

9905131-0

07 OCT 1999

1pds copy
9905/31-0

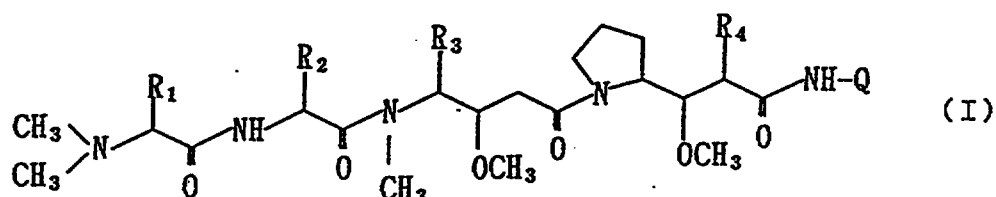
This Sheet Has Been Replaced

- 1 -
DESCRIPTION

NOVEL TETRAPEPTIDE DERIVATIVE

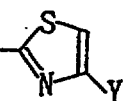
Technical Field

This invention relates to a novel tetrapeptide derivative having an antitumor activity, and relates more detailedly to a tetrapeptide derivative represented by the following formula or a salt thereof:



wherein,

R_1 , R_2 , R_3 and R_4 are the same or different and each represent a hydrogen atom, a lower alkyl group or an aralkyl group;

Q represents $-A_1-$  or a group of $-A_2-R_7$,

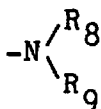
wherein,

A_1 represents a direct bond or $-\overset{\overset{R_5}{|}}{\text{CH}}-$,

Y represents a hydrogen atom or $-\text{COR}_6$,

R_5 represents a hydrogen atom, a lower alkyl group or an aralkyl group,

R_6 represents a hydroxyl group, a lower alkoxy group, an aralkyloxy group or



wherein,

THIS SHEET HAS BEEN
REPLACED



- 2 -

5 R_8 and R_9 are the same or different and each represent a hydrogen atom, a lower alkyl group, a phenyl group or a 4- to 7-membered heterocyclic group containing one or two hetero atoms selected from S, O and N, or alternatively

10 R_8 and R_9 may combine together with the nitrogen atom to which they are bonded to form a 4- to 7-membered heterocyclic ring optionally further containing one hetero atom selected from S, O and N,

A_2 represents a direct bond or a lower alkylene group, and

15 R_7 represents a cycloalkyl group, an aryl group or an indolyl group,

provided that the case is excluded where both R_1 and R_2 represent isopropyl groups, R_3 represents a sec-butyl group, R_4 represents a methyl group, and Q represents an α -(2-thiazolyl)phenethyl group.

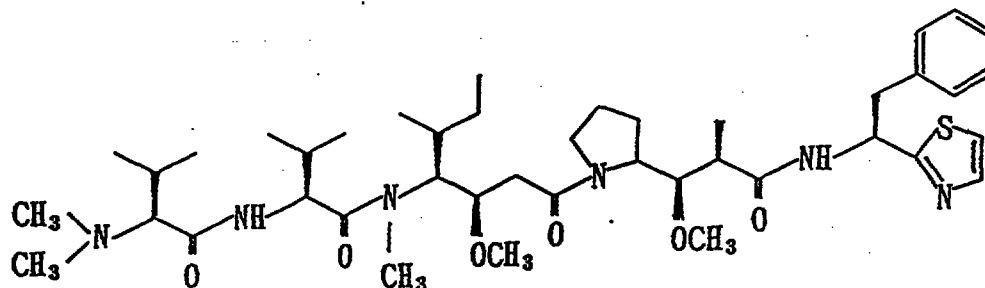
20

Background Art

Peptides having a cytostatic activity and/or an antineoplasm activity have been isolated from marine molluscs, sea hare Dolabella auricularia and these peptides are called dolastatins 1 to 15. Among them, dolastatin 10 is a pentapeptide extracted from Dolabella auricularia from the Indian Ocean in 1987 by G. R. Pettit, et al. and having the following structural formula, and is said to be the strongest cytostatic substance presently known (see G. R. Pettit, et al., J. Am. Chem. Soc., 109, 6883 (1987) and Japanese Laid-Open Patent Publication No. 167278/1992).

25

30



Dolastatin 10

Further, recently, publication was made on the total synthesis of dolastatin 10 itself (see, US Patent No. 4,978,744), but its derivatives have not so far been known at all.

The present inventors had intensely studied about derivatives of dolastatin 10, and as a result they found that certain dolastatin analogs represented by the above formula (I) have a higher cytostatic activity than dolastatin 10. They further found that many of these compounds have a larger therapeutic ratio (maximum effective dose/dose at 30% prolongation of life) and a lower toxicity than dolastatin 10, and are thus excellent as an antitumor agent.

Namely, they found not only that amino acid analogs of dolastatin 10 exhibit a higher activity than the native dolastatin 10, but that the activity is, unexpectedly, extremely increased by introducing a carboxyl derivative into the thiazole ring. Further, they found, throughly astoundingly, that derivatives wherein the thiazole ring is eliminated show extremely higher activity than dolastatin 10.

Disclosure of Invention

The embodiments of this invention completed based on these findings can be classified into the following three categories.



- 4 -

(1) Amino acid-substituted compounds of dolastatin 10 and synthesis thereof

(2) Carboxyl derivatives of the thiazole ring and synthesis thereof

5 (3) Tetrapeptide derivatives wherein the thiazole ring is eliminatd and synthesis thereof

The synthesis of compounds belonging to category (1) is described in the later-shown Flow sheets 1, 2 and 3, and synthesis of compounds belonging to category
10 (2) is described in the later-shown Flow sheets 4 and 5. Further, synthesis of compounds belonging to category (3) is described in the later-shown Flow sheet 6.

In the present description, the term "lower" means that the number of the carbon atoms of a group or
15 compound to which this term is attached is 6 or less, preferably 4 or less.

In the above formula (I), the "lower alkyl group" may be either of straight-chain and branched-chain, and there can, for example, be mentioned a methyl,
20 ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, isopentyl, neopentyl or isohexyl group, or the like and the "aralkyl group" means an aryl-lower alkyl group, and there can, for example, be mentioned a benzyl or phenethyl group, or the like. Further, the
25 "lower alkoxy group" means a lower alkyl-O-group wherein the lower alkyl part has the above meaning, an there can, for example, be mentioned a methoxy, ethoxy, n-propoxy, isopropoxy or tert-butoxy group, or the like, and the "aralkyloxy group" means an aralkyl-O-group wherein the
30 aralkyl part has the above meaning, and there can, for example, be mentioned a benzyloxy or phenethyloxy group, or the like. Further, the "lower alkylene group" may be either of straight-chain and branched-chain, and there can, for example, be mentioned a methylene, ethylene,
35 trimethylene, tetramethylene, methylmethylene, propylene, ethylethylene or 1,2-dimethylethylene group, or the like,



- 5 -

and as the "cycloalkyl group", there can be mentioned a cycloalkyl group having 3 to 7 carbon atoms such as, for example, a cyclopropyl, cyclopentyl, cyclohexyl or cycloheptyl group, and as the "aryl group", there can, for example, be mentioned a phenyl or naphthyl group, or the like.

When R_8 and R_9 represents "a 4- to 7-membered heterocyclic group containing one or two hetero atoms selected from S, O and N", as examples of the heterocyclic group, there can be mentioned azetidiny, furyl, thienyl, pyridyl, piperidiny, azepiny, thiazolyl, imidazolyl, oxazolyl, pyrimidiny and pyridazinyl groups, etc. Further, when R_8 and R_9 "combine together with the nitrogen atom to which they are bonded to form a 4- to 7-membered heterocyclic ring optionally further containing one hetero atom selected from S, O and N", as examples of the heterocyclic ring, there can be mentioned azetidino, pyrrolidino, piperidino, 1-perhydroazepiny, piperazino, morpholino and thiomorpholino groups, etc.

Thus, as examples of the group of $-N \begin{smallmatrix} R_8 \\ R_9 \end{smallmatrix}$,

there can be mentioned amino, methylamino, ethylamino, isopropylamino, tert-butylamino, dimethylamino, diethylamino, phenylamino, N-methyl-N-phenylamino, furylamino, pyridylamino, 2-thiazolylamino, imidazolylamino, pyrimidinylamino, pyrrolidino, piperidino and morpholino group, etc.

A group of compounds preferred among the compounds of the above formula (I) are compounds wherein

Q represents $\begin{array}{c} R_5 \\ | \\ -CH- \end{array} \begin{array}{c} S \\ \diagup \\ \diagdown \\ N \end{array}$ wherein R_5 has the same meaning

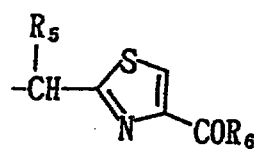
as defined above, and particularly preferable among them are compounds wherein four groups among R_1, R_2, R_3, R_4 ,



- 6 -

and R_5 represent the same groups as in dolastatin 10 (i.e., compounds wherein both R_1 and R_2 are isopropyl groups, R_3 is a sec-butyl group, R_4 is a methyl group and R_5 is a benzyl group), and only the residual one group
5 represents a group different from that in dolastatin 10.

Another group of preferred compounds are the compounds of the case where R_1 and R_2 represent isopropyl groups, R_3 represents a sec-butyl group, R_4 represents a

methyl group and Q represents  wherein

10 R_5 represents a benzyl group and R_6 has the same meaning as defined above.

Further, another group of preferred compounds are compounds of the case where Q represents $-A_2-R_7$ wherein A_2 represents a lower alkylene group and R_7 has
15 the same meaning as defined above, and particularly preferable among them are compounds wherein both R_1 and R_2 represent isopropyl groups, R_3 represents a sec-butyl group, R_4 represents a methyl group and R_7 represents an aryl group.

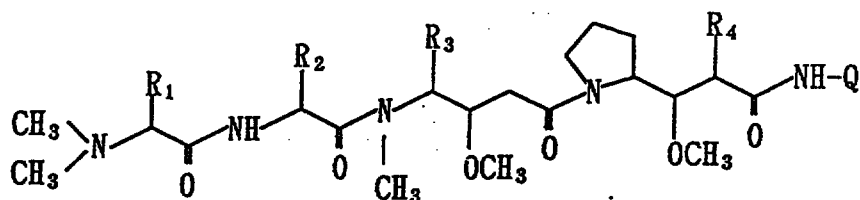
20 In the compounds of the aforesaid formula (I) of this invention, the carbon atoms to which the substituents R_1 , R_2 , R_3 , R_4 , R_5 and methoxy groups are bonded are asymmetric carbon atoms, and can take arbitrary R or S configurations, and all these are included in the scope
25 of this invention, but in view of the point of pharmacological activities, compounds which have the same configuration as dolastatin 10 are preferable.

As representative examples of the compounds of the above formula (I) provided by this invention, there
30 can be mentioned the following ones besides those mentioned in the later-described Examples.

This Sheet Has Been Replaced



- 7 -



Compound No.	R ₁	R ₂	R ₃	R ₄	Q
1	i-Pr	i-Pr	i-Bu	Me	
2	Et	"	s-Bu	"	
3	n-Pr	"	"	"	
4	i-Bu	"	"	"	
5	i-Pr	n-Pr	"	"	
6	"	i-Bu	"	"	
7	"	i-Pr	H	"	
8	"	"	Et	"	
9	"	"	t-Bu	"	
10	"	"	1-Me-Bu	"	
11	"	"	i-Pe	"	
12	"	"	Bzl	"	
13	"	"	s-Bu	Et	
14	"	"	"	n-Pr	

THIS SHEET HAS BEEN
REPLACED



- 8 -

15	"	"	"	Me	
16	"	"	"	"	
17	"	"	"	"	
18	"	"	"	"	
19	"	"	"	"	
20	"	"	"	"	
21	"	"	"	"	
22	"	"	"	"	
23	"	"	"	"	
24	"	"	"	"	
25	"	"	"	"	
26	"	"	"	"	

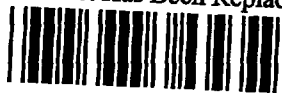


27	"	"	"	"	-Np
28	"	"	"	"	-CH ₂ - 
29	"	"	"	"	-CH(Me)CH ₂ -Ph
30	"	"	"	"	-(CH ₂) ₄ -Ph
31	"	s-Bu	"	"	-CH ₂ CH ₂ Ph
32	"	i-Pr	i-Pr	"	"
33	"	"	n-Pr	"	"
34	"	"	s-Bu	H	"
35	"	"	"	Et	"

In the above groups, Me, Et, Pr, Bu, Pe, Bzl, Ph and Np mean a methyl group, an ethyl group, a propyl group, a butyl group, a pentyl group, a benzyl group, a phenyl group and a naphthyl group, respectively.

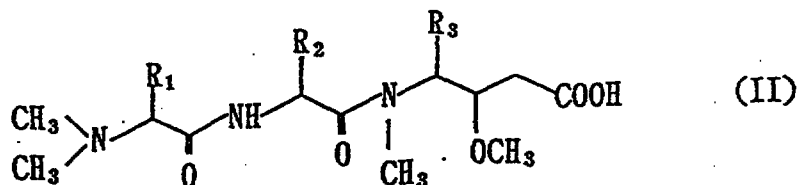
The tetrapeptide derivatives of the above formula (I) can exist as a salt, and examples of such salts, there can be mentioned hydrochlorides, hydrobromides, trifluoroacetates, p-toluenesulfonates and acetates.

According to this invention, a tetrapeptide derivative of the above formula (I) can be prepared by condensing amino acid or peptide fragments, for example according to the liquid phase synthesis method (see, E. Schröder and K. Lübke, "The Peptides", volume 1, pages 76-136, 1965, published by Academic Press) well known in the field of peptide chemistry. However, it is most preferable to synthesize these tetrapeptide derivatives by condensing a tripeptide fragment of the following



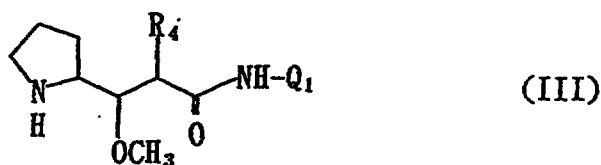
- 10 -

formula (II)

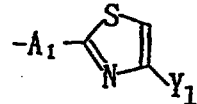


wherein R_1 , R_2 and R_3 have the same meanings as defined above,

5 with a fragment of the following formula (III)



wherein R_4 has the same meaning as defined

above, Q_1 represents a group of $-A_1$  or

10 $-A_2-R_7$ wherein Y_1 represents a hydrogen atom or a methoxycarbonyl group, and A_1 , A_2 and R_7 have the same meanings as defined above,

because each fragments of the formulae (II) and (III) is easy to synthesize and is free from racemization at their condensation step. A compound in the case where, in the
15 group Q, the substituent Y at the 4-position of the thiazole ring represents the group $-COR_6$ other than a methoxycarbonyl group, can be obtained by preparing a compound of the formula (I) wherein Y represents a methoxycarbonyl group and then converting the methoxycarbonyl
20 group to a group $-COR_6$.

The reaction can generally be carried out by treating the above fragments (II) and (III) with a condensing agent, e.g. dicyclohexylcarbodiimide (DCC), diphenyl



- 11 -

phosphoryl azide (DPPA) or diethyl phosphorocyanidate (DEPC), a so-called BOP reagent, or the like in an inert solvent such as, for example, chloroform, ethyl acetate, tetrahydrofuran (THF), dimethylformamide (DMF) or acetonitrile, if necessary in the presence of an organic base such as, for example, triethylamine, N-methylmorpholine or diisopropylethylamine (DIEA).

The reaction temperature is usually -10°C to room temperature, preferably around 0°C , and as for use rates of the compound of the formula (III), the organic base and the condensing agent based on the compound of the formula (II), it is advantageous to use the compound of the formula (III) of at least one mole, preferably of the order of 1.0 to 1.1 moles, the organic base of the order of 2 moles, and the condensing agent of the equimolar order, respectively per mole of the compound of the formula (II).

The conversion of a compound wherein the substituent Y at the 4-position of the thiazole ring in the group Q represents a methoxycarbonyl group to a compound wherein the substituent Y represents a group $-\text{CO}-\text{R}_6$ can easily be carried out, for example, when R_6 represents a hydroxyl group, by hydrolyzing the former compound with an alkali; when R_6 represents a lower alkoxy group or an aralkyloxy group, by esterifying a compound wherein R_6 represents a hydroxyl group according to

a conventional method; and when R_6 represents $-\text{N} \begin{smallmatrix} \text{R}_8 \\ \text{R}_9 \end{smallmatrix}$,

by treating the compound wherein the substituent Y represents a methoxycarbonyl group with ammonia, a primary amine or a secondary amine.

Thus, the desired tetrapeptide derivatives of the formula (I) are formed, and the isolation and purification thereof from the reaction mixtures can be carried



- 12 -

out by recrystallization, ion exchange chromatography, gel filtration, high performance liquid chromatography, etc.

5 The compounds of the above formulae (II) and (III) used as starting materials in the above reaction are novel compounds not disclosed in prior literatures, and can easily be prepared by condensing amino acids, which are constituents thereof, according to a liquid phase synthesis method.

10 The tetrapeptide derivatives of the formula (I) of this invention have a higher cytostatic activity than dolastatin 10, and are useful for treatment of acute myelocytic leukemia, acute lymphocytic leukemia, chronic melanoma, pulmonary adenocarcinoma, neuroblastoma, pulmo-
15 nary small cell carcinoma, breast cancer, colon cancer, ovary cancer, bladder cancer, etc.

 The screening of cytostatic activity was made using lymphocytic leukemia P388 cells. The results are shown in the following Table.



- 13 -

Table

<u>Example No. of Compound</u>	<u>ED₅₀ (μg/ml)</u>
1	3.1×10^{-6}
4	1.7×10^{-6}
5	3.1×10^{-5}
15	2.4×10^{-7}
16	2.7×10^{-5}
23	$< 1.0 \times 10^{-6}$
25	$< 1.0 \times 10^{-6}$
26	$< 1.0 \times 10^{-6}$
28	2.9×10^{-6}
Dolastatin 10	7.0×10^{-4}

The compounds of this invention, when used as a drug, can be used by formulating them into any dosage form of solid forms (e.g., tablets, hard capsules, soft capsules, granules, powders, fine granules, pills, troches, etc.), semi-solid forms (e.g., suppositories, ointments, etc.) and liquid forms (e.g., injections, emulsions, suspensions, lotions, sprays, etc.). As nontoxic additives usable in the above formulations, there can, for example, be mentioned starches, gelatin, glucose, lactose, fructose, maltose, magnesium carbonate, talc, magnesium stearate, methylcellulose, carboxymethylcellulose or salts thereof, gum arabic, polyethylene glycol, p-hydroxybenzoic acid alkyl esters, syrups, ethanol, propylene glycol, vaseline, carbowax, glycerol, sodium chloride, sodium sulfite, sodium phosphate, citric acid, etc. The drug can also contain another therapeutically effective drug.

The content of the compound of this invention

THIS SHEET HAS BEEN
REPLACED



- 14 -

in the drug varies depending on the dosage form, but it is generally preferable that the drug contains the compound at a concentration of 0.1 to 50 wt% in the case of solid and semi-solid forms, and at a concentration of 0.05 to 10 wt% in the case of liquid form.

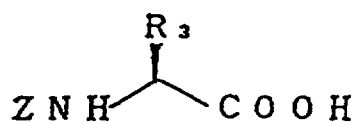
The dose of the compound of this invention can widely be varied depending on the kind of warm-blooded animals including human beings and a subject, administration routes, the seriousness of symptoms, the diagnoses of doctors, etc., but can generally be on the order of 0.01 to 50 mg/kg per day. However, it is of course possible to administer the compound in an amount smaller than the lower limit of the above range or in an amount larger than the upper limit thereof in accordance with the seriousness of the symptom of the patient and the diagnosis of the doctor as mentioned above. The above dose can be administered once a day or in divided several portions per day.

This invention is further described below according to Referential Examples and Examples.

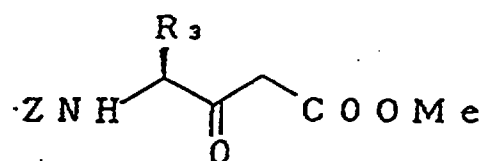
As for the structure of compounds corresponding to compound numbers used in Referential Examples and Examples, please refer to the following Flow sheets 1 to 6. Therein, Z, Me, Bu^t, Boc and Bzl represent a benzyl-oxycarbonyl group, a methyl group, a tert-butyl group, a tert-butoxycarbonyl group and a benzyl group, respectively, and R₁, R₂, R₃, R₄, R₅, R₆ and -A₂-R₇ have the aforesaid meanings.



Flow Sheet 1

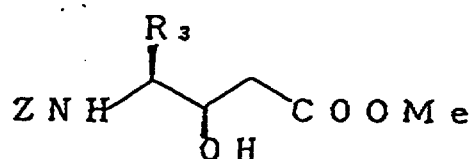


Referential
Example 1



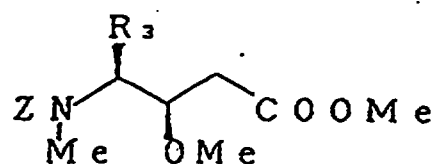
Compound 1

Referential
Example 2



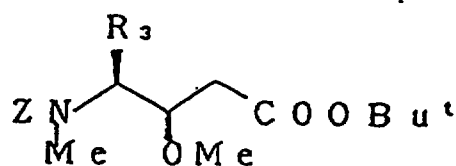
Compound 2

Referential
Example 3



Compound 3

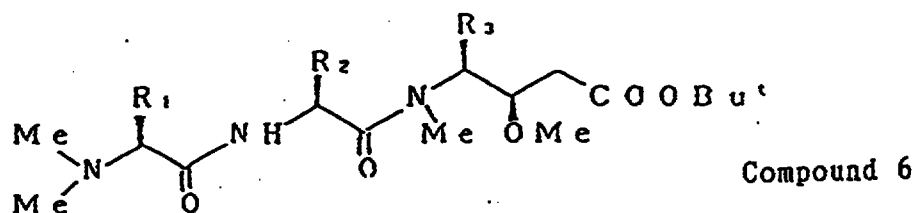
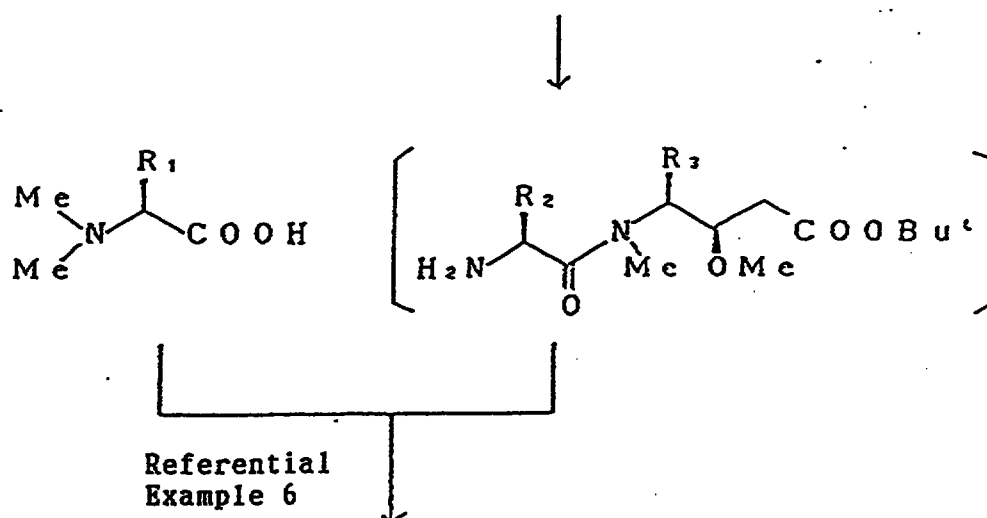
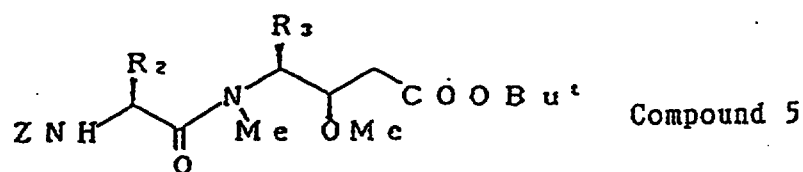
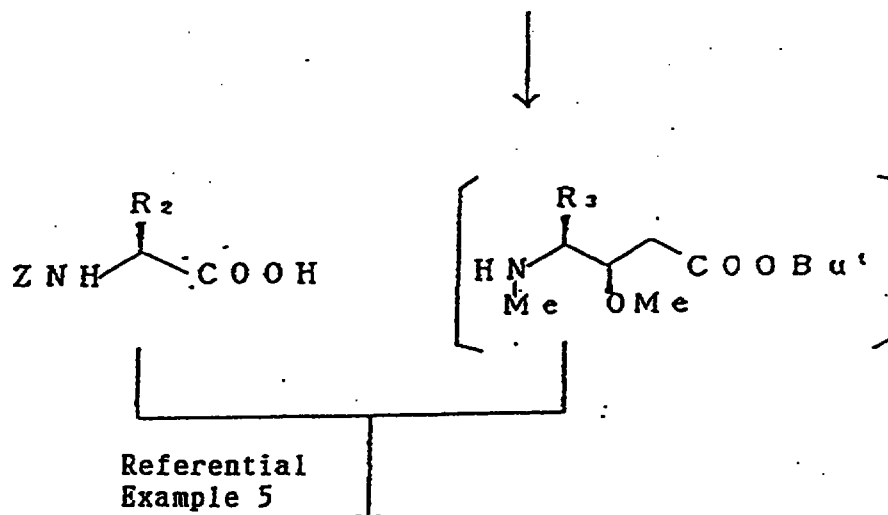
Referential
Example 4



Compound 4



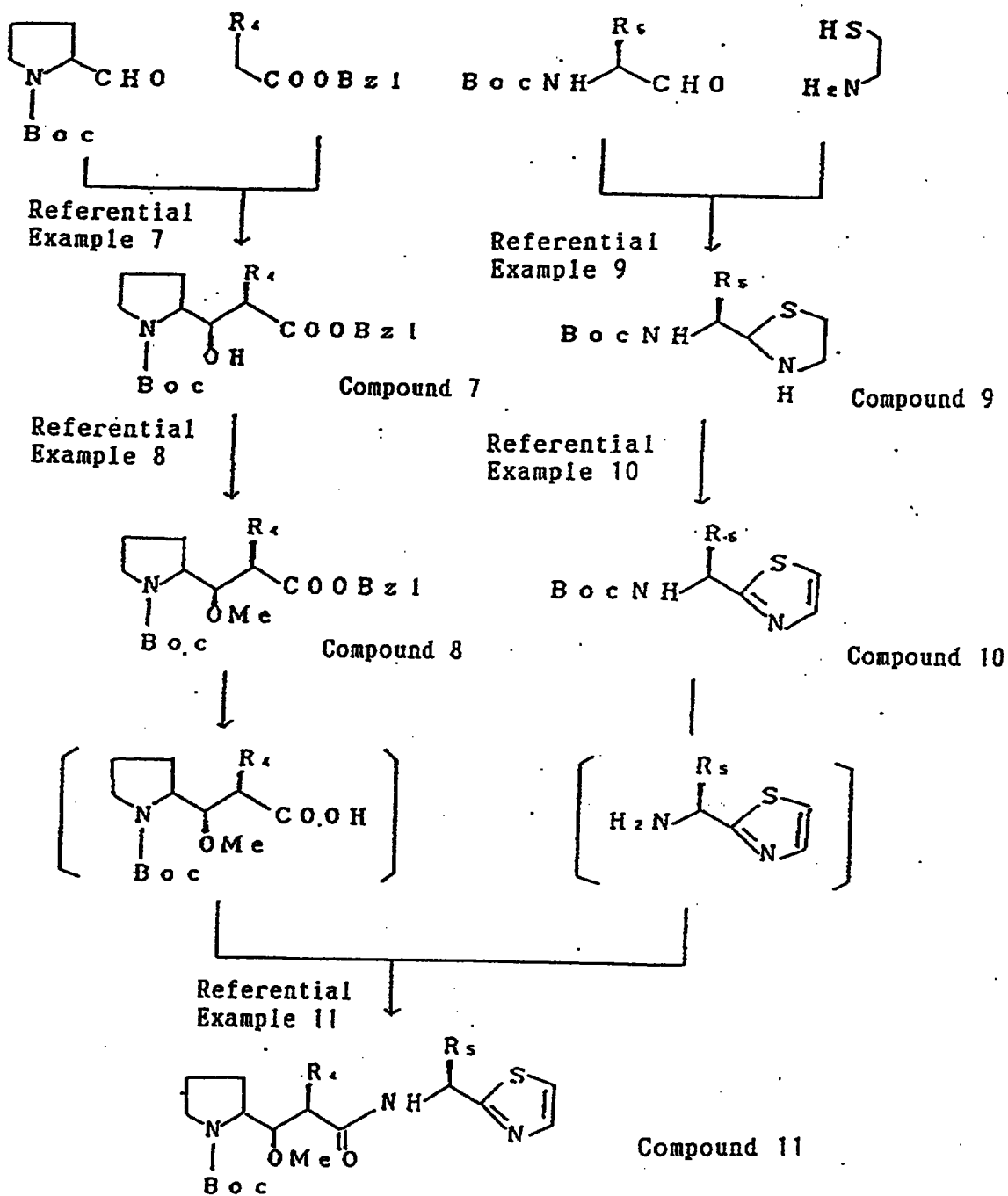
- 16 -





- 17 -

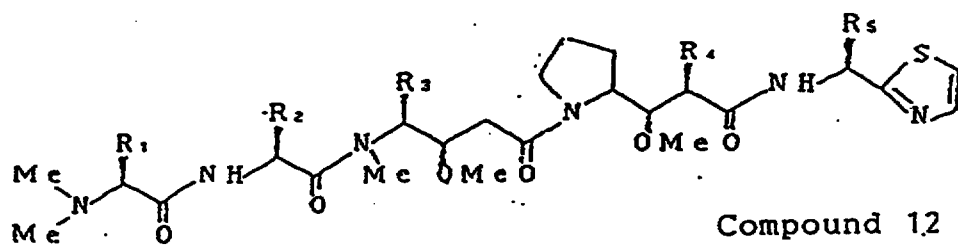
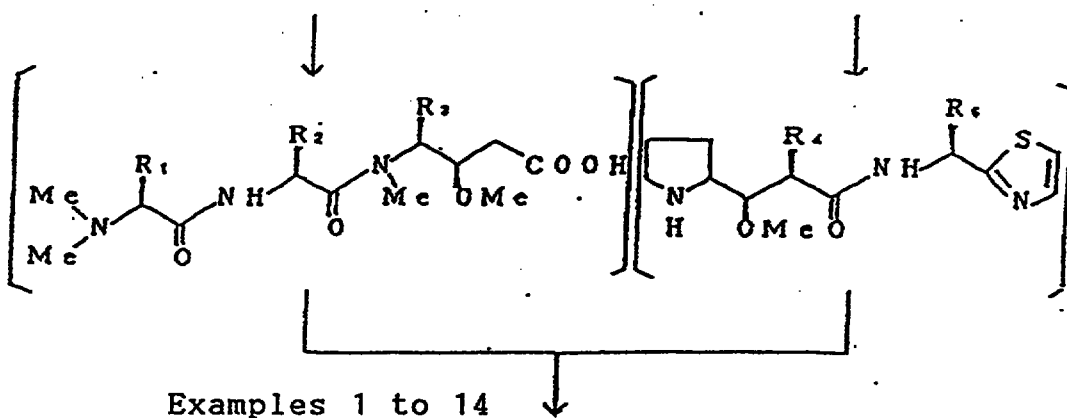
Flow sheet 2



Flow sheet 3

Compound 6

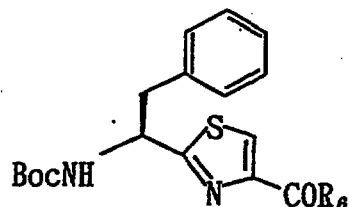
Compound 11



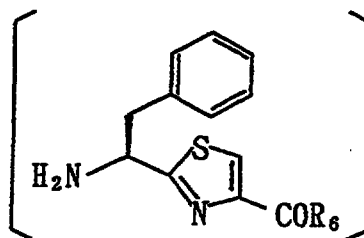
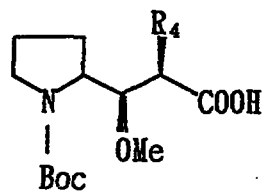


Flow sheet 4

Referential Example 12
($R_6 = \text{OMe} \rightarrow \text{OCH}_2\text{Ph}$)

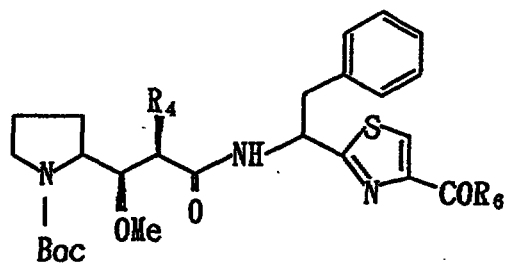


Compound 13



Compound 14

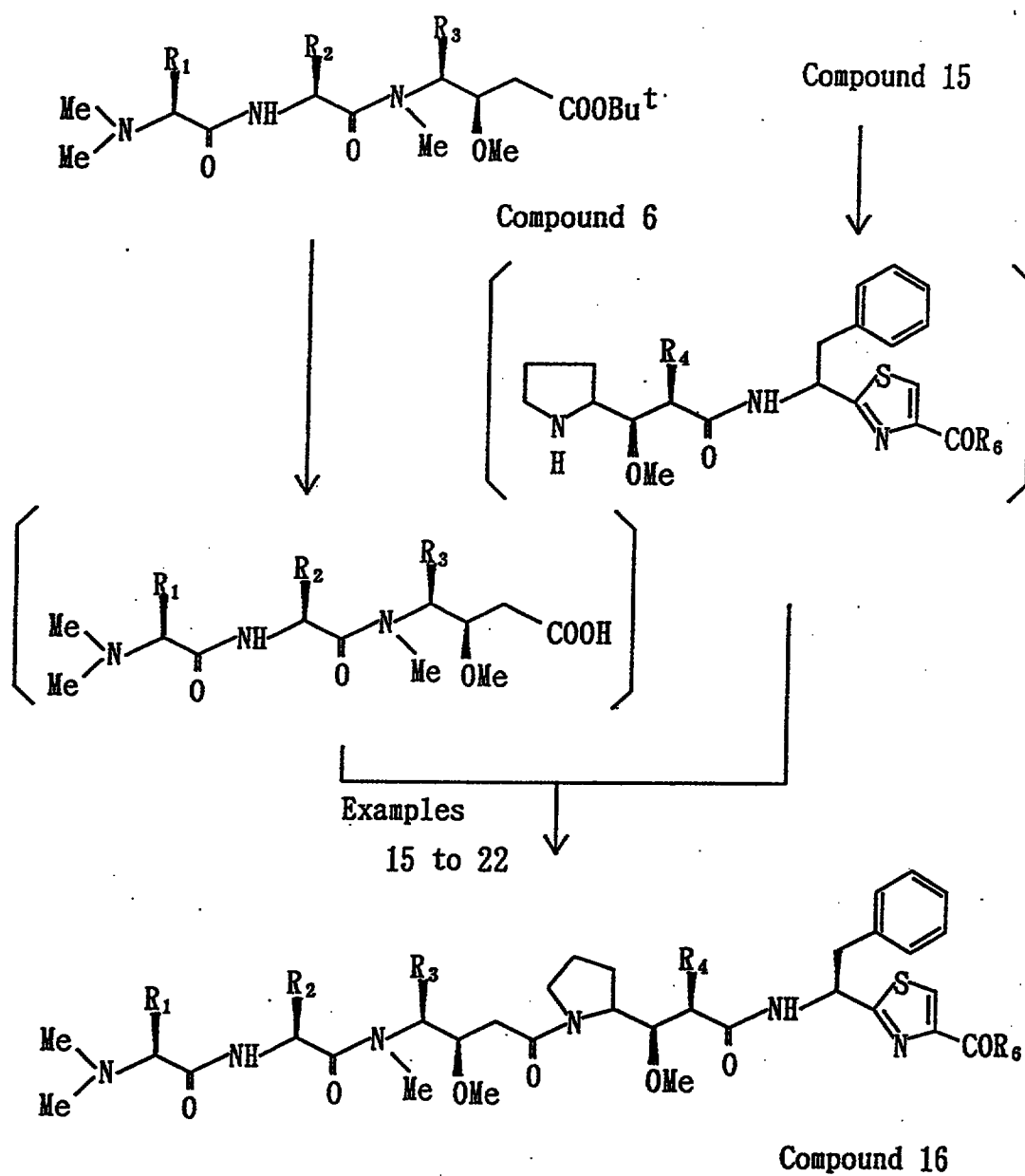
Referential Example 13

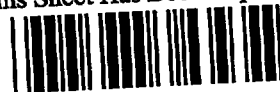


Compound 15

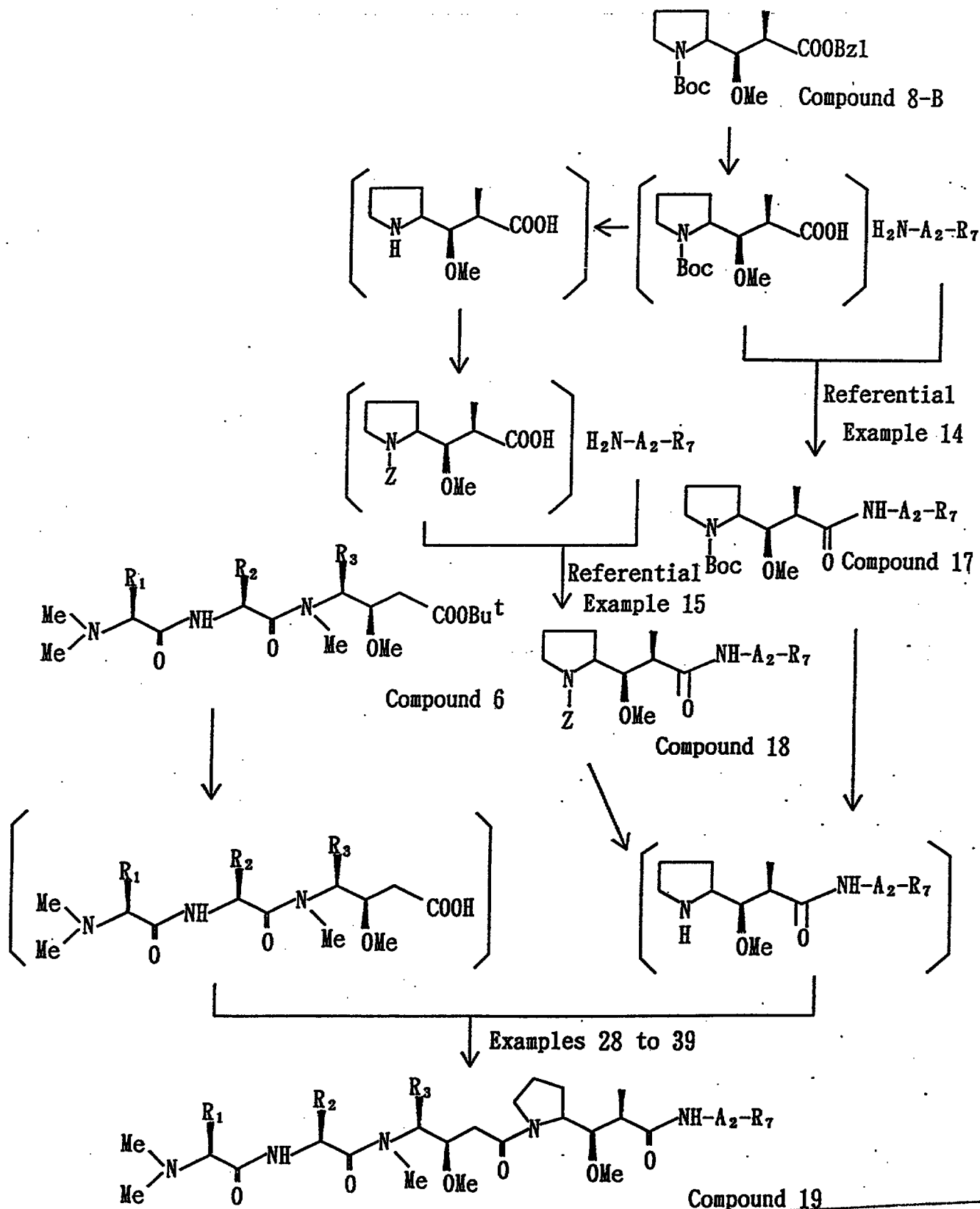


Flow Sheet 5





Flow Sheet 6





- 22 -

Referential Example 1-A

Preparation of compound 1-A (Compound 1 wherein $R_3=CH_3$)

11.15 g (50 mmoles) of Z-alanine is dissolved
5 in 140 ml of tetrahydrofuran, 9.72 g (60 mmoles) of
carbonyldiimidazole is added, and the mixture is stirred at
room temperature for 4 to 5 hours.

On the other hand, 17.16 g (110 mmoles) of
malonic acid monomethyl ester potassium salt and 7.60 g
10 (80 mmoles) of anhydrous magnesium chloride are suspended
in 150 ml of tetrahydrofuran and is stirred for 6 hours
with heating on a water bath of 55°. The reaction mix-
ture is cooled with ice, the above reaction solution is
poured thereinto all at once, the cooling bath is immedi-
15 ately removed, and the mixture is stirred at room tempe-
rature for 24 to 48 hours.

A small amount of water is added to the reac-
tion solution, and a clear supernatant is decanted from
the deposited waxy precipitate, and concentrated under
20 reduced pressure to obtain an oily matter. Ethyl acetate
and ice-cooled 4N hydrochloric acid are added to each of
the above waxy residue and this oily matter, each of the
mixtures is shaken to dissolve, the two solutions are
combined, the layers are separated, and the aqueous layer
25 is extracted again with ethyl acetate. The ethyl acetate
layer is washed with ice-cooled 2N hydrochloric acid and
saturated aqueous sodium bicarbonate and dried, and the
solvent is distilled off to obtain 13.50 g of a pale
yellow oily matter. This is purified by silica gel
30 column chromatography (eluent : ethyl acetate-n-hexane
(1 : 1)) to obtain the desired compound 1-A as a color-
less - pale yellow oily matter. 12.96 g (92.9%).

$[\alpha]_D^{26} -17.7^\circ$ (c=1.01, MeOH)

35 1H -NMR (CDCl₃, δ) 1.38(3H, d, J=7.1 Hz), 3.55(2H, s), 3.72(3
H, s), 4.45(1H, m, J=7.1 Hz), 5.11(2H, s),
5.25~5.55(1H, m), 7.34(5H, s)

This Sheet Has Been Replaced



- 23 -

Referential Examples 1-B, 1-C, 1-D and 1-E were carried out in all the same manner as in Referential Example 1-A to obtain compounds 1-B, 1-C, 1-D and 1-E as oily matters, respectively.

THIS SHEET HAS BEEN
REPLACED

Referential Example	Compound	R ₃	Yield	[α] _D [*]	¹ H-NMR (CDCl ₃ , δ)
1-B	1-B	CH ₃ CH ₂ CH ₂ -	88.9%	N. D.	0.75~1.05(6H, m)、1.1~2.1(4H, m)、 3.54(2H, s)、3.72(3H, s)、4.1~4.6(1H, m)、 5.11(2H, s)、5.2~5.5(1H, m)、7.34(5H, s)
1-C	1-C	$\begin{array}{c} \text{CH}_3 \\ \text{CH}_3 \end{array} > \text{CH}-$	87.5%	-22.3° (26°)	0.82(3H, d, J=6.8 Hz)、1.03(3H, d, J=6.8 Hz)、 2.0~2.4(1H, m)、3.54(2H, s)、3.72(3H, s)、 4.2~4.6(1H, m)、5.11(2H, s)、5.1~5.5 (1H, m)、7.34(5H, s)
1-D	1-D	$\begin{array}{c} \text{CH}_3 \\ \text{CH}_3 \end{array} > \text{CH}-\text{CH}_2-$	93.7%	-35.2° (26°)	0.8~1.05(6H, m)、1.1~1.9(3H, m)、3.55 (2H, s)、3.71(3H, s)、4.1~4.6(1H, m)、 5.11(2H, s)、7.33(5H, s)
1-E	1-E	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \text{CH}_3 \end{array} > \text{CH}-$	99.2%	-27.6° (26°)	0.6~1.5(9H, m)、3.54(2H, s)、3.71(3H, s)、 4.2~4.5(1H, m)、5.10(2H, s)、5.15~5.45 (1H, m)、7.34(5H, s)、

*) c=1.00、MeOH



Referential Example 2-A

Preparation of compound 2-A (Compound 2 wherein $R_3=CH_3$)

12.96 g (46.45 mmoles) of compound 1-A obtained
5 in Referential Example 1-A is dissolved in 380 ml of
methanol, and 3.56 g (93.67 mmoles) of sodium borohydride
is added thereto all at once under stirring at -78° .
Cooling and stirring are continued for 6 hours, ice-
cooled 1N hydrochloric acid is added gradually, and when
10 it is confirmed that the mixture becomes acidic, the
mixture is concentrated under reduced pressure, and the
deposited oily matter is extracted with ethyl acetate.
The ethyl acetate layer is washed with saturated aqueous
sodium bicarbonate and dried, and the solvent is dis-
15 tilled off to obtain 12.93 g of crystals. The crystals
are recrystallized from isopropyl ether to obtain the
desired compound 2-A as colorless needles of a melting
point of 78° . 11.09g (85.0%).

$[\alpha]_D^{28} -4.4^\circ$ (c=1.00, MeOH)

20 As $C_{14}H_{19}NO_5$

Calculated C=59.77% H=6.81% N=4.98%

Found C=59.83% H=6.92% N=5.07%

1H -NMR ($CDCl_3$, δ) 1.15(3H, d, J=6.8 Hz), 2.35~2.55(2H, m),
3.70(3H, s), 3.85~4.15(1H, m), 5.09(2H,
25 s), 7.34(5H, s)

Referential Examples 2-B, 2-C, 2-D and 2-E were
carried out in all the same manner as in Referential
Example 2-A to obtain compounds 2-B, 2-C, 2-D and 2-E,
respectively.





This Sheet Has Been Replaced

Referential Example	Compound	R ₃	Yield	Melting point	[α] _D [*]	Analytical value	¹ H-NMR (CDCl ₃ , δ)
2-B	2-B	CH ₃ CH ₂ CH ₂ -	88.1%	126°	-13.6° (27°)	C ₁₆ H ₂₃ NO ₅ ★ C 62.12% H 7.49% N 4.53% ★ C 61.99% H 7.51% N 4.75%	0.75~1.05(3H, m)、1.1~1.7 (4H, m)、2.35~2.55(2H, m)、 3.69(3H, s)、3.8~4.15 (1H, m)、4.65~4.95(1H, m)、 5.10(2H, s)、7.34(5H, s)
2-C	2-C	$\begin{array}{c} \text{CH}_3 \\ \text{CH}_3 \end{array} \text{CH-}$	80.7%	81°	+9.6° (28°)	C ₁₆ H ₂₃ NO ₅ ★ C 62.12% H 7.49% N 4.53% ★ C 62.16% H 7.51% N 4.70%	0.87(3H, d, J=6.5 Hz)、0.95 (3H, d, J=6.5 Hz)、1.9~ 2.35(1H, m)、2.4~2.6 (2H, m)、3.18(1H, br, d)、 3.69(3H, s)、4.45~4.80 (1H, m)、5.10(2H, s)、 7.34(5H, s)
2-D	2-D	$\begin{array}{c} \text{CH}_3 \\ \text{CH}_3 \end{array} \text{CH-CH}_2\text{-}$	81.9%	100°	-21.5° (26°)	C ₁₇ H ₂₅ NO ₅ ★ C 63.14% H 7.79% N 4.33% ★ C 63.05% H 7.76% N 4.62%	0.90(3H, d, J=6.2 Hz)、0.92 (3H, d, J=6.2 Hz)、1.1~1.8 (3H, m)、2.35~2.55(2H, dd)、 3.69(3H, s)、3.8~4.15 (1H, m)、4.65~4.95(1H, m)、 5.10(2H, s)、7.34(5H, s)

THIS SHEET HAS BEEN
REPLACED



This Sheet Has Been Replaced

Referential Example 2 (continued)

Referential Example	Compound	R ₃	Yield	Melting point	[α] _D *	Analytical value	¹ H-NMR (CDCl ₃ , δ)
2-E	2-E	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \text{CH}_3 \end{array} \text{CH-}$	86.4%	77°	+6.9° (27°)	$\text{C}_{17}\text{H}_{25}\text{NO}_5$ ★ C 63.14% H 7.79% N 4.33% ★ C 63.12% H 7.78% N 4.35%	0.8~1.05(6H, m)、1.3~1.95 (3H, m)、2.4~2.6(2H, m)、 3.1~3.25(1H, br, d)、 3.69(3H, s)、3.8~4.15 (1H, m)、4.66(1H, br, d)、 5.10(2H, s)、7.34(5H, s)

*) c=1.00、MeOH

★ Caluculated

★ Found

THIS SHEET HAS BEEN
REPLACED

Referential Example 3-A

Preparation of compound 3-A (Compound 3 wherein $R_3=CH_3$)

9.78 g (34.80 mmoles) of the compound obtained
5 in Referential Example 2-A is dissolved in 100 ml of
dimethylformamide, 40.0 g (172.41 mmoles) of silver oxide
and 50 ml of methyl iodide are added, and the mixture is
stirred for 5 hours in a water bath of 35°. The mixture
is filtered, silver oxide is washed with dimethylforma-
10 mide, and the filtrate and washings are combined and
concentrated under reduced pressure at 50° or below. The
residue is thoroughly extracted with ethyl acetate, the
ethyl acetate layer is washed with 5% sodium thiosulfate
and saturated aqueous sodium bicarbonate and dried, and
15 the solvent is distilled off to obtain 10.43 g of a
yellow oily matter. The oily matter is purified by
silica gel column chromatography (eluent : benzene-ethyl
acetate (5 : 1)) to obtain the desired Compound 3-A as a
pale yellow oily matter. 7.63 g (71.0%).

20 $[\alpha]_D^{25} -39.8^\circ$ (c=1.03, MeOH)

^1H-NMR (CDCl₃, δ) 1.21(3H, d, J=6.8 Hz), 2.47(2H, d, J=6.2
Hz), 2.80(3H, s), 3.38(3H, s), 3.64(3H,
s), 5.13(2H, s), 7.34(5H, s)

Referential Examples 3-B, 3-C, 3-D and 3-E were
25 carried out in all the same manner as in Referential
Example 3-A to obtain compounds 3-B, 3-C, 3-D and 3-E as
oily matters, respectively.

This Sheet Has Been Replaced



THIS SHEET HAS BEEN
REPLACED

This Sheet Has Been Replaced

THIS SHEET HAS BEEN
REPLACED

Referential Example	Compound	R ₃	Yield	[α] _D *	¹ H-NMR (CDCl ₃ , δ)
3-B	3-B	CH ₃ CH ₂ CH ₂ -	72.5%	-50.7° (25°)	0.75~1.05(3H, m)、1.05~1.8(4H, m)、2.48 (2H, t, J=5.5 Hz)、2.75(3H, s)、3.36, 3.38 (3H, s)、3.63(3H, s)、5.13(2H, s)、 7.34(5H, s)
3-C	3-C	$\begin{array}{c} \text{CH}_3 \\ \text{CH}_3 \end{array} \text{CH-}$	67.9%	-20.7° (26°)	0.8~1.15(6H, m)、1.8~2.2(1H, m)、 2.4~2.6(2H, m)、2.80(3H, s)、3.31, 3.38 (3H, s)、3.65, 3.66(3H, s)、5.13(2H, s)、 7.33(5H, s)
3-D	3-D	$\begin{array}{c} \text{CH}_3 \\ \text{CH}_3 \end{array} \text{CH-CH}_2\text{-}$	83.9%	-34.2° (26°)	0.7~1.0(6H, m)、1.25~1.6(3H, m)、2.47 (2H, t, J=5.6 Hz)、2.74(3H, s)、3.35, 3.37 (3H, s)、3.62(3H, s)、5.13(2H, s)、 7.33(5H, s)
3-E	3-E	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \text{CH}_3 \end{array} \text{CH-}$	74.4%	-4.0° (27°)	0.7~1.1(6H, m)、1.1~1.9(3H, m)、 2.4~2.6(2H, m)、2.78(3H, s)、3.29, 3.38 (3H, s)、3.66(3H, s)、3.75~4.2(2H, m)、 5.13(2H, s)、7.33(5H, s)

*) c=1.00, MeOH



- 30 -

Referential Example 4-A

Preparation of compound 4-A (Compound 4 wherein $R_3=CH_3$)

6.60 g (21.36 mmoles) of the compound 3-A
5 obtained in Referential Example 3-A is dissolved in 100 ml of dioxane, 23.5 ml (23.5 mmoles) of 1N sodium hydroxide is added, and the mixture is stirred at room temperature for 2 to 3 hours. 20% citric acid is added to the reaction solution to make the pH 4.0, the mixture
10 is concentrated under reduced pressure, and the deposited oily matter is extracted with ethyl acetate. The ethyl acetate layer is washed with saturated saline and dried, and the solvent is distilled off to give a colorless - pale yellow oily matter.

15 This is dissolved in 60 ml of dichloromethane, 0.8 ml of concentrated sulfuric acid is added, and the mixture is shaken with 25 ml of isobutene in a pressure bottle at room temperature for 48 to 96 hours. The reaction solution is poured into saturated aqueous sodium
20 bicarbonate, nitrogen gas is blown therein to remove isobutene and the greater part of dichloromethane, the deposited oily matter is extracted with ethyl acetate, and the ethyl acetate layer is washed with saturated aqueous sodium bicarbonate and dried. The solvent is
25 distilled off, and the remaining yellow oily matter (7.32 g) is purified by silica gel column chromatography (eluent : benzene-ethyl acetate (10 : 1)) to obtain 6.31 g (84.1%) of the desired compound 4-A as a colorless - pale yellow oily matter.

30 $[\alpha]_D^{27} -33.0^\circ$ (c=1.02, MeOH)

1H -NMR (CDCl₃, δ) 1.21(3H, d, J=6.8 Hz), 1.44(9H, s), 2.38(2
H, d, J=6.2 Hz), 2.82(3H, s), 3.38(3H, s),
3.5~3.85(1H, m), 3.85~4.4(1H, m), 5.13(2
H, s), 7.34(5H, s)

35 Referential Examples 4-B, 4-C, 4-D and 4-E were carried out in all the same manner as in Referential

- 31 -

Example 4-A to obtain compounds 4-B, 4-C, 4-D and 4-E as oily matters, respectively.

This Sheet Has Been Replaced



THIS SHEET HAS BEEN
REPLACED

Referential Example	Compound	R ₃	Yield	[α] _D [†]	¹ H-NMR (CDCl ₃ , δ)
4-B	4-B	CH ₃ CH ₂ CH ₂ -	84.0%	-42.0° (27°)	0.7~1.05(3H, m)、1.44(9H, s)、2.25~2.5 (2H, m)、2.77(3H, s)、3.37, 3.38(3H, s)、 3.5~3.75(1H, m)、3.75~4.25(1H, m)、 5.13(2H, s)、7.33(5H, s)
4-C	4-C	$\begin{array}{c} \text{CH}_3 \\ \text{CH}_3 \end{array} \text{CH-}$	75.2%	-17.8° (27°)	0.8~1.1(6H, m)、1.45(9H, s)、1.75~2.25 (1H, m)、2.25~2.5(2H, m)、2.81(3H, s)、 3.31, 3.39(3H, s)、3.7~4.05(2H, m)、 5.13(2H, s)、7.33(5H, s)
4-D	4-D	$\begin{array}{c} \text{CH}_3 \\ \text{CH}_3 \end{array} \text{CH-CH}_2\text{-}$	75.8%	-28.9° (26°)	0.7~1.0(6H, m)、1.44(9H, s)、2.25~2.5 (2H, m)、2.77(3H, s)、3.36(3H, s)、 3.45~3.75(1H, m)、3.8~4.4(1H, m)、 5.13(2H, s)、7.33(5H, s)
4-E	4-E	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \text{CH}_3 \end{array} \text{CH-}$	87.6%	-12.0° ** (27°)	0.7~1.05(8H, m)、1.45(9H, s)、1.65~1.75 (1H, m)、2.3~2.45(2H, m)、2.79(3H, s)、 3.29, 3.39(3H, s)、3.75~4.2(2H, m)、 5.13(2H, s)、7.33(5H, s)

*) c=1.00、MeOH

**) CHCl₃

THIS SHEET HAS BEEN
REPLACED

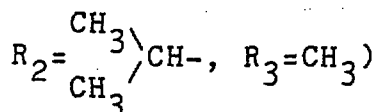




- 33 -

Referential Example 5-A

Preparation of compound 5-A (Compound 5 wherein



0.70 g (2.00 mmoles) of compound 4-A obtained
 5 in Referential Example 4-A is dissolved in 20 ml of
 t-butanol-water (9 : 1), 0.1 g of 5% palladium carbon is
 added, and the mixture is stirred under a hydrogen stream
 for 2 hours. After the reaction, the catalyst is fil-
 10 tered and washed, and the filtrate and the washings are
 concentrated under reduced pressure. The remaining oily
 matter is dissolved in 30 ml of benzene, the solution is
 concentrated again under reduced pressure, and the opera-
 tion is repeated once more. The resultant oily matter is
 15 dissolved together with 0.56 g (2.23 mmoles) of Z-valine
 in 10 ml of acetonitrile, and 0.43 g (2.09 mmoles) of DCC
 is added under ice cooling and stirring. Soon, crystals
 appear. Stirring is continued at 0° for at least 3
 hours, and then overnight during which time the tempera-
 20 ture is allowed to rise as the ice melts, the reaction
 mixture is diluted with ethyl acetate, and the crystals
 are filtered and washed with ethyl acetate. The filtrate
 and the washings are concentrated under reduced pressure,
 the syrupy residue is dissolved in ethyl acetate, the
 insoluble matters, if any, are removed by filtration, the
 25 ethyl acetate solution is washed with ice-cooled 2N
 hydrochloric acid and saturated aqueous sodium bicarbo-
 nate and dried, and the solvent is distilled off to
 obtain 1.01 g of a colorless oily matter. This is puri-
 fied by silica gel column chromatography (eluent : ben-
 30 zene-ethyl acetate (5 : 1)) to obtain 0.67 g (74.4%) of
 the desired compound 5-A as a colorless oily matter..

$[\alpha]_D^{25} -31.4^\circ$ (c=1.02, MeOH)

$^1\text{H-NMR}$ (CDCl_3 , δ) 0.8~1.1(6H, m), 1.17(3H, d, J=6.8 Hz),
 1.45(9H, s), 2.25~2.45(2H, m), 3.00(3H, s),

This Sheet Has Been Replaced



- 34 -

3.37(3H, s)、3.68(1H, dd, J=12.1 Hz, 6.2

Hz)、4.35~4.75(2H, m)、5.09(2H, s)、5.56

(1H, br, d)、7.33(5H, s)

The following compounds were obtained in all
5 the same manner as in Referential Example 5-A.

THIS SHEET HAS BEEN
REPLACED

Referential Example	Compound	R ₂	R ₃	Yield	[α] _D *	¹ H-NMR (CDCl ₃ , δ)
5-B	5-B	$\begin{array}{c} \text{CH}_3 \\ \text{CH}_3 \end{array} \text{CH-}$	$\text{CH}_3\text{CH}_2\text{CH}_2\text{-}$	80.2%	-46.2° (27°)	0.7~1.1(9H, m)、1.45(9H, s)、 2.25~2.45(2H, m)、2.98(3H, s)、 3.37(3H, s)、3.6~3.8(1H, m)、 4.3~4.7(2H, m)、5.10(2H, s)、 5.50(1H, br, d)、7.33(5H, s)
5-C	5-C	$\begin{array}{c} \text{CH}_3 \\ \text{CH}_3 \end{array} \text{CH-}$	$\begin{array}{c} \text{CH}_3 \\ \text{CH}_3 \end{array} \text{CH-}$	73.6%	-32.9° (25°)	0.75~1.1(12H, m)、1.46(9H, s)、 2.25~2.45(2H, m)、2.97(3H, s)、 3.35(3H, s)、3.7~4.0(1H, m)、 4.3~4.7(2H, m)、5.09(2H, s)、 5.48(1H, br, d)、7.32(5H, s)
5-D	5-D	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \text{CH}_3 \end{array} \text{CH-}$	$\begin{array}{c} \text{CH}_3 \\ \text{CH}_3 \end{array} \text{CH-}$	76.6%	-33.6° (25°)	0.7~1.2(14H, m)、1.46(9H, s)、 2.25~2.45(2H, m)、2.99(3H, s)、 3.34(3H, s)、3.6~4.0(1H, m)、4.35~ 4.65(2H, dd, J=9.5Hz, 6.6Hz)、5.09 (2H, s)、5.41(1H, br, d)、7.32(5H, s)
5-E	5-E	$\begin{array}{c} \text{CH}_3 \\ \text{CH}_3 \end{array} \text{CH-}$	$\begin{array}{c} \text{CH}_3 \\ \text{CH}_3 \end{array} \text{CH-CH}_2\text{-}$	85.4%	-41.1° (27°)	0.7~1.1(12H, m)、1.46(9H, s)、 2.3~2.45(2H, m)、2.97(3H, s)、 3.35(3H, s)、3.5~3.8(1H, m)、 4.4~4.7(2H, m)、5.08(2H, s)、 5.3~5.6(1H, br, d)、7.34(5H, s)

Referential Example 5 (continued)

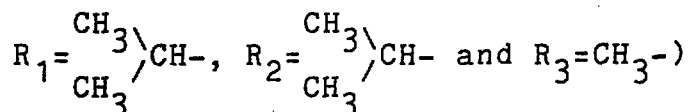
Referential Example	Compound	R ₂	R ₃	Yield	[α] _D *	¹ H-NMR (CDCl ₃ , δ)
5-F	5-F	H	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \text{CH}_3 \end{array} \text{CH-}$	58.7%	-11.0° (27°)	0.7~1.1(6H, m)、1.45(9H, s)、 2.3~2.45(2H, m)、2.82(3H, s)、 3.36(3H, s)、3.75~4.15(3H, m)、 5.12(2H, s)、5.8(1H, br)、 7.34(5H, s)
5-G	5-G	$\begin{array}{c} \text{CH}_3 \\ \text{CH}_3 \end{array} \text{CH-}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \text{CH}_3 \end{array} \text{CH-}$	81.3%	-22.2° (26°)	0.65~1.1(12H, m)、1.45(9H, s)、 2.25~2.45(2H, m)、2.96(3H, s)、 3.34(3H, s)、3.75~4.05(1H, m)、 4.35~4.7(2H, m)、5.10(2H, s)、 5.50(1H, br, d)、7.33(5H, s)
5-H	5-H	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \text{CH}_3 \end{array} \text{CH-}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \text{CH}_3 \end{array} \text{CH-}$	62.6%	-26.5° (24°)	0.7~1.1(12H, m)、1.45(9H, s)、 2.25~2.45(2H, m)、2.97(3H, s)、 3.34(3H, s)、3.7~4.05(1H, m)、 4.35~4.7(2H, m)、5.09(2H, s)、 5.43(1H, br, d)、7.32(5H, s)
5-I	5-I	PhCH ₂ -	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \text{CH}_3 \end{array} \text{CH-}$	59.3%	-4.8° (26°)	0.65~1.1(9H, m)、1.48(9H, s)、 2.1~2.3(2H, m)、2.9~3.1(2H, m)、 3.31(3H, s)、3.5~4.0(2H, m)、 4.3~4.7(1H, m)、5.06(2H, s)、 5.55(1H, br, d)、7.25(5H, s)、7.31(5H, s)

* c=1.00、MeOH



Referential Example 6-A

Preparation of compound 6-A (Compound 6 wherein

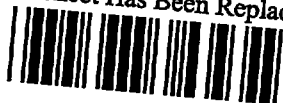


0.65 g (1.44 mmoles) of compound 5-A obtained
 5 in Referential Example 5-A is dissolved in 15 ml of
 t-butanol-water (9 : 1), 50 mg of 5% palladium carbon is
 added, and the mixture is stirred under a hydrogen stream
 for 2 hours. After the reaction, the catalyst is removed
 by filtration and washed, and the filtrate and the wash-
 10 ings are concentrated under reduced pressure. The oily
 residue is dissolved in 30 ml of benzene and the solution
 is concentrated again under reduced pressure, and this
 operation is further repeated once more. The resultant
 oily matter is dissolved in 6 ml of dimethylformamide,
 15 0.25 g (1.72 mmoles) of N,N-dimethylvaline and 0.29 g
 (1.78 mmoles) of DEPC are added, the mixture is stirred
 at room temperature until clear solution is obtained,
 then the solution is cooled with ice, and the solution of
 0.17 g (1.68 mmoles) of triethylamine in 1 ml of dimeth-
 20 ylformamide is added dropwise over a period of 4 minutes.
 Stirring is continued at 0° for at least 4 hours and then
 overnight during which time the temperature is allowed to
 rise as the ice melts, the resultant clear reaction
 mixture is diluted with ethyl acetate, and the ethyl
 25 acetate solution is thoroughly washed with saturated
 aqueous sodium bicarbonate and then dried. The solvent
 is distilled off, and 0.66 g of the remaining pale brown
 oily matter is purified by silica gel column chromatogra-
 phy (eluent : ethyl acetate-hexane (1 : 1)) to obtain
 30 0.46 g (71.9%) of the desired compound 6-A as a colorless
 oily matter.

$[\alpha]_D^{27} -56.5^\circ$ (c=1.00, MeOH)

$^1\text{H-NMR}$ (CDCl_3 , δ) 0.8~1.1(12H, m), 1.15(3H, d, J=7.0 Hz),
 1.45(9H, s), 2.27(6H, s), 3.05(3H, s),

This Sheet Has Been Replaced



- 38 -

3.38(3H, s)、3.55~3.85(1H, m)、4.35~
4.65(1H, m)、4.65~4.95(1H, m)、0.88(1H,
br, d)

The following compounds were obtained in all
5 the same manner as in Referential Example 6-A.

THIS SHEET HAS BEEN
REPLACED

Referential Example	Compound	R ₁	R ₂	R ₃	Yield	Melting point	[α] _D [*]	¹ H-NMR (CDCl ₃ , δ)
6-B	6-B	H	$\begin{array}{c} \text{CH}_3 \\ \diagup \\ \text{CH}- \\ \diagdown \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \diagup \\ \text{CH}-\text{CH}_2- \\ \diagdown \\ \text{CH}_3 \end{array}$	58.1%	oil	-52.7° (26°)	0.7~1.1(12H, m)、1.46(9H, s)、 2.28(6H, s)、3.01(3H, s)、3.37 (3H, s)、3.5~3.85(1H, m)、 4.45~4.95(3H, m)、 7.65(1H, br, d)
6-C	6-C	CH ₃	$\begin{array}{c} \text{CH}_3 \\ \diagup \\ \text{CH}- \\ \diagdown \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \diagup \\ \text{CH}- \\ \diagdown \\ \text{CH}_3 \end{array}$	61.8%	oil	-42.9° (26°)	0.75~1.05(12H, m)、1.25 (3H, d, J=7.0 Hz)、1.46(9H, s)、 2.25(6H, s)、3.01(3H, s)、 3.35(3H, s)、3.7~4.05(1H, m)、 4.73(1H, dd, J=9.5Hz, 6.6Hz)、 7.62(1H, br, d)
6-D	6-D	$\begin{array}{c} \text{CH}_3 \\ \diagup \\ \text{CH}- \\ \diagdown \\ \text{CH}_3 \end{array}$	H	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \diagup \\ \text{CH}- \\ \diagdown \\ \text{CH}_3 \end{array}$	90.3%	oil	-14.5° (26°)	0.7~1.1(12H, m)、1.46(9H, s)、 2.30(6H, s)、2.85(3H, s)、 3.37(3H, s)、3.8~4.0(1H, m)、 4.0~4.2(2H, m)、4.4~4.65 (1H, m)、7.0~7.15(1H, br)





Referential Example 6 (continued)

Referential Example	Compound	R ₁	R ₂	R ₃	Yield	Melting point	[α] _D	¹ H-NMR (CDCl ₃ , δ)
6-E	6-E	$\begin{array}{c} \text{CH}_3 \\ \diagup \\ \text{CH} \\ \diagdown \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \diagup \\ \text{CH} \\ \diagdown \\ \text{CH}_3 \end{array}$	CH ₃ CH ₂ CH ₂ -	70.3%	oil	-62.8° (28°)	0.7~1.1(15H, m), 1.45(9H, s), 2.25(6H, s), 3.02(3H, s), 3.37(3H, s), 3.5~3.85(1H, m), 4.35~4.65(1H, m), 4.74(1H, dd, J=9.2Hz, 6.4Hz), 6.84(1H, br, d)
6-F	6-F	$\begin{array}{c} \text{CH}_3 \\ \diagup \\ \text{CH} \\ \diagdown \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \diagup \\ \text{CH} \\ \diagdown \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \diagup \\ \text{CH} \\ \diagdown \\ \text{CH}_3 \end{array}$	77.5%	122°	-51.0° (27°)	0.7~1.15(18H, m), 1.46(9H, s), 2.25(6H, s), 3.02(3H, s), 3.35(3H, s), 3.7~4.0(1H, m), 4.3~4.6(1H, m), 4.65~4.9(1H, m), 6.86(1H, br, d)
6-G	6-G	$\begin{array}{c} \text{CH}_3 \\ \diagup \\ \text{CH} \\ \diagdown \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \diagup \\ \text{CH} \\ \diagdown \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \diagup \\ \text{CH} \\ \diagdown \\ \text{CH}_3 \end{array}$ -CH ₂ -	79.2%	oil	-57.8° (26°)	0.7~1.1(18H, m), 1.46(9H, s), 2.25(6H, s), 3.01(3H, s), 3.36(3H, s), 3.5~3.8(1H, m), 4.80(1H, dd, J=9.2Hz, 6.2Hz), 6.85(1H, br, d)

Referential Example 6 (continued)

Referential Example	Compound	R ₁	R ₂	R ₃	Yield	Melting point	[α] _D	¹ H-NMR (CDCl ₃ , δ)
6-H	6-H	$\begin{array}{c} \text{CH}_3 \\ \diagup \\ \text{CH}- \\ \diagdown \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \diagup \\ \text{CH}- \\ \diagdown \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \diagup \\ \text{CH}- \\ \diagdown \\ \text{CH}_3 \end{array}$	84.7%	112°	-44.4° (27°)	0.65~1.15(18H, m)、1.46 (9H, s)、2.26(6H, s)、3.01 (3H, s)、3.35(3H, s)、 3.7~4.05(1H, m)、4.80 (1H, dd, J=9.2 Hz, 6.4Hz)、 6.89(1H, br, d)
6-I	6-I	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \diagup \\ \text{CH}- \\ \diagdown \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \diagup \\ \text{CH}- \\ \diagdown \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \diagup \\ \text{CH}-\text{CH}_2- \\ \diagdown \\ \text{CH}_3 \end{array}$	80.1%	oil	-59.2° (26°)	0.7~1.1(18H, m)、1.46(9H, s)、 2.24(6H, s)、3.01(3H, s)、 3.36(3H, s)、3.5~3.85(1H, m)、 4.5~4.95(2H, m)、6.95 (1H, br, d)
6-J	6-J	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \diagup \\ \text{CH}- \\ \diagdown \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \diagup \\ \text{CH}- \\ \diagdown \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \diagup \\ \text{CH}- \\ \diagdown \\ \text{CH}_3 \end{array}$	68.6%	93°	-43.3° (27°)	0.7~1.1(18H, m)、1.46(9H, s)、 2.25(6H, s)、3.01(3H, s)、 3.34(3H, s)、3.7~4.0(1H, m)、 4.5~4.95(2H, m)、6.90 (1H, br, d)



This Sheet Has Been Replaced

- 41 -

THIS SHEET HAS BEEN
REPLACED

Referential Example 6 (continued)

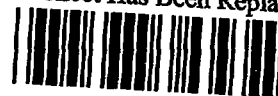
Referential Example	Compound	R ₁	R ₂	R ₃	Yield	Melting point	[α] _D	¹ H-NMR(CDCl ₃ , δ)
6-K	6-K	H	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH} \\ \diagup \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \diagdown \\ \text{CH} \\ \diagup \\ \text{CH}_3 \end{array}$	72.7%	Oil	-25.7° (24°) **	0.7-1.1(12H, m), 1.46(9H, s), 2.42(6H, s), 3.00(3H, s), 3.35(3H, s), 3.7-4.0(1H, m), 4.74(1H, dd, J=9.0Hz, J=6.4Hz), 7.75(1H, br. d)
6-L	6-L	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH} \\ \diagup \\ \text{CH}_3 \end{array}$	PhCH ₂ -	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \diagdown \\ \text{CH} \\ \diagup \\ \text{CH}_3 \end{array}$	86.0%	Oil	-18.0° (27°)	0.7-1.0(15H, m), 1.48(9H, s), 2.19(6H, s), 2.76(3H, s), 3.31(3H, s), 3.6-3.9(1H, m), 4.3-4.7(1H, m), 5.26(1H, dd, J=8.8Hz, 7.7Hz), 6.87(1H, br. d), 7.26(5H, s)
6-M	6-M	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH} \\ \diagup \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \diagdown \\ \text{CH} \\ \diagup \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \diagdown \\ \text{CH} \\ \diagup \\ \text{CH}_3 \end{array}$	72.5%	104°	-45.7° (27°)	0.7-1.1(18H, m), 1.46(9H, s), 2.27(6H, s), 3.01(3H, s), 3.34(3H, s), 3.7-4.0(1H, m), 4.82(1H, dd, J=9.2Hz, J=7.0Hz), 6.80(1H, br. d)

* c=1.00, MeOH

** CHCl₃(c=0.315)

THIS SHEET HAS BEEN
REPLACED

This Sheet Has Been Replaced



- 43 -

Referential Example 7

Preparation of compound 7-A (Compound 7 wherein $R_4=H$)

7 ml (15.5 mmoles) of a 23.8% LDA tetrahydro-
5 furan : hexane (1 : 1) solution is added dropwise to 10
ml of anhydrous tetrahydrofuran at -20°C under a nitrogen
atmosphere and stirring. Then the mixture is cooled to
 -78°C in a dry ice-acetone bath. 2.3 g (15 mmoles) of
benzyl acetate is added dropwise over a period of 30
10 minutes, the mixture is stirred at -78°C for 5 minutes,
and a solution of 2.0 g (10 mmoles) of Boc-L-prolinal in
10 ml of tetrahydrofuran is added dropwise over a period
of 1 hour. The mixture is stirred at -78°C for 10
minutes, 30 ml of ice-cooled 1N hydrochloric acid is
15 added, and the temperature is elevated up to room tempe-
rature. The mixture is extracted with ethyl acetate, the
extract is washed with water and dried, the solvent is
distilled off under reduced pressure, and the residue is
purified by silica gel flash chromatography using hexane
20 : ethyl acetate (5 : 1) as an eluent to obtain 1.12 g
(32.0%) of the desired compound 7-A as an oily matter.

$[\alpha]_D^{25} -23.7^{\circ}$ ($c=1.26$, CHCl_3)

MS 331, 276

$^1\text{H-NMR}$ (CDCl_3 , δ) 1.46(9H, s), 2.47(2H, d, $J=6.8$ Hz), 3.7~
25 4.3(2H, m), 5.15(2H, s), 7.2~7.4(5H, m)

Referential Example 8

Preparation of compound 8-A (Compound 8 wherein $R_4=H$)

560 mg (1.6 mmoles) of compound 7-A obtained in
30 Referential Example 7 is dissolved in 27 ml of dichloro-
methane, 202 μl (1.6 mmoles) of $\text{BF}_3 \cdot \text{Et}_2\text{O}$ is added thereto
under cooling in ice-salt bath, and an ether solution of
diazomethane (32 mmoles) is added dropwise over a period
of 30 minutes. After stirring for further two hours
35 under cooling in ice-salt bath, 2 ml of saturated aqueous
sodium bicarbonate is added. The insoluble matter is



- 44 -

removed by filtration, the filtrate is extracted with ethyl acetate, and the extract is washed with water and dried. The solvent is distilled off under reduced pressure, and the residue is purified by silica gel flash chromatography using hexane : ethyl acetate (5 : 1) as an eluent to obtain 378 mg (65.0%) of the desired compound 8-A as an oily matter.

$[\alpha]_D^{25}$ -58.7° (c=0.52, CHCl₃)

MS 241, 218

¹H-NMR (CDCl₃, δ) 1.46(9H, s), 2.47(2H, d, J=7.5 Hz), 3.34(3H, s), 3.6~4.3(2H, m), 5.14(2H, s), 7.2~7.4(5H, m)

Referential Example 9

Preparation of compound 9-A (Compound 9 wherein

R₅=CH₃)

6.82 g (60 mmoles) of cysteamine hydrochloride is dissolved in 50 ml of dimethylformamide, and the solution is neutralized with 8.4 ml of triethylamine. The solution is mixed with a solution of crude Boc-L-alaninal obtained by oxidizing 8.75 g (50 mmoles) of Boc-alaninol in 50 ml of dimethylformamide, and mixture is stirred overnight at room temperature. The solvent is distilled off under reduced pressure, the formed crystals are dissolved in ethyl acetate, the solution is washed with 10% citric acid and saturated aqueous sodium bicarbonate, and the ethyl acetate layer is dried. The solvent is distilled off under reduced pressure to obtain 4.93 g (42.6%) of the desired compound 9-A as white crystals.

Melting point 81.0-84.1°C

MS 232 (M⁺), 159

¹H-NMR (CDCl₃, δ) 1.24(3H, d, J=6.6 Hz), 1.45(9H, s), 2.1(1H, br), 4.42(1H, d, J=8.1 Hz)

Referential Example 10

Preparation of compound 10-A (Compound 10 wherein R₃=CH₃)



- 45 -

1.34 g (5.77 mmoles) of the compound 9-A obtained in Referential Example 9 and 12.5 g of manganese dioxide are stirred in 58 ml of benzene at 55°C for 1.5 hours. The suspension is filtered, the solvent is distilled off under reduced pressure, and the residue is purified by silica gel flash chromatography using hexane : ethyl acetate (5 : 1) as an eluent to obtain 118 mg (9.0%) of the desired compound 10-A as an oil matter.

$[\alpha]_D^{22} - 36.0^\circ$ (c=1.29, CH_2Cl_2)

MS 228(M^+) 172

$^1\text{H-NMR}$ (CD_2Cl_2 , δ) 1.43(9H, s), 1.56(3H, d, $J=7.2$ Hz), 4.9 ~5.2(2H, m), 7.26(1H, d, $J=3.3$ Hz), 7.67 (1H, d, $J=3.3$ Hz)

Referential Example 11-A

Preparation of compound 11-A (Compound 11 wherein $\text{R}_4=\text{H}$ and $\text{R}_5=\text{PhCH}_2$)

220 mg (0.604 mmole) of compound 8-A obtained in Referential Example 8 is dissolved in 9 ml of t-butanol : water (9 : 1), 50 mg of palladium carbon is added, and the mixture is stirred under a hydrogen stream. After completion of the reaction, the reaction solution is filtered, and the solvent is distilled off under reduced pressure to obtain 165 mg (0.604 mmole) of a solid matter. This is dissolved in 3 ml of acetonitrile, 267 mg (0.604 mmole) of the BOP reagent and 192 mg (0.604 mmole) of a trifluoroacetate salt obtained from known compound 10-B (compound 10 wherein $\text{R}_5=\text{CH}_2\text{Ph}$) are added, and then 195 mg (1.51 mmoles) of diisopropylethylamine is added dropwise under ice cooling. The mixture is stirred overnight at room temperature, the solvent is distilled off under reduced pressure, the residue is dissolved in dichloromethane, and the solution is washed successively with 10% citric acid, saturated aqueous sodium bicarbonate and saturated saline and dried. The solvent is distilled off under reduced pressure, and the resultant crude product is purified by silica gel column chromato-



- 46 -

graphy using dichloromethane-methanol (50 : 1)) as an eluent to obtain 262 mg (94.4%) of the desired compound 11-A as amorphous powder.

$[\alpha]_D^{27} - 86.4^\circ$ (c=0.43, MeOH)

5 MS 428, 368

$^1\text{H-NMR}$ (CDCl_3 , δ) 1.47(9H, s), 2.29(2H, d, J=6.2 Hz), 3.27(3H, s), 5.63(1H, m), 7.1~7.3(6H, m), 7.40(1H, d, J=3.3Hz)

Referential Example 11-B

10 Preparation of compound 11-B (Compound 11 wherein $\text{R}_4 = \text{CH}_3$ and $\text{R}_5 = \text{CH}_3$)

Compound 8-B (compound 8 wherein $\text{R}_4 = \text{CH}_3$), known compound, and compound 10-A obtained in Referential Example 10 are treated in all the same manner as in

15 Referential Example 11-A to obtain the desired compound 11-B. Yield 62.0%, an oily matter.

$[\alpha]_D^{26} - 100^\circ$ (c=1.3, MeOH)

MS 365, 324, 309

20 $^1\text{H-NMR}$ (CDCl_3 , δ) 1.26(3H, d, J=6.7 Hz), 1.48(9H, s), 1.63(3H, d, J=6.7 Hz), 3.43(3H, s), 5.2~5.6(1H, m), 7.23(1H, d, J=3.3 Hz), 7.69(1H, s, J=3.3 Hz)

Referential Example 12-A

25 Preparation of compound 13-C (Compound 13 wherein $\text{R}_6 = \text{OCH}_2\text{Ph}$)

870 mg (2.4 mmoles) of compound 13-A (compound 13 wherein $\text{R} = \text{OCH}_3$), known compound, is dissolved in 5 ml of ethanol, and 3 ml of 1N sodium hydroxide is added.

The mixture is stirred at room temperature for 30 minutes, the solvent is distilled off under reduced pressure, water is added, the mixture is acidified with citric acid, and the mixture is extracted with ethyl acetate. The extract is washed with water and dried over anhydrous sodium sulfate, and the solvent is distilled off under reduced pressure to obtain 680 mg (83.3%) of crystals of compound 13-B (compound 13 wherein $\text{R}_6 = \text{OH}$).

THIS SHEET IS
REPLACED



- 47 -

70 mg (0.2 mmole) of the crystals are dissolved in 0.5 ml of dichloromethane, 2.4 mg (0.02 mmole) of 4-dimethylaminopyridine and 30 mg (2.4 mmoles) of benzyl alcohol are added, and 50 mg (2.4 mmoles) of DCC is added under ice cooling. The mixture is stirred under ice cooling for 1 hour and then overnight at room temperature. The deposited crystals are removed by filtration, and the filtrate is diluted with ethyl acetate, washed with saturated aqueous sodium bicarbonate and saturated saline, and dried. The solvents are distilled off under reduced pressure, and the resultant crude product is purified by preparative TLC (developing solvent, hexane : ethyl acetate = 2 : 1) to obtain 79.6 mg (91.0%) of crystals (compound 13-C) having a melting point of 111.5 to 113.4°.

$[\alpha]^{26}_D +8.38^\circ$ (c=0.37, MeOH)

MS 438(M⁺), 382, 365

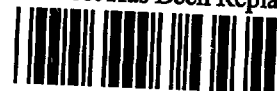
¹H-NMR (CDCl₃, δ) 1.38(9H, s), 3.31(2H, br. d, J=5.5 Hz), 5.1~5.3(2H, m), 5.4(2H, s), 7.0~7.5(10H, m), 8.04 (1H, s)

Referential Example 12-B

Preparation of compound 13-D (Compound 13 wherein R₆=NHPh)

28.2 mg (0.0809 mmole) of compound 13-B is dissolved in 0.5 ml of dichloromethane, 35.8 mg (1.0 equivalent) of the BOP reagent and 9 mg (1.2 equivalents) of aniline are added, and 15.7 mg (1.5 equivalents) of diisopropylethylamine is added dropwise under water cooling. The reaction solution is stirred overnight at room temperature and then concentrated under reduced pressure. The residue is dissolved in dichloromethane, and the solution is washed successively with 10% aqueous citric acid, saturated aqueous sodium bicarbonate and saturated saline, and dried. The crude product is purified by preparative TLC using hexane-ethyl acetate (2 : 1) as a developing solvent to obtain 35 mg (100%) of

This Sheet Has Been Replaced



- 48 -

the desired compound as crystals.

$[\alpha]^{26}_D$ -15.7° (c=0.305, MeOH)

MS 423, 368

$^1\text{H-NMR}$ (CDCl_3 , δ) 1.42(9H, s), 3.31(2H, d J=6.4), 4.9-5.4
(2H, m), 7.0~7.8(11H, m), 8.09(1H, s)

5

Referential Examples 12-C and 12-D are carried out in the same manner as above to obtain the following compounds.

THIS SHEET HAS BEEN
REPLACED

This Sheet Has Been Replaced

Referential Example	Compound	R ₆	Yield	[α] _D	MS	¹ H-NMR(CDCl ₃ , δ)
12-C	13-E	-NH-Bu ^t	100%	-21.0° (c=0.315, CHCl ₃) (25°)	404 348	1.40(9H, s), 1.49(9H, s), 3.27(2H, d, J=5.9Hz), 5.0-5.4(2H, m), 7.0-7.4 (6H, m), 7.93(1H, s)
12-D	13-F		96.4%	-28.2° (c=0.355, CHCl ₃) (25°)	417 361	1.41(9H, s), 3.25(2H, d, J=6.4Hz), 3.76(8H, br.), 5.0-5.5(2H, m), 6.9-7.3 (6H, m), 7.84(1H, s)

THIS SHEET HAS BEEN
REPLACED.

Referential Example 13-A

Preparation of compound 15-A (Compound 15, wherein $R_4=CH_3$ and $R_6=OCH_3$)

330 mg (0.91 mmole) of compound 13-A, known compound, is dissolved in 1.4 ml of dichloromethane, 0.6 ml of trifluoroacetic acid is added under ice cooling, and the mixture is stirred at room temperature for 1.5 hours. The solvent is distilled off under reduced pressure, and ether is added to cause crystallization. The white crystals are collected by filtration and dried. Yield : 339 mg (98.9%).

314 mg (0.835 mmole) of the crystals are dissolved in 4.2 ml of acetonitrile, 369 mg (0.835 mmole) of the BOP reagent and 240 mg (0.835 mmole) of compound 14 ($R_4=CH_3$), known compound, are added, and 270 mg (2.09 mmoles) of diisopropylethylamine is added dropwise under ice cooling. The reaction solution is stirred overnight at room temperature, diluted with ethyl acetate, washed successively with 10% aqueous citric acid, saturated aqueous sodium bicarbonate and saturated saline, and dried. The crude product is purified by silica gel column chromatography using dichloromethane-methanol (50 : 1) as an eluent to obtain 431 mg (97.1%) of the desired compound 15-A as powder.

$[\alpha]^{20}_D$ -61.5° (c=1.02, MeOH)

MS 499, 440

1H -NMR ($CDCl_3$, δ) 1.12(3H, d, $J=7.0$ Hz), 1.47(9H, s), 3.35(3H, s), 3.95(3H, s), 5.4~5.7(1H, m), 7.22(6H, m), 8.05(1H, s)

The following compounds were obtained in the same manner as in Referential Example 13-A.

This Sheet Has Been Replaced



THIS SHEET HAS BEEN
REPLACED

Referential Example	Compound	R ₄	R ₆	Yield	[α] _D (MeOH)	MS	¹ H-NMR(CDCl ₃ , δ)
13-B	15-B	CH ₃	OCH ₂ Ph	86.8%	-55.8° (c=1.11) (25°)	607 575	1.21(3H, d, J=7.0Hz)、 1.46(9H, s)、3.34(3H, s)、 5.40(2H, s)、5.56(1H, m)、 7.1-7.5(11H, m)、8.05(1H, m)
13-C	15-C	H	OCH ₃	83.7%	-67.2° (c=1.96) (25°)	518 490	1.46(9H, s), 3.27(3H, s)、 3.95(3H, s)、5.60(1H, m)、 7.0-7.3(6H, m)、8.05(1H, s)
13-D	15-D	CH ₃	NHPh	99.7%	-38.5° (c=0.33) (27°)	593 560	1.15(3H, d, J=7.0Hz)、 1.48(9H, s)、3.38(3H, s)、 7.1-7.8(11H, m)、8.08(1H, s)



(Continued)

Referential Example	Compound	R ₄	R ₆	Yield	[α] _D (MeOH)	MS	¹ H-NMR(CDCl ₃ , δ)
13-E	15-E	CH ₃	NH-Bu ⁺	96.9%	-62.3° (c=0.326) (27°)	572 540	1.14(3H, d, J=7.0Hz)、 1.48(18H, s)、2.05(2H, br.)、 3.36(3H, s)、7.0-7.4(6H, m)、 7.92(1H, s)
13-F	15-F	CH ₃		100%	-57.5° (c=0.323) (26°)	587 554	1.14(3H, d, J=6.8Hz)、 1.48(9H, s)、3.36(3H, s)、 7.0-7.4(6H, m)、7.82(1H, s)

- 52 -

THIS SHEET HAS BEEN
REPLACED

This Sheet Has Been Replaced

Referential Example 14-A

Preparation of compound 17-A (Compound 17 wherein $-A_2-R_7=CH_2CH_2-Ph$)

30.5 mg (0.106 mmole) of a carboxylic acid
5 obtained from known compound 8-B (compound 8 wherein $R_4=CH_3$) according to Referential Example 11 is dissolved in 1 ml of acetonitrile, 51.6 mg (1.1 equivalents) of the BOP reagent and 14.1 mg (1.1 equivalents) of phenethyl-
10 amine are added, and 20.6 mg (1.5 equivalents) of diisopropylethylamine is added dropwise under ice cooling. The reaction solution is stirred overnight at room temperature, and concentrated under reduced pressure. The residue is dissolved in dichloromethane, and the solution is washed successively with 10% aqueous citric acid,
15 saturated aqueous sodium bicarbonate and saturated saline, and dried. The crude product is purified by preparative TLC using dichloromethane-methanol (10 : 1) as a developing solvent to obtain 38.3 mg (92.5%) of the desired compound 17-A as powder.

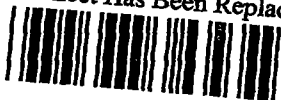
20 $[\alpha]^{26}_D -21.6^\circ$ ($c=1.02$, MeOH)

MS 358, 317

^1H-NMR ($CDCl_3$, δ) 1.19(3H, d, $J=7.0$ Hz), 1.48(9H, s), 3.37
(3H, s), 7.1-7.4(5H, m)

The following compounds were obtained in all
25 the same manner as in Referential Example 14-A.

This Sheet Has Been Replaced



THIS SHEET HAS BEEN
REPLACED

Referential Example	Compound	-A ₂ -R ₇	Yield	[α] _D (MeOH)	MS	¹ H-NMR(CDCl ₃ , δ)
14-B	17-B	-Ph	80.6%	-59.4° (c=0.204) (29°)	330 289	1.33(3H, d, J=7.0Hz)、 1.48(9H, s)、3.51(3H, s)、 7.0-7.7(5H, m)
14-C	17-C	-CH ₂ Ph	86.6%	-19.2° (c=0.285) (29°)	344 303	1.26(3H, d, J=7.0Hz)、 1.47(9H, s)、3.42(3H, s)、 4.43(2H, d, J=5.7Hz)、 7.30(5H, s)
14-D	17-D	-CH ₂ CH ₂ CH ₂ Ph	87.2%	-36.1° (c=0.23) (28°)	372 331	1.22(3H, d, J=7.0Hz)、 1.47(9H, s)、3.44(3H, s)、 7.1~7.4(5H, m)
14-E	17-E	$ \begin{array}{c} \text{CH}_3 \\ \diagup \\ \text{(S)} \\ \text{---CH}_2\text{---} \\ \diagdown \\ \text{Ph} \end{array} $	87.7%	-111.6° (c=0.865) (25°)	391 358	1.23(3H, d, J=7.0Hz)、 1.45(9H, s)、3.40(3H, s)、 7.1~7.4(5H, m)

(Continued)

Referential Example	Compound	-A ₂ -R ₇	Yield	[α] _D (MeOH)	MS	¹ H-NMR(CDCl ₃ , δ)
14-F	17-F	<chem>CH2CH2C1CCCCC1</chem>	66.8%	-36.5° (c=1.145) (26°)	397 364	1.23(3H, d, J=6.8Hz), 1.48(9H, s), 3.44(3H, s)
14-G	17-G	<chem>CH2CH2C1=CC=C2C(=C1)C3CCCCC3=C2</chem>	91.2%	-27.2° (c=0.328) (29°)	440 408	1.20(3H, d, J=7.0Hz), 1.47(9H, s), 3.37(3H, s), 7.2-8.2(7H, m)
14-H	17-H	<chem>C1=CN=C(S1)C=C1</chem>	71.7%	-73.2° (c=0.342) (29°)	337 296	1.35(3H, d, J=7.0Hz), 1.47(9H, s), 3.51(3H, s), 6.97(1H, d, J=3.7Hz), 7.45(1H, d, J=3.7Hz)

This Sheet Has Been Replaced

- 55 -

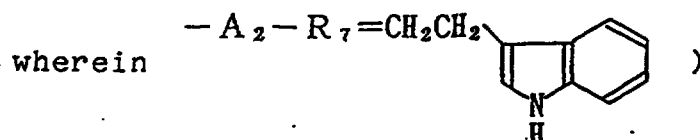
THIS SHEET HAS BEEN
REPLACED



- 56 -

Referential Example 15

Preparation of compound 18-A (Compound 18



Compound 18-A was obtained in the same manner
 5 as in Referential Example 14-A from tryptamine and a
 benzyloxycarbonylated carboxylic acid obtained from
 compound 8 as a starting material through the steps of
 debenylation (see Referential Example 11), de-tert-
 butoxycarbonylation and benzyloxycarbonylation as shown
 10 in Flow sheet 6.

$[\alpha]^{28}_D$ -6.4° ($c=1.41$, MeOH)

MS 593, 560

1H -NMR ($CDCl_3$, δ) 1.0-1.3(3H, m), 3.28(3H, s), 6.9-8.0(6H, m)

Example 1

15 Preparation of compound 12-A (Compound 12

wherein R_1 , $R_2 = \begin{matrix} CH_3 \\ \diagup \\ CH \end{matrix}$, $R_3 = \begin{matrix} CH_3 \\ \diagup \\ CH \end{matrix}-CH_2$, $R_4=CH_3$ and
 $R_5=PhCH_2$)

1 ml of concentrated hydrochloric acid is added
 with ice cooling to 108 mg (0.222 mmole) of compound 6-G
 20 obtained in Referential Example 6-G, and the mixture is
 stirred for 1 hour. The mixture is evaporated to dryness
 under reduced pressure, the residue is dissolved in 2 ml
 of dimethylformamide, and 0.15 ml of triethylamine is
 added under ice cooling. Although triethylamine hydro-
 25 chloride is deposited, the mixture is evaporated to
 dryness under reduced pressure and dried. On the other
 hand, 105 mg (0.222 mmole) of known compound 11-C (com-
 pound 11 wherein $R_4=CH_3$ and $R_5=PhCH_2$) is dissolved in 0.4
 ml of ethyl acetate, and 3.3 ml of 2N hydrogen chloride/-
 30 ethyl acetate is added under ice cooling. The mixture is
 stirred at room temperature for 1 hour, the solvent is

THIS SHEET HAS BEEN
REPLACED



- 57 -

distilled off under reduced pressure, and the residue is dried. The resultant hygroscopic crystals are dissolved in 1.6 ml of dimethylformamide and is added to the above tripeptide carboxylic acid, and 40 mg (0.222 mmole) of 5 90% DEPC and 62 μ l (0.444 mmole) of triethylamine are added under ice cooling. The mixture is stirred for 1 hour under ice cooling and then overnight at room temperature. The solvent is distilled off under reduced pressure, the residue is dissolved in dichloromethane, 10 and the solution is washed with saturated aqueous sodium bicarbonate and saturated saline and dried. The solvent is distilled off, the residue is purified by silica gel flash chromatography using dichloromethane-methanol (20 : 1) as an eluent, and the fractions containing the 15 desired compound is further purified by Sephadex LH-20 chromatography using hexane : dichloromethane : methanol (2 : 7.5 : 2.5) as an eluent to obtain 137 mg (78.4%) of the desired compound 12-A as amorphous powder.

$[\alpha]_D^{27} - 89.0^\circ$ (c=0.60, MeOH)

20 MS 741, 693

$^1\text{H-NMR}$ (CDCl_3 , δ) 2.32(6H, s), 2.96(3H, s), 3.32(3H, s),
3.40(3H, s), 5.56(1H, m), 6.8~7.3(9H, m),
7.73(1H, d, $J=3.3$ Hz)

Examples 2-14

25 The following compounds were obtained by carrying out Examples 2-14 in all the same manner as in Example 1.

THIS SHEET HAS BEEN
REPLACED

Example	Compound	R ₁	R ₂	R ₃	R ₄	R ₅	[α] _D (MeOH)	MS	¹ H-NMR (CD ₂ Cl ₂ , δ)
2	12-B	$\begin{array}{c} \text{CH}_3 \\ \text{CH}_3 \end{array} \text{CH-}$	H	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \text{CH}_3 \end{array} \text{CH-}$	CH ₃	PhCH ₂ -	-61.6° (25°) c=1.30	742(M+) 699 667	2.29(6H, s), 2.82 (3H, s), 3.31(3H, s), 3.33(3H, s), 5.52(1H, m), 6.9~7.4(9H, m), 7.73 (1H, d, J=3.3 Hz)
3	12-C	$\begin{array}{c} \text{CH}_3 \\ \text{CH}_3 \end{array} \text{CH-}$	$\begin{array}{c} \text{CH}_3 \\ \text{CH}_3 \end{array} \text{CH-}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \text{CH}_3 \end{array} \text{CH-}$	CH ₃	CH ₃	-84.9° (26°) c=1.02	665	1.24(3H, d, J=7.0 Hz), 1.60(3H, d, J=7.0 Hz), 2.23(6H, s), 2.99(3H, s), 3.30(3H, s), 3.39(3H, s), 6.75(1H, d, J=9.2 Hz), 7.12(1H, d, J=8.1 Hz), 7.25(1H, d, J=3.3 Hz), 7.66(1H, d, J=3.3 Hz),
4	12-D	$\begin{array}{c} \text{CH}_3 \\ \text{CH}_3 \end{array} \text{CH-}$	$\begin{array}{c} \text{CH}_3 \\ \text{CH}_3 \end{array} \text{CH-}$	$\begin{array}{c} \text{CH}_3 \\ \text{CH}_3 \end{array} \text{CH-}$	CH ₃	PhCH ₂ -	-79.4° (23°) c=0.33	770(M+) 727 679	2.48(6H, s), 3.02 (3H, s), 3.32(6H, s), 7.0~7.4(9H, s), 7.71(1H, d, J=3.3 Hz)

This Sheet Has Been Replaced



Examples 2 to 14 (continued)

Example	Compound	R ₁	R ₂	R ₃	R ₄	R ₅	[α] _D (MeOH)	MS	¹ H-NMR (CD ₂ Cl ₂ , δ)
5	12-E	$\begin{array}{c} \text{CH}_3 \diagdown \\ \text{CH} \\ \text{CH}_3 \diagup \end{array}$	$\begin{array}{c} \text{CH}_3 \diagdown \\ \text{CH} \\ \text{CH}_3 \diagup \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \diagdown \\ \text{CH} \\ \text{CH}_3 \diagup \end{array}$	H	PhCH ₂ -	-76.1° (28°) c=0.47	770(M+) 727 679	2.27(6H, s), 3.02(3H, s), 3.25(3H, s), 3.29(3H, s), 5.62(1H, m), 6.88 (1H, d, J=9.0 Hz), 7.1~7.3(6H, m), 7.59 (1H, d, J=7.9 Hz), 7.73(1H, d, J=3.3 Hz),
6	12-F	$\begin{array}{c} \text{C}_2\text{H}_5 \diagdown \\ \text{CH} \\ \text{CH}_3 \diagup \end{array}$	$\begin{array}{c} \text{CH}_3 \diagdown \\ \text{CH} \\ \text{CH}_3 \diagup \end{array}$	$\begin{array}{c} \text{CH}_3 \diagdown \\ \text{CH}-\text{CH}_2- \\ \text{CH}_3 \diagup \end{array}$	CH ₃	PhCH ₂ -	-87.5° (24°) c=0.33	798(M+) 755 741	2.21(6H, s), 2.96(3H, s), 3.32(3H, s), 3.38(3H, s), 5.52(1H, m), 6.85 (1H, d, J=8.8 Hz), 7.0~7.3(7H, m), 7.72(1H, d, J=3.3 Hz),
7	12-G	$\begin{array}{c} \text{C}_2\text{H}_5 \diagdown \\ \text{CH} \\ \text{CH}_3 \diagup \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \diagdown \\ \text{CH} \\ \text{CH}_3 \diagup \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \diagdown \\ \text{CH} \\ \text{CH}_3 \diagup \end{array}$	CH ₃	PhCH ₂ -	-80.6° (24°) c=0.37	812(M+) 755 721	2.21(6H, s), 3.01(3H, s), 3.31(6H, s), 5.51(1H, m), 6.79(1H, d, J=9.2 Hz), 7.1~7.5(7H, m), 7.71 (1H, d, J=3.3 Hz),

This Sheet Has Been Replaced

Examples 2 to 14 (continued)

Example	Compound	R ₁	R ₂	R ₃	R ₄	R ₅	[α] _D (MeOH)	MS	¹ H-NMR(CDCl ₃ , δ)
8	12-H	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH-} \\ \diagup \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH-} \\ \diagup \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH-CH}_2- \\ \diagup \\ \text{CH}_3 \end{array}$	H	PhCH ₂ -	-90.2° (c=0.34) (27°)	770 727	2.97(3H, s), 3.24 (3H, s), 3.36(3H, s), 5.63(1H, m), 7.1-7.3 (7H, m), 7.73(1H, d, J=3.3Hz)
9	12-I	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH-} \\ \diagup \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \diagdown \\ \text{CH-} \\ \diagup \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \diagdown \\ \text{CH-} \\ \diagup \\ \text{CH}_3 \end{array}$	CH ₃	PhCH ₂ -	-70.4° (c=0.26) (27°)	798 755	3.02(3H, s), 3.33 (6H, s), 5.56(1H, m), 7.1-7.3(7H, m), 7.73 (1H, d, J=3.1Hz)
10	12-J	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH-} \\ \diagup \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH-} \\ \diagup \\ \text{CH}_3 \end{array}$	CH ₃ CH ₂ CH ₂ -	CH ₃	PhCH ₂ -	-82.9° (c=0.21) (26°)	770 727	2.97(3H, s), 3.33 (6H, s), 5.56(1H, m), 7.1-7.3(7H, m), 7.73 (1H, d, J=3.1Hz)
11	12-K	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH-} \\ \diagup \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH-} \\ \diagup \\ \text{CH}_3 \end{array}$	CH ₃	CH ₃	PhCH ₂ -	-79.1° (c=0.665) (25°)	742 699	3.28(3H, s), 3.33(6H, s), 5.56(1H, m), 7.1 -7.3(7H, m), 7.73(1H, d, J= 3.3Hz)

- 60 -

THIS SHEET HAS BEEN
REPLACED



Examples 2 to 14 (Continued)

Example	Compound	R ₁	R ₂	R ₃	R ₄	R ₅	[α] _D (MeOH)	MS	¹ H-NMR(CDCl ₃ , δ)
12	12-L	H	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH}- \\ \diagup \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \diagdown \\ \text{CH}- \\ \diagup \\ \text{CH}_3 \end{array}$	CH ₃	PhCH ₂ -	-79.3° (c=0.545) (24°)	742 710	2.65(6H, s), 3.01 (3H, s), 3.33(6H, s), 5.55(1H, m), 7.1- 7.3(7H, m), 7.73(1H, d, J=3.1Hz)
13	12-M	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH}- \\ \diagup \\ \text{CH}_3 \end{array}$	PhCH ₂ -	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \diagdown \\ \text{CH}- \\ \diagup \\ \text{CH}_3 \end{array}$	CH ₃	PhCH ₂ -	-67.8° (c=0.905) (25°)	832 789	2.90(3H, s), 3.33 (3H, s), 3.34(3H, s), 5.56(1H, m), 7.1- 7.3(7H, m), 7.72(1H, d, J=3.3Hz)
14	12-N	CH ₃	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH}- \\ \diagup \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \diagdown \\ \text{CH}- \\ \diagup \\ \text{CH}_3 \end{array}$	CH ₃	PhCH ₂ -	-80.6° (c=0.17) (26°)	756 665	2.44(6H, s), 3.02 (3H, s), 3.33(6H, s), 5.40(1H, m), 7.1- 7.3(7H, m), 7.73(1H, d, J=3.1Hz)



- 62 -

Example 15

Preparation of compound 16-A (Compound 16

wherein $R_1 = \begin{matrix} \text{CH}_3 \\ \diagup \quad \diagdown \\ \text{CH} \end{matrix}$ -, $R_2 = \begin{matrix} \text{CH}_3 \\ \diagup \quad \diagdown \\ \text{CH} \end{matrix}$ -, $R_3 = \begin{matrix} \text{C}_2\text{H}_5 \\ \diagup \quad \diagdown \\ \text{CH} \end{matrix}$ -, $R_4 = \text{CH}_3$ and $R_6 = \text{OCH}_3$)

5 0.5 ml of concentrated hydrochloric acid is added to 93 mg (0.192 mmole) of compound 6-H under ice cooling, and the mixture is stirred for 1 hour. The mixture is evaporated to dryness under reduced pressure, the residue is dissolved in 2 ml of dimethylformamide, and 0.15 ml of triethylamine is added under ice cooling. Although triethylamine hydrochloride is deposited, the mixture is evaporated to dryness under reduced pressure and dried.

15 On the other hand, 102 mg (0.192 mmole) of compound 15-A obtained in Referential Example 13-A is dissolved in 0.4 ml of ethyl acetate, and 3.3 ml of 2N hydrogen chloride/ethyl acetate is added under ice cooling. The mixture is stirred at room temperature for 1 hour, the solvent is distilled off under reduced pressure, and the residue is dried. The resultant hygroscopic crystals are dissolved in 0.8 ml of dimethylformamide, the solution is added to the above tripeptide carboxylic acid, and 35 mg (0.192 mmole) of 90% DEPC and 54 μ l (0.384 mmole) of triethylamine are added under ice cooling. The mixture is stirred under ice cooling for 1 hour and then overnight at room temperature.

25 The solvent is distilled off under reduced pressure, the residue is dissolved in dichloromethane, and the solution is washed with saturated aqueous sodium bicarbonate and saturated saline and dried. The solvent is distilled off, the residue is purified by silica gel flash chromatography using dichloromethane : methanol (30 : 1) as an eluent, and the fraction containing the desired product are further purified by Sephadex LH-20.

chromatography using hexane : dichloromethane : methanol
(2 : 7.5 : 2.5) as an eluent to obtain 100 mg (62.0%) of
the desired compound 16-A as amorphous powder.

$[\alpha]^{27}_D$ -64.7° (c=0.66, MeOH)

5 MS 799, 751

$^1\text{H-NMR}$ (CDCl_3 , δ) 2.33(6H, s), 3.02(3H, s), 3.32(6H, s),
3.95(3H, s), 5.53(1H, m), 6.8~7.5(7H, m),
8.05(1H, s)

Examples 16-22

10 The following compounds were obtained by carry-
ing out Examples 16 to 22 according to Example 15.

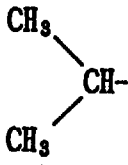
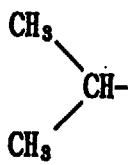
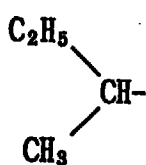
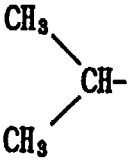
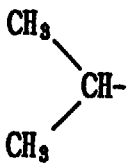
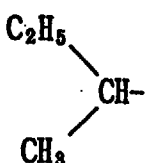
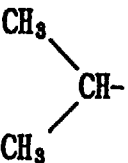
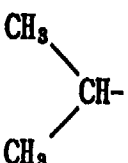
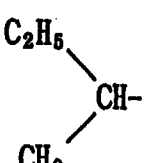
This Sheet Has Been Replaced



THIS SHEET HAS BEEN
REPLACED

Example	Compound	R ₁	R ₂	R ₃	R ₄	R ₆	[α] _D (MeOH)	MS	¹ H-NMR(CDCl ₃ , δ)
16	16-B	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH-} \\ \diagup \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH-} \\ \diagup \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \diagdown \\ \text{CH-} \\ \diagup \\ \text{CH}_3 \end{array}$	CH ₃	OCH ₂ Ph	-61.6° (c=0.83) (25°)	886 875 843	2.51(6H, br.), 3.02 (3H, s), 3.32(6H, s), 5.39(2H, s), 5.52 (1H, m), 7.0-7.5 (12H, m), 8.04(1H, s)
17	16-C	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH-} \\ \diagup \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH-} \\ \diagup \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \diagdown \\ \text{CH-} \\ \diagup \\ \text{CH}_3 \end{array}$	H	OCH ₃	-73.3° (c=0.30) (26°)	828 785	3.02(3H, s), 3.26 (6H, s), 3.95(3H, s), 7.2-7.3(6H, m), 8.05 (1H, s)
18	16-D	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH-} \\ \diagup \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH-} \\ \diagup \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH-CH}_2- \\ \diagup \\ \text{CH}_3 \end{array}$	CH ₃	OCH ₃	-71.0° (c=0.465) (26°)	842 799	2.32(6H, s), 2.97 (3H, s), 3.32(3H, s), 3.40(3H, s), 3.95 (3H, s), 7.2-7.3 (6H, m), 8.05(1H, s)
19	16-E	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH-} \\ \diagup \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH-} \\ \diagup \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH-CH}_2- \\ \diagup \\ \text{CH}_3 \end{array}$	H	OCH ₃	-76.8° (c=0.50) (27°)	828 785	2.98(3H, s), 3.24 (3H, s), 3.34(3H, s), 3.95(3H, s), 5.60(1H, m), 7.1~ 7.3(6H, m), 8.05 (1H, s)

(Continued)

Example	Compound	R ₁	R ₂	R ₃	R ₄	R ₆	[α] _D (MeOH)	MS	¹ H-NMR(CDC1 ₃ , δ)
20	16-F				CH ₃	-NHPh	-42.9° (c=0.385) (27°)	903 860	3.00(3H, s), 3.33 (3H, s), 3.34(3H, s), 5.55(1H, m), 5.55 (1H, m), 7.1-7.8 (1H, m), 8.07(1H, s)
21	16-G				CH ₃	-NHBu ^t	-60.7° (c=0.29) (28°)	883 868	1.48(9H, s), 2.51 (6H, br.), 3.03(3H, s), 3.33(6H, s), 5.49(1H, m), 7.1- 7.3(6H, m), 7.90 (1H, s)
22	16-H				CH ₃		-64.4° (c=0.341)	897 854	2.44(6H, br.), 3.03 (3H, s), 3.33(6H, s), 5.56(1H, m), 7.1- 7.3(6H, m), 7.82 (1H, s)

- 65 -

THIS SHEET HAS BEEN
REPLACED

This Sheet Has Been Replaced



Example 23

Preparation of compound 16-I (Compound 16

wherein $R_1 = \begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH} \\ \diagup \\ \text{CH}_3 \end{array}$ -, $R_2 = \begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH} \\ \diagup \\ \text{CH}_3 \end{array}$ -, $R_3 = \begin{array}{c} \text{C}_2\text{H}_5 \\ \diagdown \\ \text{CH} \\ \diagup \\ \text{CH}_3 \end{array}$ -, $R_4 = \text{CH}_3$ and $R_6 = \text{OBu}^t$)

- 5 1. 120 mg (0.142 mmole) of compound 16-A is dissolved in 5 ml of dimethylformamide, and 0.16 ml of 1N sodium hydroxide is added under ice cooling. The mixture is stirred under ice cooling for 30 minutes and then at room temperature for 90 minutes. 0.16 ml of 1N hydro-
- 10 chloric acid is added under ice cooling, the solvent is distilled off under reduced pressure, the residue is dissolved in dichloromethane, and the deposited sodium chloride is removed by filtration. The filtrate is
- 15 purified by Sephadex LH-20 chromatography using hexane : dichloromethane : methanol (2 : 7.5 : 2.5) as an eluent. The desired product is dissolved again in water, and the solution is lyophilized to obtain 114 mg (96.5%) of compound 16 carboxylic acid ($R_6 = \text{OH}$) as powder.
- 20 2. 30 mg (0.024 mmole) of the above carboxylic acid is dissolved in 0.5 ml of dichloromethane, 10 μ l of concentrated sulfuric acid and 1 ml of isobutene are added under cooling in dry ice-acetone bath, and the mixture is stirred for 2 days in a sealed tube. 5 ml of
- 25 saturated aqueous sodium bicarbonate is added to the reaction solution, the mixture is extracted with dichloromethane, and the extract is washed with water and dried. The solvent is distilled off under reduced pressure, and the residue is purified by Sephadex LH-20
- 30 chromatography using hexane : dichloromethane : methanol (2 : 7.5 : 2.5) as an eluent to obtain 13 mg (62.5%) of the desired product compound 16-I as amorphous powder.

$[\alpha]^{25}_D -60.6^\circ$ ($c=0.35$, MeOH)

MS 841, 840, 793



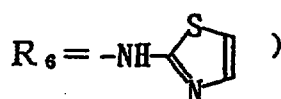
- 67 -

$^1\text{H-NMR}$ (CD_2Cl_2 , δ) 1.58(9H, s), 2.57(6H, br.), 3.02(3H, s),
3.31(6H, s), 5.46(1H, m), 7.0~7.4(7H, m), 7.93(1H, s)

Example 24

5 Preparation of compound 16-J (Compound 16

wherein $\text{R}_1 = \begin{array}{c} \text{CH}_3 \\ \diagup \\ \text{CH} \\ \diagdown \\ \text{CH}_3 \end{array}$ -, $\text{R}_2 = \begin{array}{c} \text{CH}_3 \\ \diagup \\ \text{CH} \\ \diagdown \\ \text{CH}_3 \end{array}$ -, $\text{R}_3 = \begin{array}{c} \text{C}_2\text{H}_5 \\ \diagup \\ \text{CH} \\ \diagdown \\ \text{CH}_3 \end{array}$ -, $\text{R}_4 = \text{CH}_3$ and



21.5 mg (0.026 mmole) of compound 16 carboxylic acid ($\text{R}_6 = \text{OH}$) is dissolved in 0.5 ml of acetonitrile, 11.5 mg (1 equivalent) of the BOP reagent and 2.6 mg (1 equivalent) of 2-aminothiazole and added, and 5 mg (1.5 equivalents) of diisopropylethylamine is added dropwise under ice cooling. The mixture is stirred overnight at room temperature, and the reaction solution is concentrated under reduced pressure. The concentrate is dissolved in dichloromethane, and the solution is washed successively with 10% aqueous citric acid, saturated aqueous sodium bicarbonate and saturated saline and dried. The crude product is subjected to preparative TLC using dichloromethane-methanol (10 : 1) as a developing solvent, and are further purified by Sephadex LH-20 chromatography using hexane : dichloromethane : methanol (2 : 7.5 : 2.5) as an eluent to obtain 13.7 mg (57.7%) of the desired compound 16-J as amorphous powder.

25 $[\alpha]^{25}_D -48.8^\circ$ ($c=0.25$, MeOH)

MS 910, 867

$^1\text{H-NMR}$ (CDCl_3 , δ) 3.01(3H, s), 3.33(3H, s), 3.37(3H, s),
5.52(1H, m), 7.05(1H, d, $J=3.5\text{Hz}$), 7.2~7.3 (6H, m), 7.50(1H, d, $J=3.3\text{Hz}$), 8.16(1H, s)

Example 25

Preparation of compound 16-K (Compound 16

wherein $R_1 = \begin{matrix} \text{CH}_3 \\ \diagup \\ \text{CH} \\ \diagdown \\ \text{CH}_3 \end{matrix}$ -, $R_2 = \begin{matrix} \text{CH}_3 \\ \diagup \\ \text{CH} \\ \diagdown \\ \text{CH}_3 \end{matrix}$ -, $R_3 = \begin{matrix} \text{C}_2\text{H}_5 \\ \diagup \\ \text{CH} \\ \diagdown \\ \text{CH}_3 \end{matrix}$ -, $R_4 = \text{CH}_3$ and $R_6 = \text{NH}_2$)

- 5 21 mg (0.025 mmole) of compound 16-A is dissolved in 3 ml of ammonia-saturated methanol, and the solution is allowed to stand at room temperature for 1 hour. The solvent is distilled off under reduced pressure, and the residue is purified by Sephadex LH-20 using
10 hexane : dichloromethane : methanol (2 : 7.5 : 2.5) as an eluent to obtain 20 mg (96.0%) of the desired compound 16-K as amorphous powder.

$[\alpha]^{25}_D$ -65.8° (c=0.41, MeOH)

MS 784, 736

- 15 $^1\text{H-NMR}$ (CDCl_3 , δ) 2.48(6H, s), 3.06(3H, s), 3.33(6H, s),
5.50(1H, m), 7.0~7.6(7H, m), 8.04(1H, s)

Examples 26 and 27

- Compounds shown in the following table were obtained in the same manner as in Example 25 except that
20 70% aqueous ethylamine solution or 5% aqueous dimethylamine solution was used in place of ammonia-saturated methanol.

This Sheet Has Been Replaced



THIS SHEET HAS BEEN
REPLACED

Example	Compound	R ₁	R ₂	R ₃	R ₄	R ₆	[α] _D (MeOH)	MS	¹ H-NMR(CDCl ₃ , δ)
26	16-L	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH-} \\ \diagup \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH-} \\ \diagup \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \diagdown \\ \text{CH-} \\ \diagup \\ \text{CH}_3 \end{array}$	CH ₃	-NHC ₂ H ₅	-63.5° (c=0.32) (25°)	812 764	2.48(6H, s), 3.02 (3H, s), 3.32(6H, s), 5.46(1H, m), 7.0- 7.5(7H, m), 7.92 (1H, s)
27	16-M	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH-} \\ \diagup \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH-} \\ \diagup \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \diagdown \\ \text{CH-} \\ \diagup \\ \text{CH}_3 \end{array}$	CH ₃	$\begin{array}{c} \text{CH}_3 \\ \diagup \\ \text{-N} \\ \diagdown \\ \text{CH}_3 \end{array}$	-67.1° (c=0.255) (28°)	885 812	1.67(6H, s), 3.16 (3H, s), 3.33(3H, s), 7.1-7.3(6H, m), 7.26(1H, s)



Example 28

Preparation of compound 19-A (Compound 19

wherein $R_1 = \begin{array}{c} \text{CH}_3 \\ \diagup \\ \text{CH} \\ \diagdown \\ \text{CH}_3 \end{array} -$, $R_2 = \begin{array}{c} \text{CH}_3 \\ \diagup \\ \text{CH} \\ \diagdown \\ \text{CH}_3 \end{array} -$, $R_3 = \begin{array}{c} \text{C}_2\text{H}_5 \\ \diagup \\ \text{CH} \\ \diagdown \\ \text{CH}_3 \end{array} -$,
 $-A_2-R_7 = \text{CH}_2\text{CH}_2\text{Ph}$)

5 27.7 mg (0.057 mmole) of compound 6-H is dis-
 solved in 0.3 ml of dichloromethane, and 0.3 ml of tri-
 fluoroacetic acid is added under ice cooling. The mix-
 ture is stirred at room temperature for 1 hour, the
 solvent is distilled off under reduced pressure, and the
 10 residue is sufficiently dried under reduced pressure. On
 the other hand, 22.3 mg (0.057 mmole) of compound 7-A is
 dissolved in 2N hydrogen chloride/ethyl acetate under ice
 cooling, and the mixture is stirred at room temperature
 for 1 hour. The solvent is distilled off under reduced
 15 pressure, the residue is dried and dissolved in 0.5 ml of
 dimethylformamide, the solution is added to the above
 tripeptide carboxylic acid, and 9.8 mg (1.0 equivalent)
 of 95% DEPC and 16 μ l (2 equivalents) of triethylamine
 are added under ice cooling. The mixture is stirred
 20 under ice cooling for 1 hour and then overnight at room
 temperature.

The solvent is distilled off under reduced
 pressure, and the residue is dissolved in dichlorometh-
 ane, washed successively with saturated aqueous sodium
 25 bicarbonate and saturated saline, and then dried. The
 solvent is distilled off, the residue is subjected to
 preparative TLC using dichloromethane-methanol (10 : 1) as
 a developing solvent, and the fractions of the desired
 product are further purified by Sephadex LH-20 chromato-
 30 graphy using hexane : dichloromethane : methanol (2 :
 7.5 : 2.5) as an eluent to obtain 35.8 mg (89.5%) of the
 desired compound 19-A as amorphous powder.

This Sheet Has Been Replaced



THIS SHEET HAS BEEN
 REPLACED

$[\alpha]^{25}_D$ -38.0° (c=0.566, MeOH)

MS 701, 658

$^1\text{H-NMR}$ (CD_2Cl_2 , δ) 1.16(3H, d, $J=7.0\text{Hz}$), 2.23(6H, s), 3.00
(3H, s), 3.30(3H, s), 3.34(3H, s)

5 The following compounds were obtained in the same manner as in Example 28.

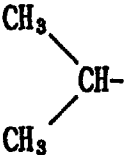
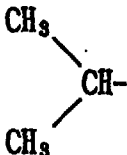
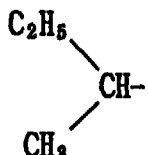
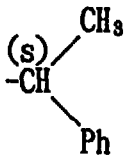
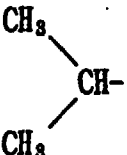
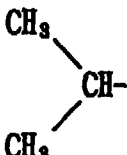
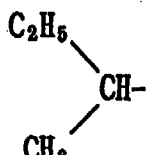
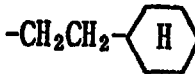
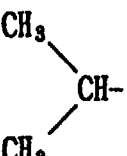
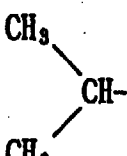
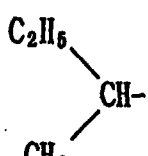
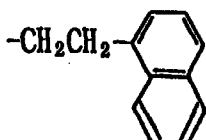
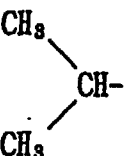
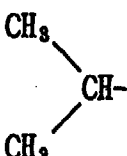
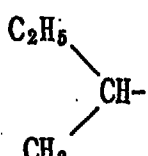
This Sheet Has Been Replaced



THIS SHEET HAS BEEN
REPLACED

Example	Compound	R ₁	R ₂	R ₃	-A ₂ R ₇	[α] _D (MeOH)	MS	¹ H-NMR(CDCl ₃ , δ)
29	19-B	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH-} \\ \diagup \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH-} \\ \diagup \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \diagdown \\ \text{CH-} \\ \diagup \\ \text{CH}_3 \end{array}$	-Ph	-52.6° (c=0.352) (28°)	673 630	1.36(3H, d, J=7.0Hz)、 3.02(3H, s)、3.34 (3H, s)、3.47(3H, s)、 7.0-7.7(6H, m)
30	19-C	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH-} \\ \diagup \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH-} \\ \diagup \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \diagdown \\ \text{CH-} \\ \diagup \\ \text{CH}_3 \end{array}$	-CH ₂ Ph	-35.8° (c=0.330) (28°)	688 644	1.28(3H, d, J=7.0Hz)、 2.33(6H, br.)、3.01 (3H, s)、3.30(3H, s)、 3.39(3H, s)、7.2- 7.4(5H, m)
31	19-D	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH-} \\ \diagup \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH-} \\ \diagup \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \diagdown \\ \text{CH-} \\ \diagup \\ \text{CH}_3 \end{array}$	-CH ₂ CH ₂ CH ₂ Ph	-47.1° (c=0.312) (27°)	716 672	1.28(3H, d, J=7.0Hz)、 2.36(6H, s)、3.00 (3H, s)、3.33(3H, s)、 3.41(3H, s)、7.1- 7.3(5H, m)

(Continued)

Example	Compound	R ₁	R ₂	R ₃	-A ₂ R ₇	[α] _D (MeOH)	MS	¹ H-NMR(CDCl ₃ , δ)
32	19-E					-94.8° (c=0.335) (28°)	658	1.49(3H, d, J=7.0Hz), 2.44(6H, br.), 3.02 (3H, s), 3.33(3H, s), 3.38(3H, s), 7.2- 7.4(5H, m)
33	19-F					-48.3° (c=0.265) (28°)	644	2.48(6H, br.), 3.02 (3H, s), 3.33(3H, s), 3.41(3H, s)
34	19-G					-34.1° (c=0.347) (29°)	708	3.00(3H, s), 3.29 (3H, s), 3.34(3H, s), 7.2-8.3(7H, m)
35	19-H					-61.9° (c=0.315) (29°)	680 637	1.40(3H, d, J=7.0Hz), 2.51(6H, s), 3.15(3H, s), 3.37(3H, s), 3.42 (3H, s), 6.93(1H, d, J= 3.5Hz), 7.48(1H, d, J= 3.7Hz)

This Sheet Has Been Replaced



- 73 -

THIS SHEET HAS BEEN
REPLACED

(Continued)

Example	Compound	R ₁	R ₂	R ₃	-A ₂ R ₇	[α] _D (MeOH)	MS	¹ H-NMR(CDCl ₃ , δ)
36	19-I	H	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH}- \\ \diagup \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CHCH}_2- \\ \diagup \\ \text{CH}_3 \end{array}$	-CH ₂ CH ₂ Ph	-54.7° (c=0.625) (29°)	659 644 627	1.21(3H, d, J=7.0Hz)、 2.42(6H, s)、2.98 (3H, s)、3.35(6H, s)、 7.1-7.4(5H, m)
37	19-J	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH}- \\ \diagup \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH}- \\ \diagup \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CHCH}_2- \\ \diagup \\ \text{CH}_3 \end{array}$	-CH ₂ CH ₂ Ph	-53.3° (c=0.368) (28°)	701 658	1.21(3H, d, J=7.3Hz)、 2.44(6H, s)、2.98 (3H, s)、3.35(6H, s)、 3.38(3H, s)、7.1-7.3 (5H, m)
38	19-K	$\begin{array}{c} \text{CH}_3 \\ \diagdown \\ \text{CH}- \\ \diagup \\ \text{CH}_3 \end{array}$	PhCH ₂ -	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \diagdown \\ \text{CH}- \\ \diagup \\ \text{CH}_3 \end{array}$	-CH ₂ CH ₂ Ph	-18.7° (c=0.307) (30°)	749 706	2.92(3H, s)、3.31 (3H, s)、3.36(3H, s)、 7.1-7.4(10H, m)

THIS SHEET HAS BEEN
REPLACED

- 74 -

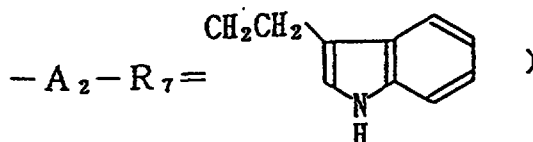


This Sheet Has Been Replaced

Example 39

Preparation of compound 19-L (Compound 19

wherein $R_1 = \begin{array}{c} \text{CH}_3 \\ \diagup \\ \text{CH}- \\ \diagdown \\ \text{CH}_3 \end{array}$, $R_2 = \begin{array}{c} \text{CH}_3 \\ \diagup \\ \text{CH}- \\ \diagdown \\ \text{CH}_3 \end{array}$, $R_3 = \begin{array}{c} \text{C}_2\text{H}_5 \\ \diagup \\ \text{CH}- \\ \diagdown \\ \text{CH}_3 \end{array}$, and



5 The desired compound 19-L is obtained by reacting a product obtained by removing of Z of compound 18-A according to Referential Example 5-A with compound 6-H according to Example 28.

$[\alpha]^{27}_D -25.9^\circ$ (c=0.255, MeOH)

10 MS 740, 697

$^1\text{H-NMR}$ (CDCl_3 , δ) 2.64(6H, br.), 3.03(3H, s), 3.30(6H, s), 7.0-7.7(5H, m), 8.4(1H, m)

This Sheet Has Been Replaced

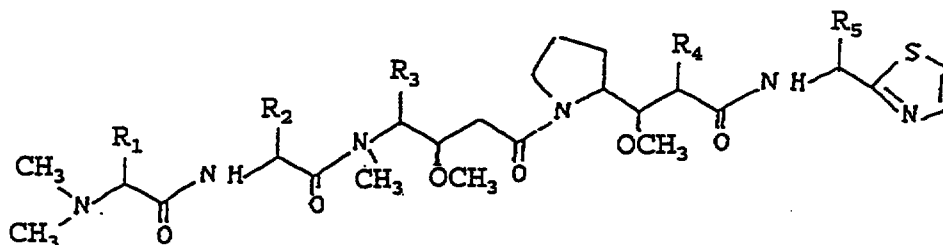


THIS SHEET HAS BEEN
REPLACED



Claims

1. A tetrapeptide derivative represented by the following formula or a salt thereof



wherein

- (a) $R_1, R_2 = \begin{matrix} \text{CH}_3 \\ \diagup \\ \text{CH} \\ \diagdown \\ \text{CH}_3 \end{matrix} -$, $R_3 = \begin{matrix} \text{CH}_3 \\ \diagup \\ \text{CH} - \text{CH}_2 - \\ \diagdown \\ \text{CH}_3 \end{matrix}$, $R_4 = \text{CH}_3 -$ and $R_5 = \text{PhCH}_2 -$

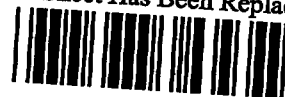
- (b) $R_1, R_2, R_3 = \begin{matrix} \text{CH}_3 \\ \diagup \\ \text{CH} \\ \diagdown \\ \text{CH}_3 \end{matrix} -$, $R_4 = \text{CH}_3 -$ and $R_5 = \text{PhCH}_2 -$;

This Sheet Has Been Replaced

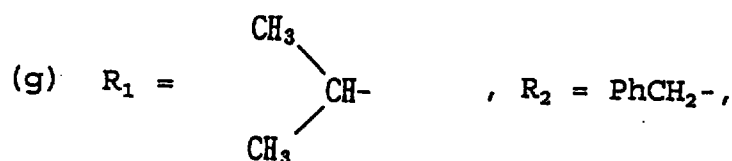
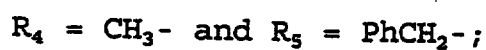
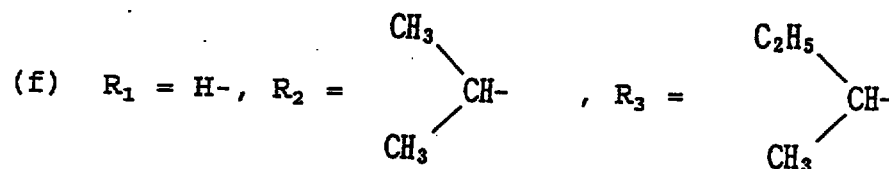
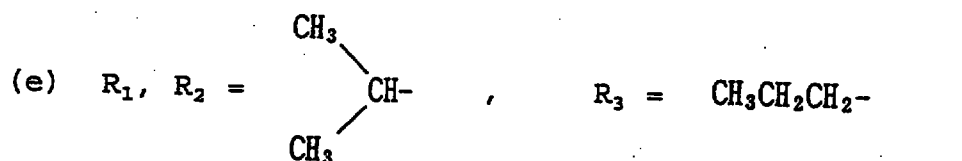
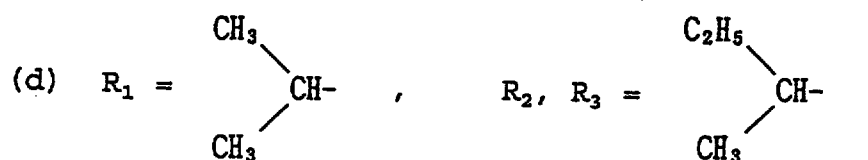
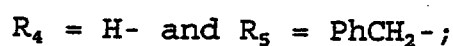
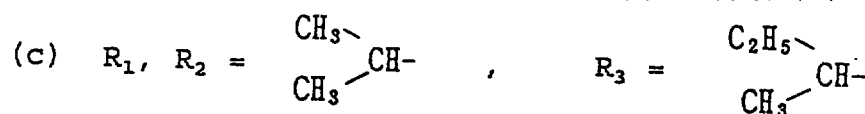
THIS SHEET HAS BEEN
REPLACED



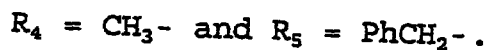
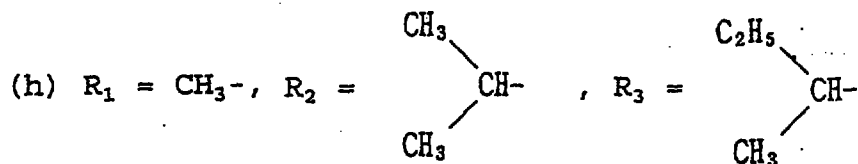
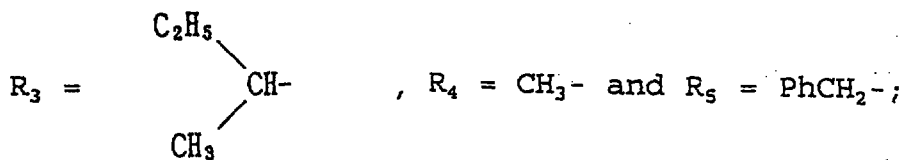
This Sheet Has Been Replaced



- 77 -



THIS SHEET HAS BEEN
REPLACED



2. An antitumor agent containing a tetrapeptide derivative or a salt thereof according to claim 1 as an effective ingredient.

This Sheet Has Been Replaced



THIS SHEET HAS BEEN
REPLACED