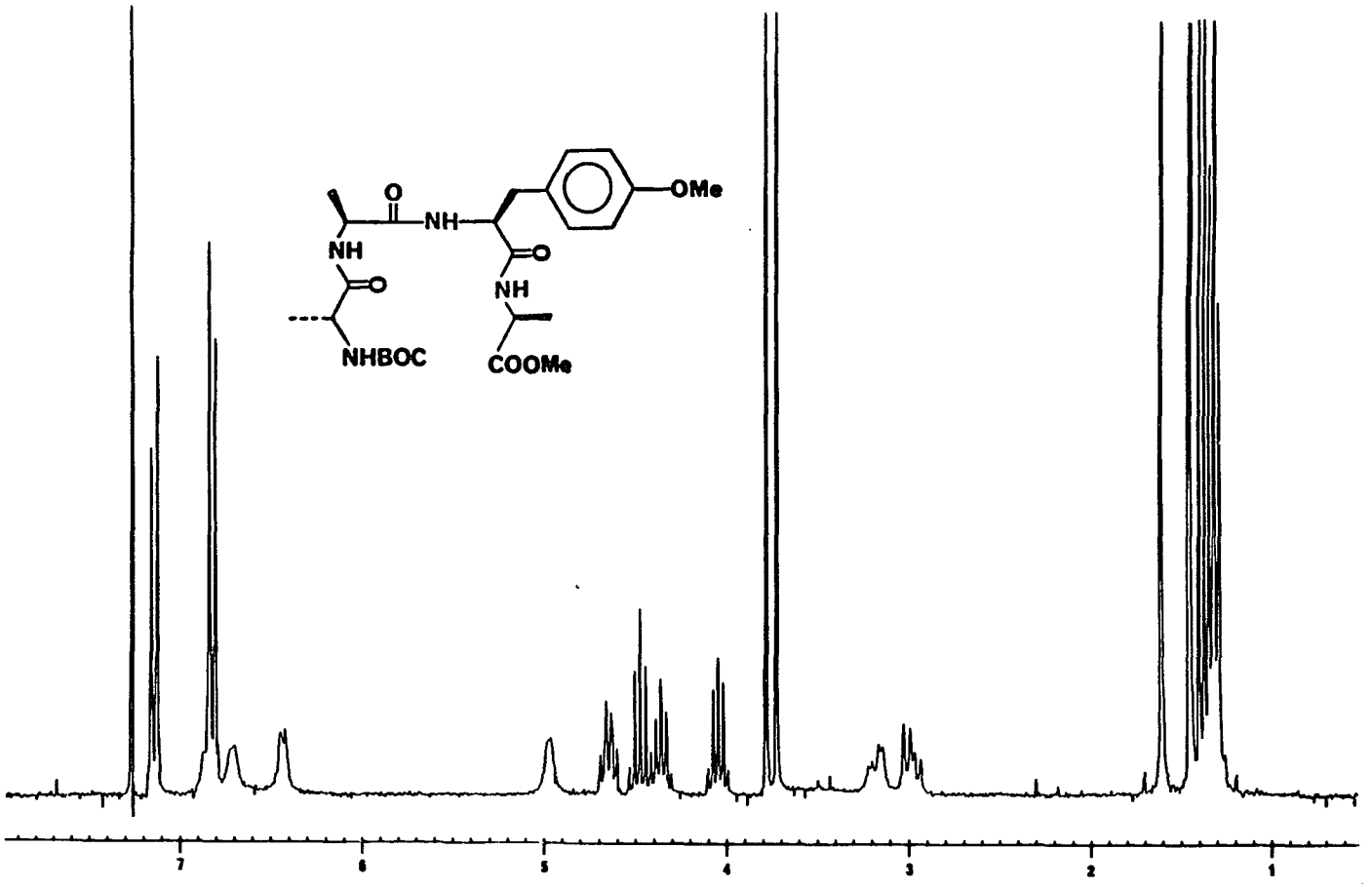


Fig. 21. ^{1}H NMR Spectrum ($CDCl_3$) of 25

stirring for 1 h the slurry had turned orange. The complex was precipitated by slowly adding a solution of sodium tetrafluoroborate (110 mg, 1 mmol) in water (1 mL) over 2 h. After stirring at 25°C for 1 h the crystals were filtered off and washed with water (2 x 3 mL).

27

15 (1.2 g, 1.8 mmol) and **26** (0.2 g, 264 μmol) were dissolved in t-butyl alcohol and placed in a high pressure reaction vessel. The reaction mixture was flushed with hydrogen gas and exposed to pressure (1700 lbs) and heat (70°C) for 26 h. The brown sludge which remained was filtered and run through an LC column using 300 mL of 7:3 ethyl acetate:hexane, 200 mL of ethyl acetate and 500 mL of methanol, all collected at 50 mL intervals. Most of the fractions collected contained mixtures of products; fraction eleven contained pure **28** (Fig. 22; ^{1}H NMR ($CDCl_3$): δ1.16d (6.2), 1.22d (6.2), 1.42s, 2.22m, 3.03m, 3.71s, 3.81s, 4.53m, 4.98m, 6.84d (8.7), 6.94m, 7.04d (8.4). An attempt was made to obtain pure **27** using MPLC with chloroform as the solvent, but it failed to separate **27** from **15**.

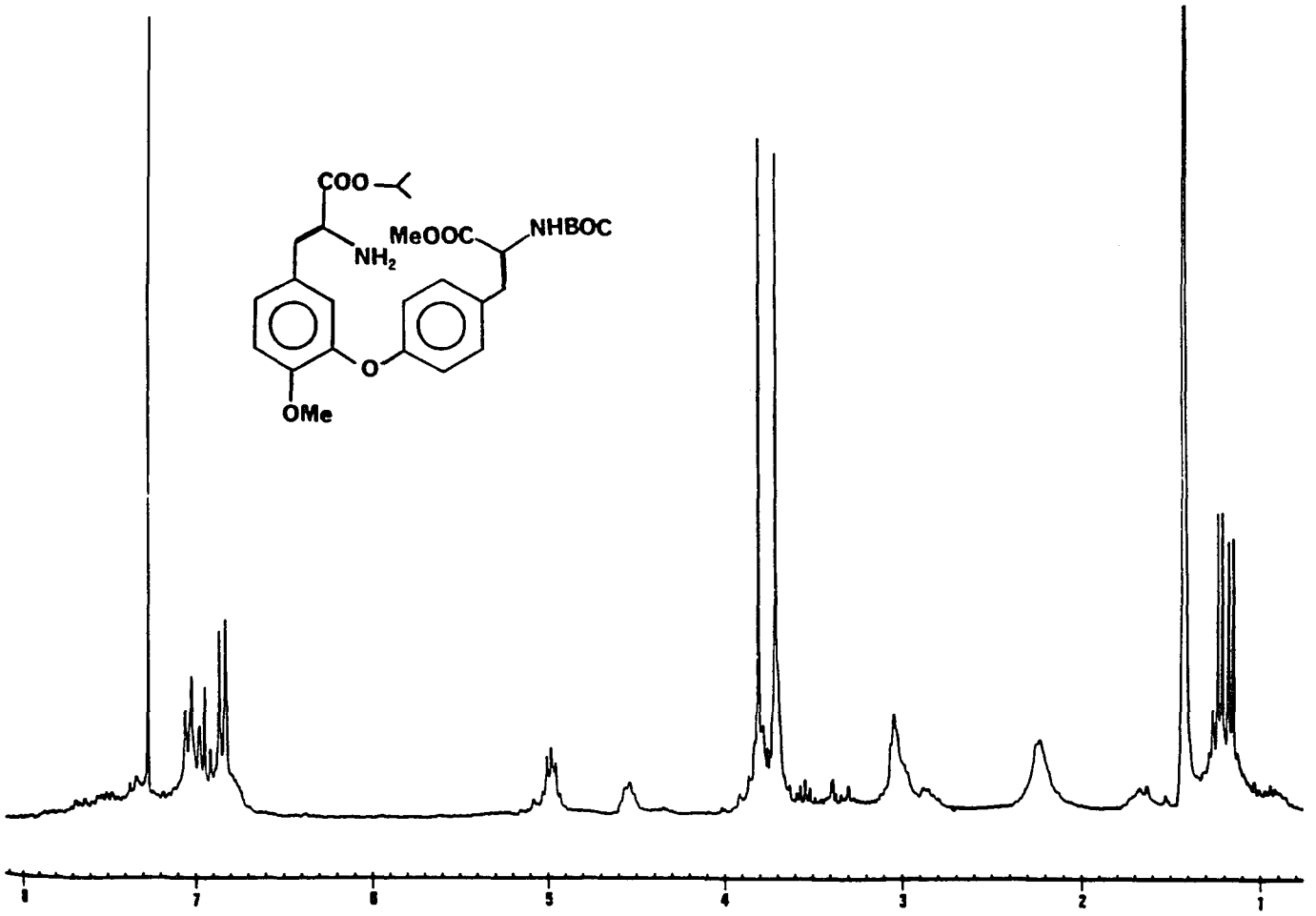


Fig. 22. ^{1}H NMR Spectrum ($CDCl_3$) of 28

Boc-L-tyrosine

Boc-L-tyr methyl ester (517 mg, 1.7 mmol) was stirred in dioxane (2.5 mL) and 2 N sodium hydroxide (2.5 mL) for 2 h. The solution was then washed with dichloromethane (3 x 10 mL), acidified with 1 N hydrochloric acid (pH = 2), extracted with ethyl acetate (4 x 25 mL), dried over magnesium sulfate, filtered, evaporated using rotary evaporation and vacuum pumped overnight leaving a white solid (542 mg, > 100% yield). The NMR showed that some methyl ester still remained so the product was dissolved in ethyl acetate (30 mL), extracted with potassium bicarbonate (3 x 10 mL) and water (1 x 10 mL), acidified with 1 N hydrochloric acid and extracted with ethyl acetate (3 x 50 mL). After drying over magnesium sulfate, evaporating using rotary evaporation, and placing under vacuum (1 mm) overnight, Boc-L-tyr (445 mg, 93% yield) remained. The ^{1}H NMR ($CDCl_3$): δ1.42s, 3.03m, 4.55m, 5.11d (8.2), 5.95bs, 6.72d (8.0), 6.94d (8.0) showed the presence of two rotamers.

Boc-L-tyr Methyl Ester

Diazald (686 mg, 3.2 mmol) in ether was slowly added through a dropping funnel to sodium hydroxide (2 g, 50 mmol), water (4 mL) and ethanol (15 mL) in a distilling flask which was warmed to 65°C in an oil bath. One hour after the addition started diazomethane began distilling into a receiving flask containing Boc-L-tyr (445 mg, 1.6 mmol) dissolved in ether (30 mL). After excess diazomethane was detected by a light green color in the receiving flask, the solution was quenched with a drop of acetic acid and evaporated using rotary evaporation, leaving solid Boc-L-tyr methyl ester (445 mg, 100% yield; ^1H NMR ($CDCl_3$): δ1.42s, 3.0m, 3.71s, 4.53m, 5.00d (7.4), 6.73d (8.3), 6.97d (8.5); α_D = 6.1°, 9.4 mg/mL of methanol).

Boc-L-tyrosine

Boc-tyr methyl ester (4.2 mg, 13.8 mmol) was dissolved in ethanol (0.2 mL). Buffer (pH = 8, 1 mL) and esterase from porcine liver (10 μL) were added to the solution and stirred for 22 h. The reaction mixture was then acidified with citric acid (pH = 3), extracted with ethyl acetate (3 x 5 mL), washed with water (3 x 5 mL), dried over magnesium sulfate, filtered, rotary evaporated

and vacuum pumped overnight. The product (95% yield) was characterized by ^1H NMR ($CDCl_3$): δ1.43s, 3.03m, 4.55m, 5.05d (8.4), 5.83bs, 6.72d (8.0), 6.99d (7.8).

28

27 (3 mg, 4.5 μmol) was dissolved in ethyl acetate (1 mL). 10% palladium on activated carbon catalyst (3 mg) was added and the solution was stirred 24 h at 25°C under a hydrogen atomosphere. The reaction mixture was then filtered and washed through Celite to remove the catalyst. The product (100% yield) was identified by ^1H NMR ($CDCl_3$): δ1.16d (6.2), 1.22d (6.2), 1.42s, 2.22m, 3.03m, 3.71s, 3.81s, 4.53m, 4.98m, 6.84d (8.7), 6.94m, 7.04d (8.4).

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