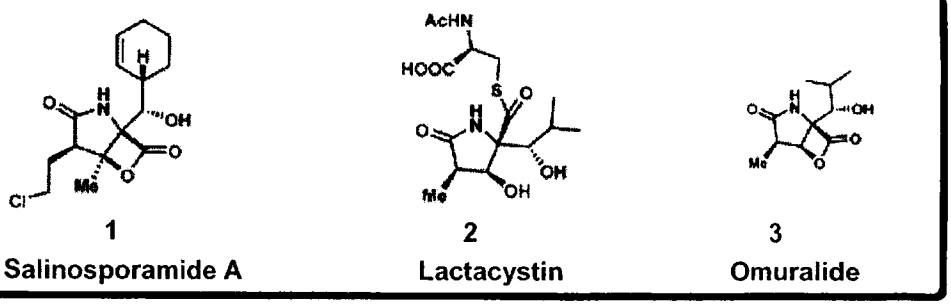
Total Synthesis of Proteasome Inhibitor : Salinosporamide A and its Biological Aspects

Figure1:
Proteasome inhibitor



1 Salinosporamide A | 2 Lactacystin | 3 Omuralide

	Salinosporamide A	Lactacystin	Omuralide
isolated from	bioactive product of a marine microorganism	Secondary metabolite derived from a Streptomyces in 1991	Key intermediate
Biological activity	Proteasome inhibitor IC_{50} = 1.3 nM Antitiumor against HCT-166 IC_{50} = 11 $ngML^{-1}$	Proteasome inhibitor IC_{50} = 49 nM (Omuralide)	
First total synthesis	JACS.2004.126. 6230 (E. J. Corey) JSCS.2005.127. 8298 (Danishefsky, S. J.)	JACS.1992.114. 10677 (E. J. Corey) JACS.1993.115. 5302 (Omura-Smith) JACS.1994.116. 2139 etc...	TL.1993.34. 6977 (Key intermediate) (E. J. Corey)

Aaron Ciechanover Avram Hershko Irwin Rose

The Nobel Prize in Chemistry 2004
" Ubiquitin-Proteasome system"

Today's contents

A: Total Synthesis of Salinosporamide A

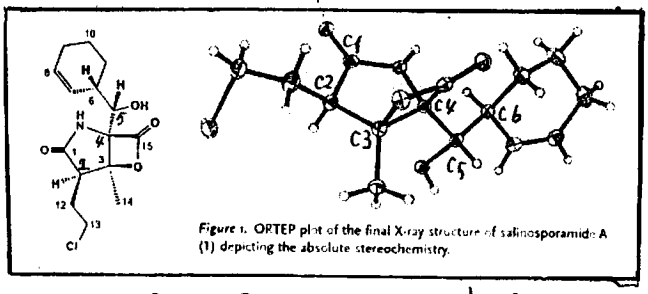


Figure 1. ORTEP plot of the final X-ray structure of salinosporamide A (1) depicting the absolute stereochemistry.

Fig. 2. Salinosporamide A

* Structual feutures:
  - small molecule and it has five asymmetric carbons and functional groups.
  - C2, C3, C4, C5, C6 ··· asymmetric center.
  - C3, C4 ···· quaternary center
  - C5, C6 ···· asymmetric center on side chain C4.

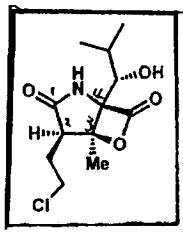


Fig 3. Omuralide-Salinosporamide A Hybrid.

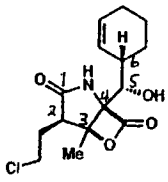
* The Side chain on C4 was isopropyl group.

* Reported synthesis.
  - E. J. Corey. JACS. 2004, 126, 6230
  - Danishefsky. JACS. 2005, 127, 8298

Fig. 3. * Reported by E.J. Corey et al. JACS, 2005, 127, 8974

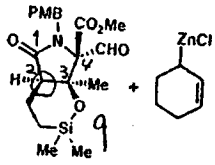
(A-1) Retro Synthesis.

Corey's method



Salinosporamide A ⇨ 9

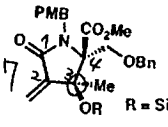
C5, C6 ··asymmetric center



9 ⇨ 7 Key Reaction

* C2, C3: Stereo relationship → radical-chain cyclization

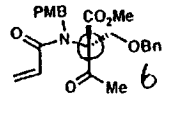
Key ⇩



7 ⇨ 6 Key Reaction

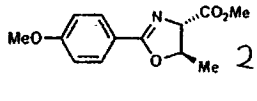
R = $Si(Me)_2CH_2Br$

Key ⇩ * C(3): asymmetric center



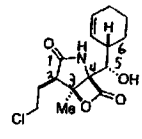
Baylis-Hilman -aldol reaction

6 ⇨ 2



C(4): tertiary stereocenter

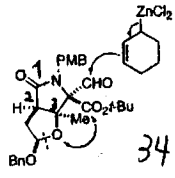
Danishefsky's method



Salinosphoramide A ⇩ 34

C5, C6 ··asymmetric center

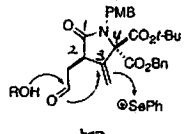
↖ same strategy as corey's case.



34 ⇨ 32 Key Reaction

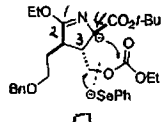
34 C3: quatanary center

Key ⇩ C2, C3: stereo relationship → unusual cationic hemiacetal seleno cyclization



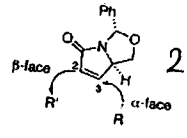
32 ⇨ 27

C4: quaternary center



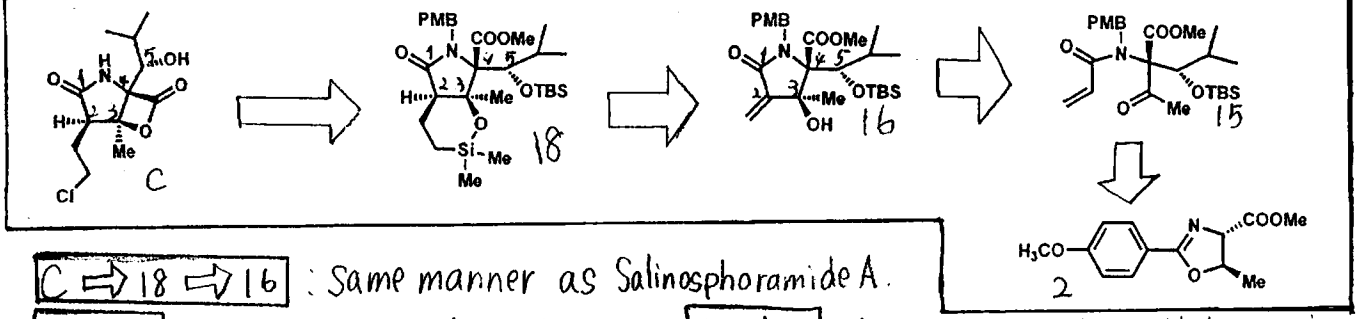
→ lactone formation and the use of nucleophilic selenium species.

27 ⇨ 20



20 C2, C3: asymmetric center.

Retro Synthesis : Hybrid type.



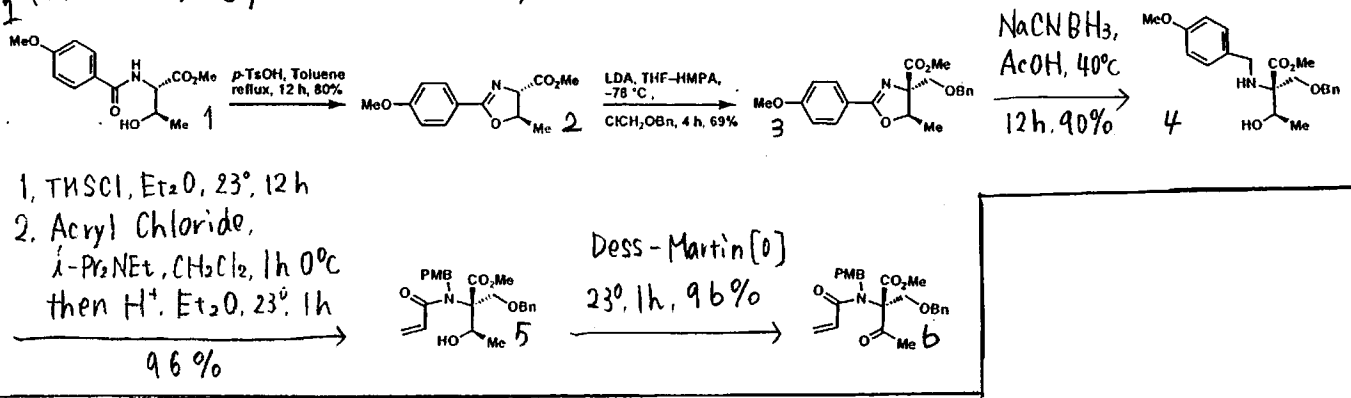
C ⇨ 18 ⇨ 16 : Same manner as Salinosphoramide A.

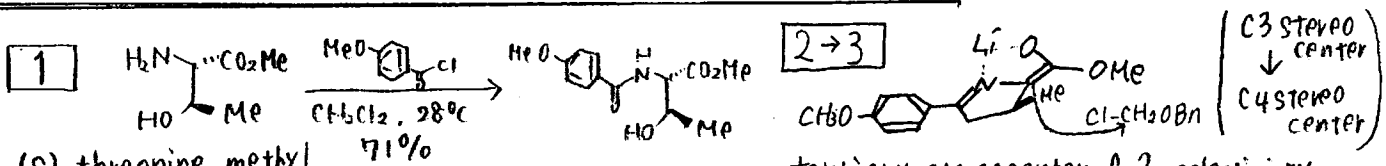
16 ⇨ 15 : more improved cyclization 15 ⇨ 2 diastereoselective aldol reaction.

⟨A-2⟩ Corey's Method

⟨A-2-1⟩ Synthesis of key intermediate b.

Scheme 1

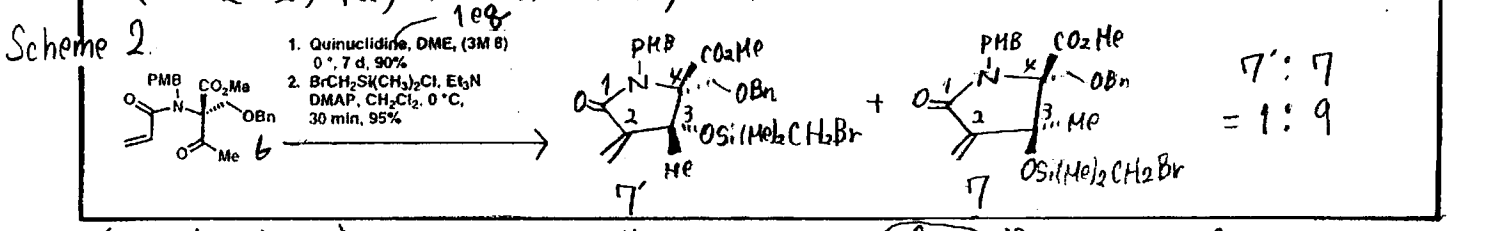




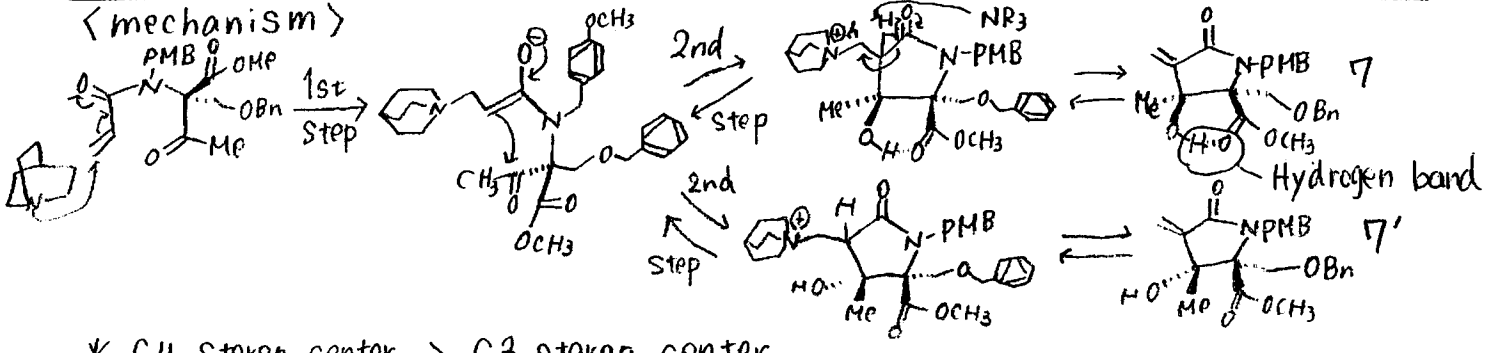
tertiary stereocenter of 3 selectivity.

⟨A-2-2⟩ Key Reaction : Baylis Hilman - aldol Reaction

Scheme 2



⟨mechanism⟩



* C4 Stereo center → C3 stereo center

* 7, 7' : diastereomer were separated at this stage by column.

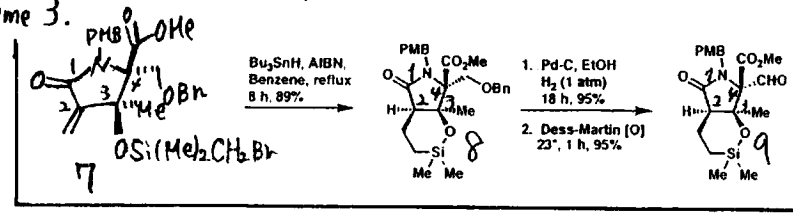
* Condition : 20°C for 9h ➡ 7' : 7 = 1 : 4 90% yield

0 °C for 7day ➡ 7' : 7 = 1 : 9 90% yield

long time ➡ Overcome this problem : P5, ⟨A-3-2⟩

〈A-2-3〉 Key Reaction: Radical-Chain Cyclization

Scheme 3.



7→8 〈mechanism〉

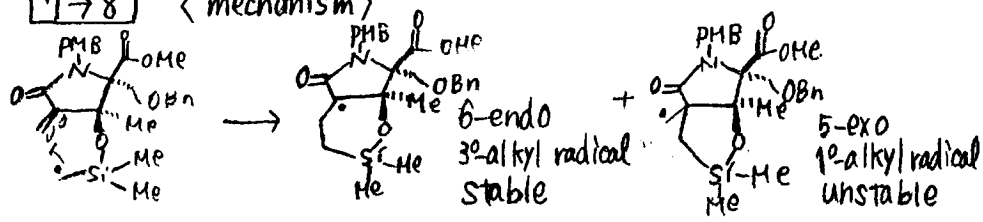
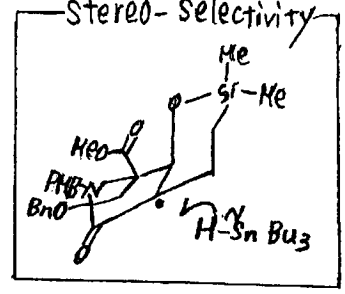
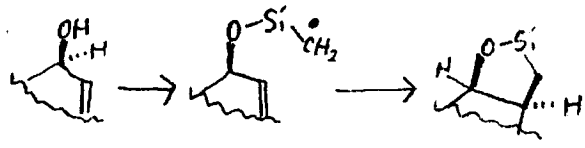


Fig. 4.



* cyclic allylic alchohols → cis bicyclic ether



(Ref: Stork, JACS: 1985, 107, 501
: Nishiyama, Joc: 1984, 49, 2299
: Chem Rev: 1995, 1253/1997: 2193)

* Si-effect (Ref: Tetrahedron, 1985, 3979)
  - Silicon-induced stereoelectronic factor
  - Silyl radical were stabilized. And it would decrease their cyclization potency.

"Baldwin-Beckwith rules" (Radical cyclization)

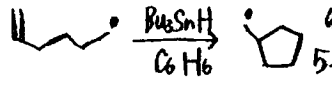
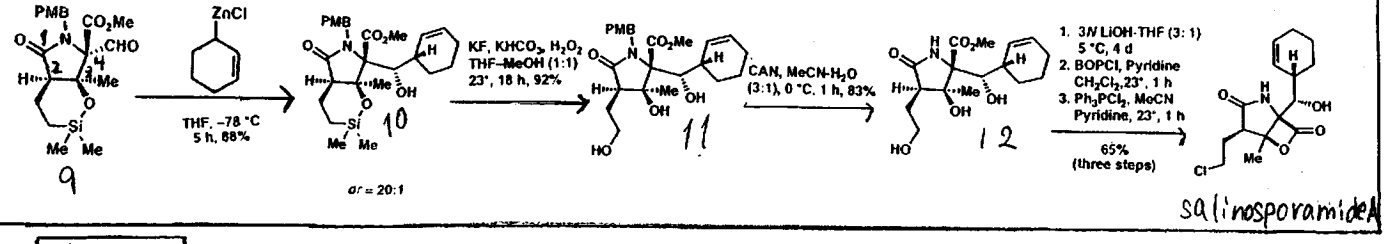
ex) 

Table 2. Rate Constants for the Cyclization of 5-Hexenyl and Some Substituted 5-Hexenyl Radicals at 25 °C[20]

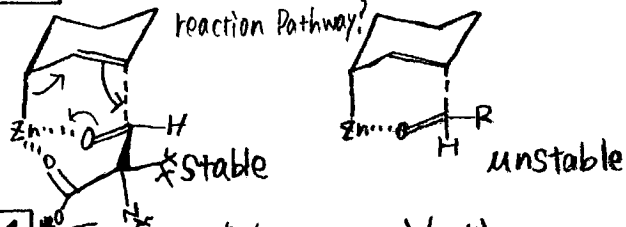
Radical	$10^{-5}k_{exo}$, s^{-1}	$10^{-5}k_{endo}$, s^{-1}
		
 45	>200	~0.2
 46	0.7	1.6

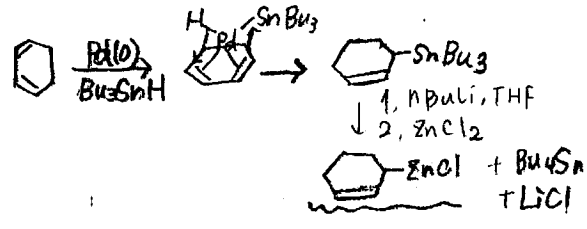
〈A-2-4〉 Synthesis of Salinosporamide A

Scheme 4

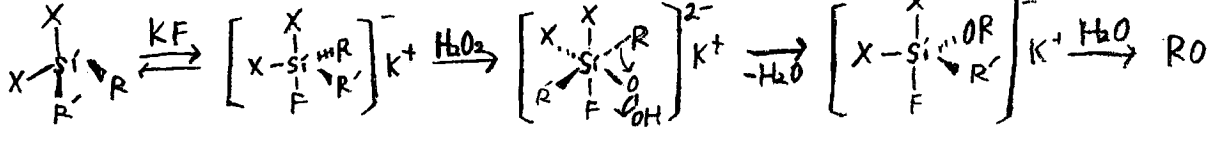


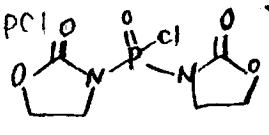
9→10 δ-selective reaction Pathway?





10→11 Tamao-Fleming oxidation



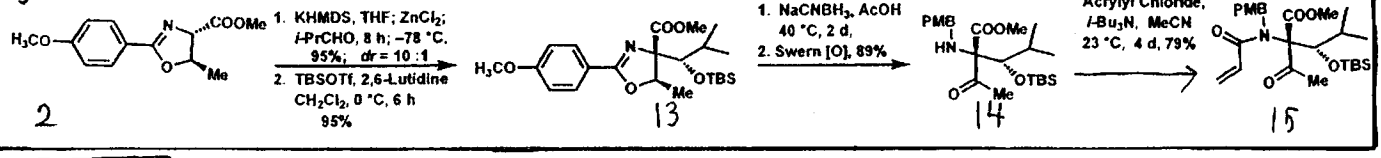
(BOPCl )

Total 17steps, 13.7%yield.

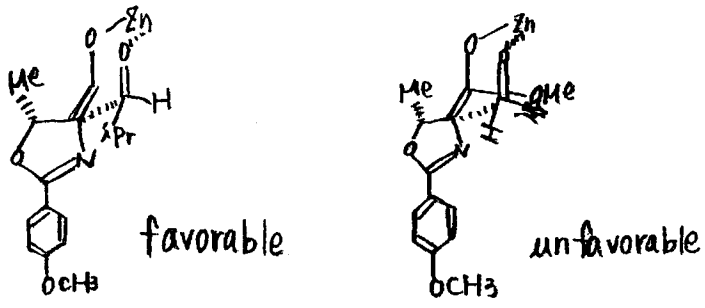
⟨A-3⟩ Omuralide-Salinosporamide A Hybrid

⟨A-3-1⟩ Synthesis of key intermediate 15.

Scheme 5

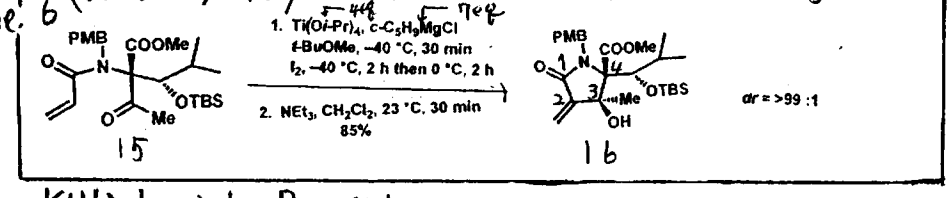


2 → 13 diastereo selective aldol reaction

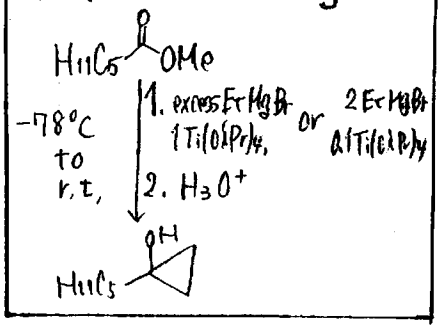


⟨A-3-2⟩ Key Reaction : Kulinkovich Reagent

Scheme 6

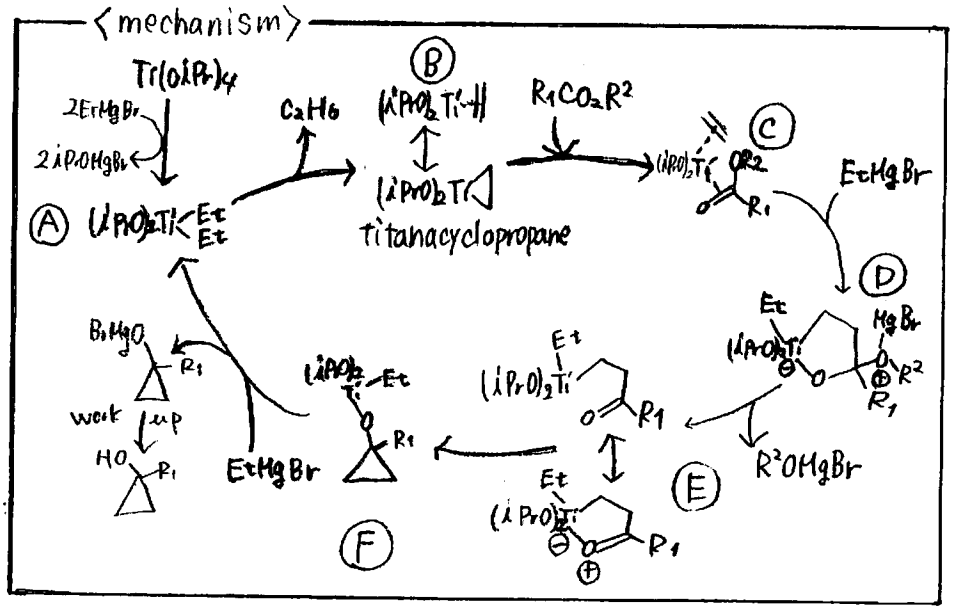


Kulinkovich Reagent

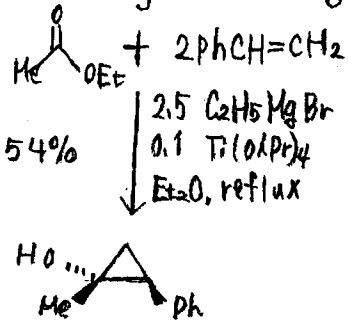


(Ref : Chem Rev, 2003, vol 103, 2597
Adv. Synth. Catal, 2001, 343,
JACS, 1996, 118, 3182

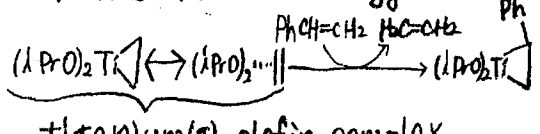
⟨mechanism⟩



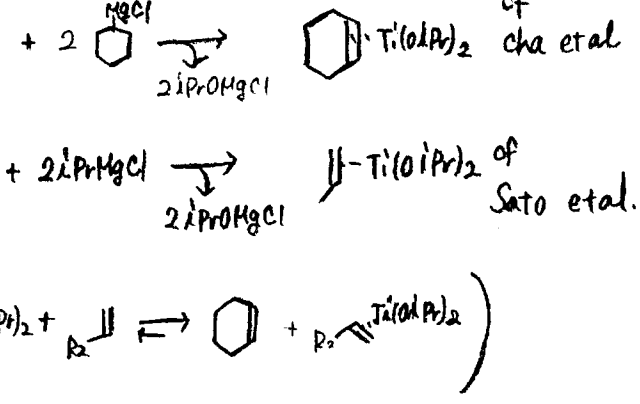
……* Olefin-ligand exchange……



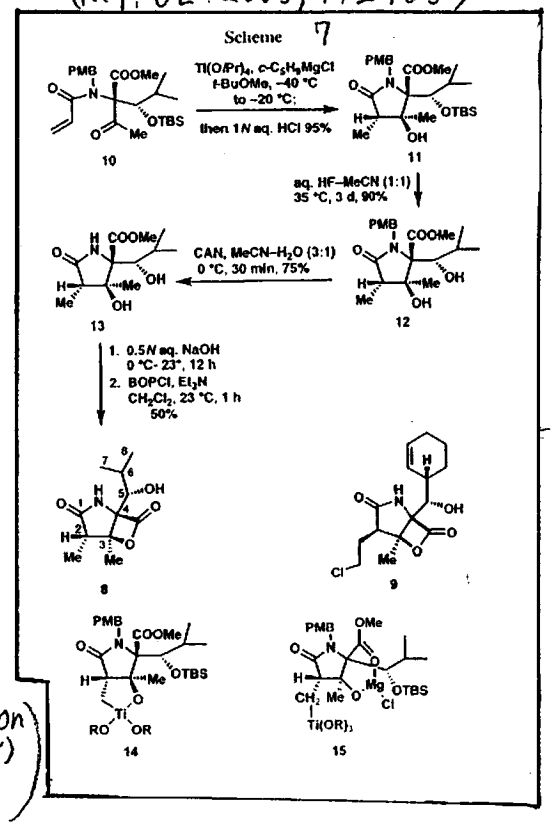
→ This result was suggested

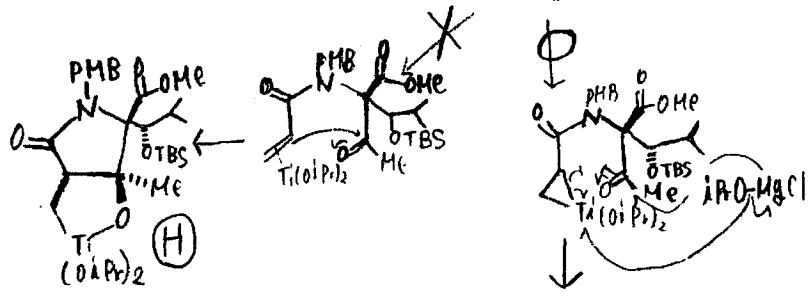


* To facilitate ligand exchange.

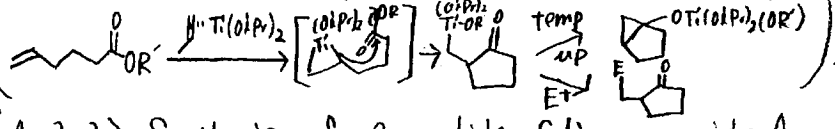


(Ref. OL. 2005, 7. 2703)

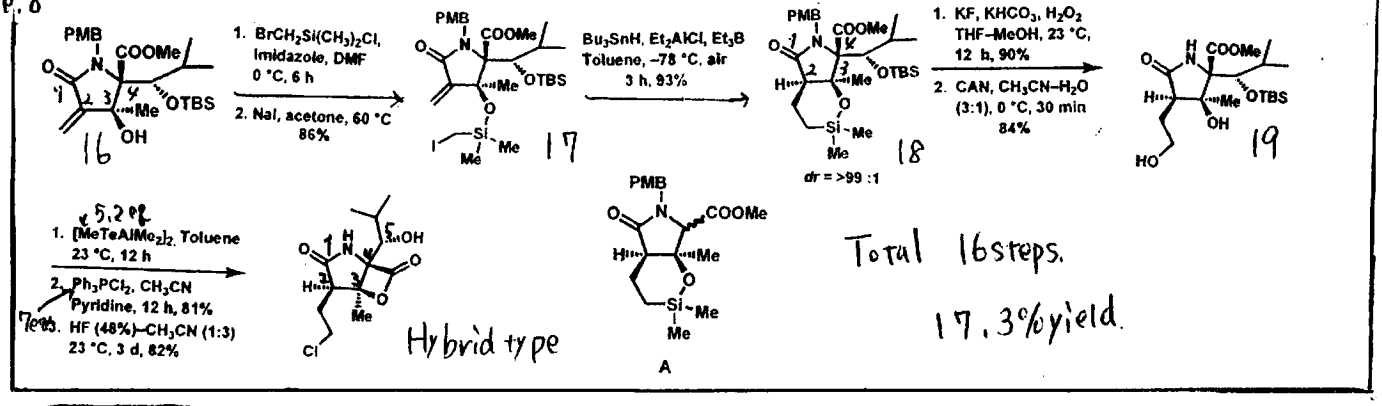




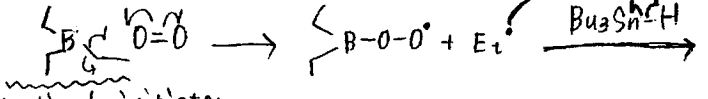
(Intramolecular Nucleophilic Acyl Substitution Reaction)



Scheme 8 (A-3-3) Synthesis of Omuralide-Salinosporamide A Hybrid.



17→18 • Bu3SnH/AIBN ··· condition : heat 80~100°C
Bu3SnH/Et3B/air ··· condition : 0°C (water) mild condition (JACS. 1987. 109. 2549)



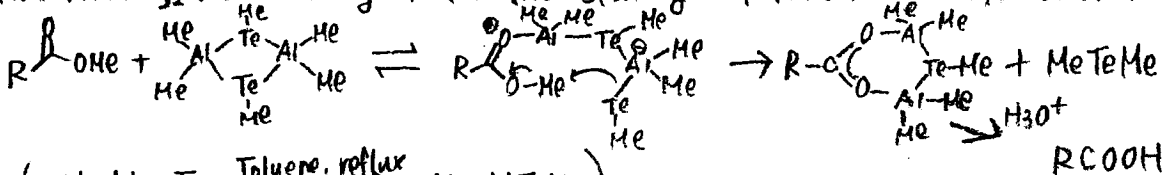
radical initiator

• Et2AlCl : • Lewis Acid affects the reaction rates and stereoselectivity. (OL. 2001, 67)
• Et2AlCl increased the electron-withdrawing nature of the carbonyl group thus making the conjugate addition pathway via a low energy process.

19→ Hybridtype

Cleavage of methyl esters of 19 : complete failure under variety of conditions and compound A was obtained.

[MeTeAlMe2]2 : New Reagent for the cleavage of hindered methyl ester. (Ref, TL, 2005 46, 4589)

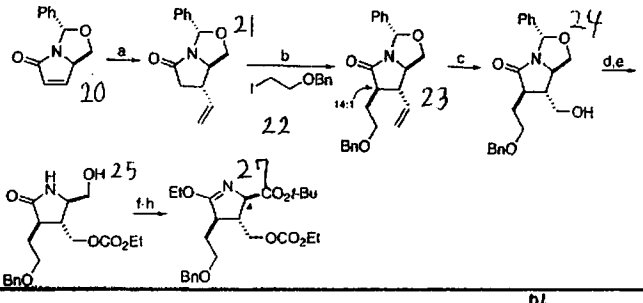


(Me3Al + Te → Me3AlTeMe)
1eq 1.1eq Toluene, reflux 6h

〈A-4〉 Danishefsky method

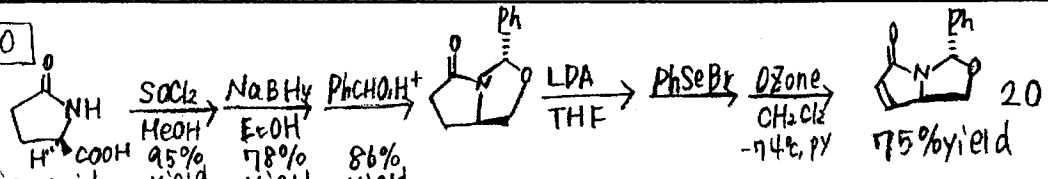
〈A-4-1〉 Synthesis of key intermediate 27

Scheme 9



Key: (a) vinylmagnesium bromide, TMSCl, CuI, THF, −78 °C (75%); (b) **22**,[10] LDA, THF, room temperature (rt) (77%, *dr* = 14:1); (c) O_3, CH_2Cl_2–MeOH (3:1), −78 °C then $NaBH_4$, 0 °C (86%); (d) $ClCO_2Et$, pyridine, rt (96%); (e) TfOH, THF–H_2O (9:1), rt (quant); (f) Jones reagent, acetone, rt; (g) $Me_2NCH(Ot\text{-}Bu)_2$, toluene, reflux (72% in two steps); (h) Et_3OBF_4, K_2CO_3, CH_2Cl_2, rt (88%);

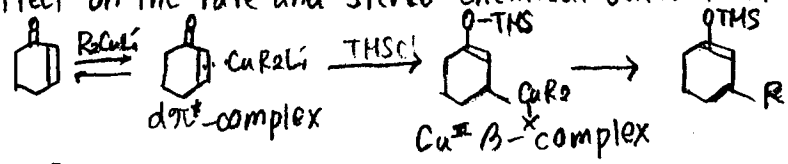
20



(S)-progultamic acid — $SOCl_2$, MeOH, 95% yield — $NaBH_4$, EtOH, 78% yield — $PhCHO, H^+$, 86% yield — LDA, THF — PhSeBr — Ozone, CH_2Cl_2, −78 °C, py — 75% yield — **20**

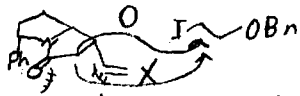
20→21 * TMSCl has effect on the rate and stereo chemical outcome of many cuprate reactions.

C(β) stereo center → C(α) stereo: O-Si strong > O-Li



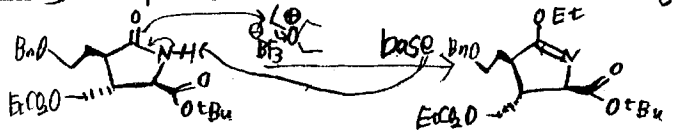
$d\pi^*$-complex — Cu^{III} β-X complex

21→23



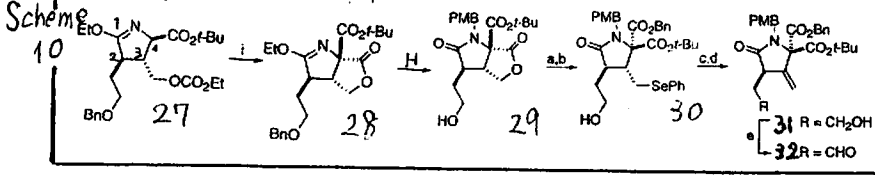
C(α) stereo center ← Ph group and C(β) group

25→27 Step (h): Et_3OBF_3 : Meerwein Reagent



〈A-4-2〉 Synthesis of intermediate 32

Scheme 10



(i) LHMDS, THF, −20 °C (82%); (j) 1 M HCl aq, THF, 0 °C (90%); (k) PMBCl, NaH, DMF, rt (61%); (l) $Pd(OH)_2$–C, H_2, EtOH, rt (quant).

(a) $PhSeSePh$, $NaBH_4$, EtOH, 60 °C; (b) BnBr, K_2CO_3, DMF, rt (65% in 2 steps); (c) 30% H_2O_2 aq, THF, rt; (d) toluene, 100 °C (94% in two steps, 72% **31** + 22% **32**); (e) Dess–Martin periodinane, CH_2Cl_2, rt (92%, 89% in three steps from **30**);

27→28 C(3) stereo → C(4) stereo : Using intramolecular acylation.

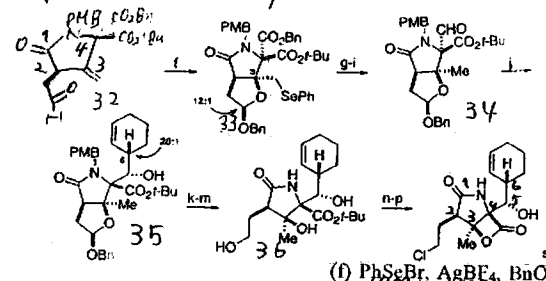
29→30 Step (a): nucleophilic ring opening (Ref: JACS, 1980, 3904, JOC, 1981, 46, 2605)

$PhSe\text{-}SePh + 2NaBH_4 \xrightarrow{EtOH} 2NaSePh[BH_3] + H_2$ ····(1)

ex)  80% yield

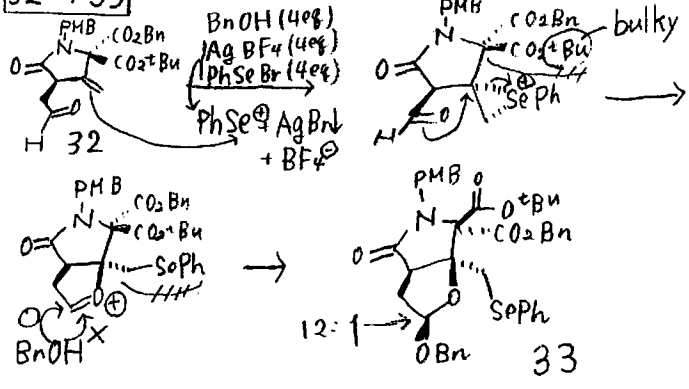
〈A-4-3〉 Synthesis of Salinosporamide A

Scheme 11



(f) PhSeBr, AgBF4, BnOH, CH_2Cl_2, −20 to 0 °C (74% as an anomeric mixture, 12:1); (g) AIBN, *n*-Bu_3SnH, toluene, 100 °C (98%); (h) $NaBH_4$, THF–EtOH (3:1), rt (85%); (i) Dess–Martin periodinane, CH_2Cl_2, rt (95%); (j) 8, THF, −78 °C (88% for **35**, *dr* = 20:1); (k) ceric ammonium nitrate (CAN), CH_3CN–H_2O, 0 °C (90%); (l) Na, liq NH_3, −78 °C; (m) $NaBH_4$, THF–H_2O (2:1), rt (97% in two steps); (n) BCl_3, CH_2Cl_2, 0 °C; (o) BOPCl, TEA, CH_2Cl_2, rt; (p) Ph_3PCl_2, pyridine, CH_3CN, rt (51% in three steps).

32→33 acetal Mediate cationic cyclization

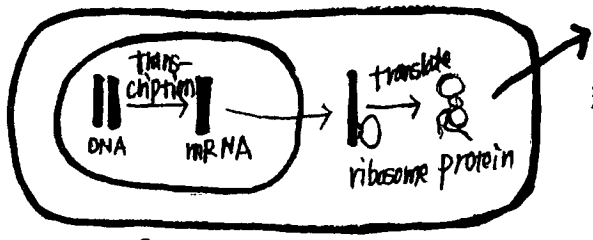


Total 28 steps, < 1.8% yield.

Ref: ・ユビキチンがわかる. 羊土社, 田中啓二
・有機合成協会誌 Vol 56, No10, 2000, 968
・Annu. Rev. Biochem, 67, 425, 1998

〈B〉Ubiqutin-Proteasome system

〈B-1〉Introduction.



Protein control life phenomenon.

*Abnomolly folded protein cause human Diseases.
ex) Alzheimer's disease.
Parkinson's disease. etc....

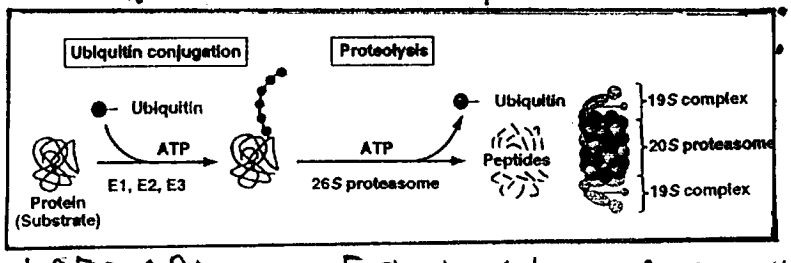
—System of protein degradation.

Outside cell .... Protease

Inside cell
- lysosome
- Ubiqutin-Proteasome System ... selective protein degradation

—Ubiqutin-Proteasome system

Fig. 1



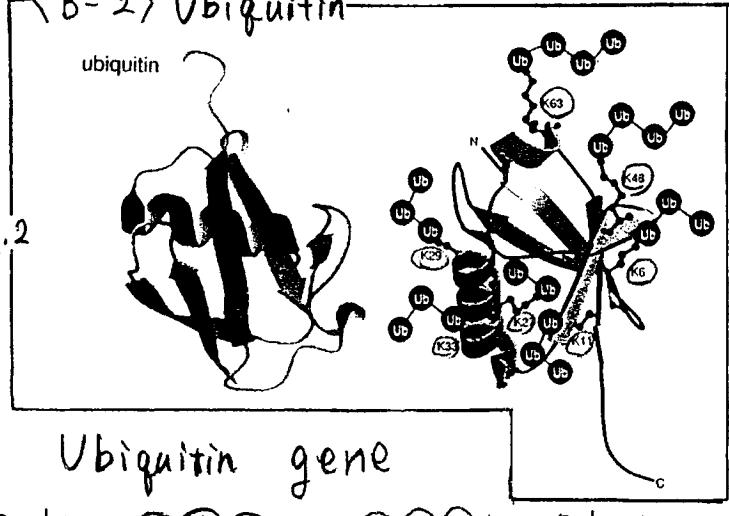
- Ubiqutin ....Degradation-signal
- Proteasome
  - a protein destruction machine
  - a complex protease

* ATP依存的タンパク質分解システム

-history- 1953 : Simpson 「The breakdown of the cell's own proteins does require energy」
1977 : Goldberg "A first step towards an explanation of this enegy-dependent protein degradation"
late 1970 and early 1980s
Nobel prize winner succeeded in showing that protein was destroyed being labelled with the ubiqutin and this regulation requires energy.

〈B-2〉Ubiquitin

Fig. 2



- 76-amino-acid residue.
- Secondary structure ... βββαββ
- 熱安定性タンパク質
- Strongly concerved in evolution.
- Ubiquitin has seven Lys residue. (K6, K11, K27, K29, K33, K48, K63)

Ubiquitin gene

- code two type
  - (Ub)(Ub)(Ub) ....(Ub)(Ub)(Ub): Polyubiquitin gene
  - (Ub)[LRP]
  - (Ub)[SRP]

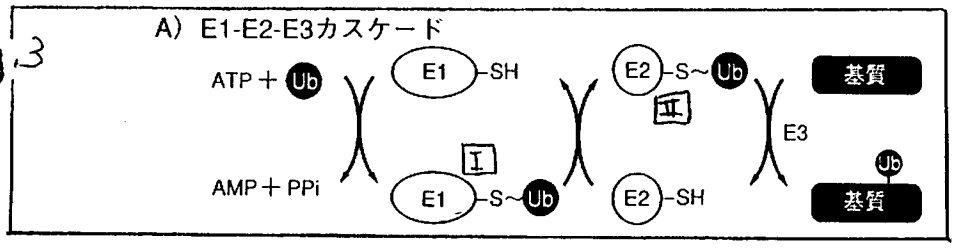
・ユビキチン
・リボソームタンパク質
融合遺伝子

(LSP: 大リボソームサブユニットタンパク質
SRP: 小リボソームサブユニットタンパク質)

・熱ショック応答遺伝子 ・脱ユビキチン化酵素により単体機能分子に成熟

〈B-3〉 Ubiquitin System

Fig. 3



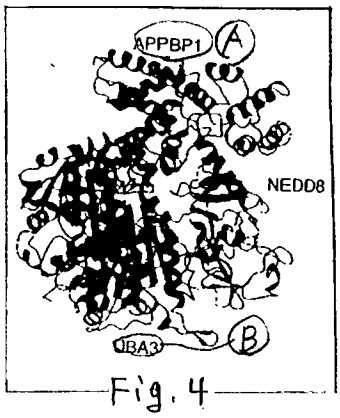


Fig. 4

- **E1 (Ubiquitin activating enzyme)**
  - 110~120 KDa
  - strongly conserved in evolution.
  - two domain ← Adenylation domain → (A)
    Catalytic Cys domain → (B)

Ub-C(=O)OH (C-terminal) —ATP, E1SH → AMP+PPi → Ubiquitin-adenylate intermediate → (Ub)-C(=O)-S-E1 (Fig 3. I)
E1-ubiquitin thiol ester.

- **E2 (Ubiquitin conjugating enzyme)**
  - core domain … 150 amino residue
  - E2 mediate the transfer of Ubiquitin from E1 to the protein substrate, with or without the participation of ubiquitin-protein ligase E3.

- **E3 (Ub-protein ligase)**

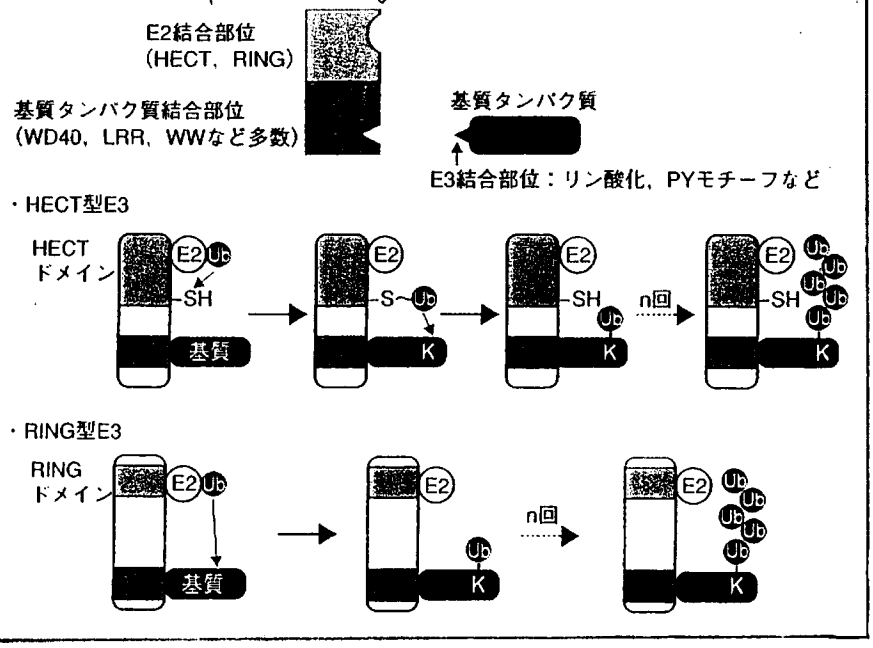
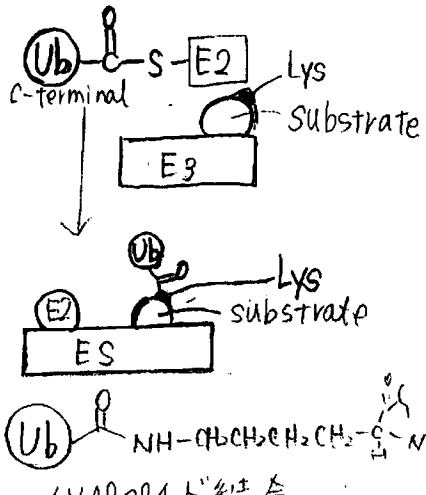


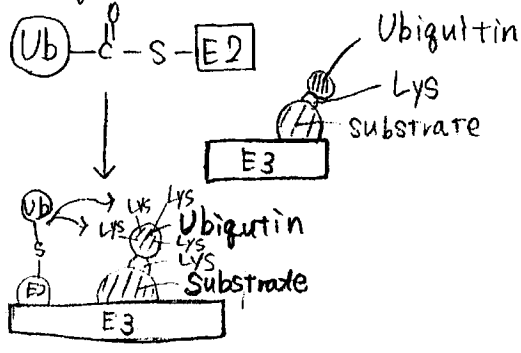
Fig 5

- hundreds of different E3 proteins existed.
- two type
  - HECT type
  - RING type



Ub—NH—CH₂CH₂CH₂CH₂—C(=O)—NH
イソペプチド結合

- **Polyubiquitin**



* Ubiquitin has seven Lys residue.

- K48 → protein degradation.
- K63 → DNAの修復 etc
  not protein degradation.

→ ユビキチンの修飾は多様性をもつ

〈B-4〉 Proteasome.

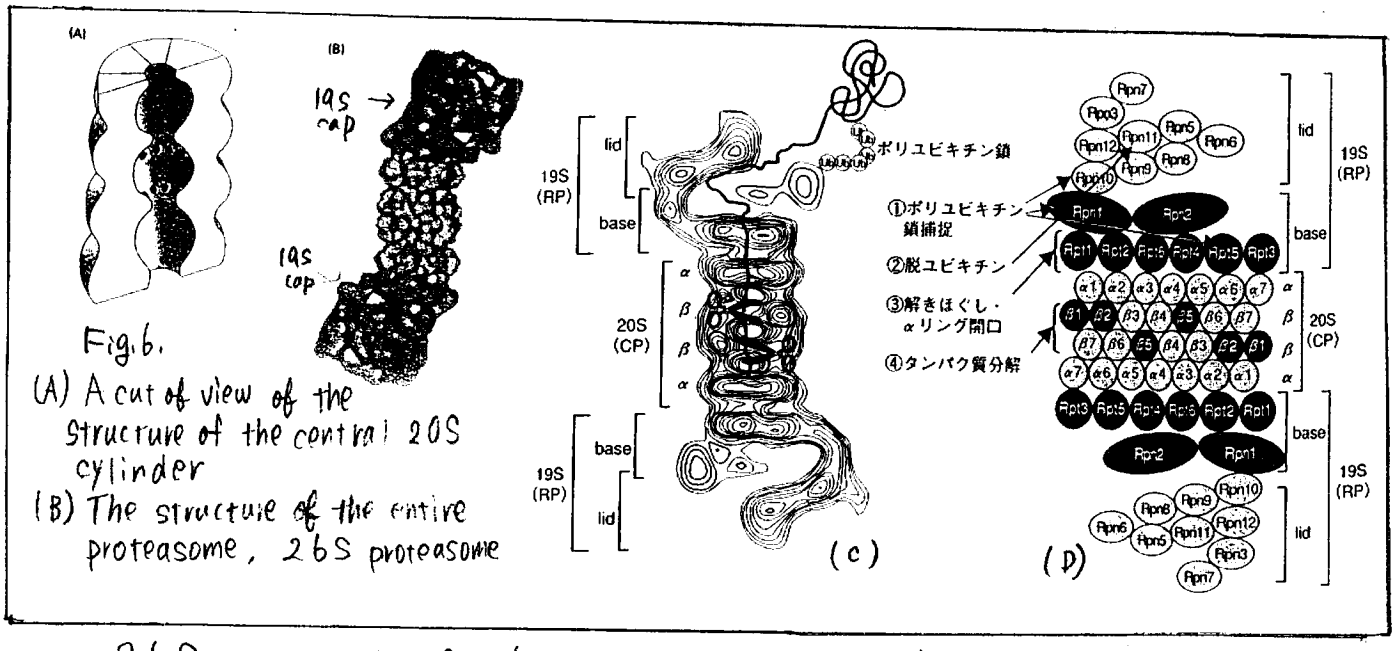


Fig.6.
(A) A cut of view of the structure of the central 20S cylinder
(B) The structure of the entire proteasome, 26S proteasome

26S proteasome (Fig: 6 - (B), (C), (D))

- ~2500KDa complex • made up of at least 32 different subunit
- highly conserved among all eukaryotes.
- two major subcomplexes
  - 20S (CP): contains the protease subunit
  - 19S (RP): regulatory particle (regulates the function of the former)

* 20S (CP) (Fig: 6 - (A))

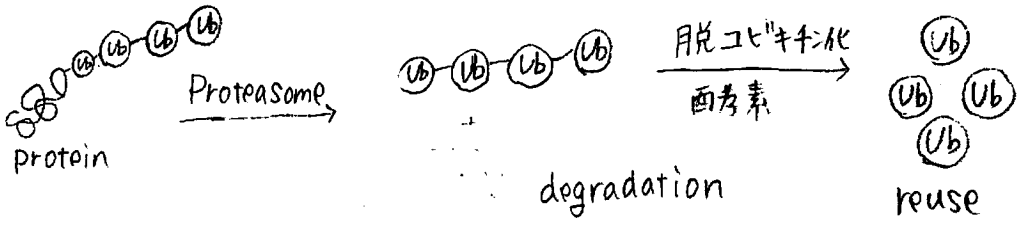
- barrel-shaped structure • length: 15nm • Diameter 11nm • 700KDa
- made up of four rings of seven subunits each
- Two inner β-ring contain the proteolytic active sites facing inward into a sequestered proteolytic camber.
- β1, β2, β5 subunit .... Active site
  chymotrypsin-like, trypsin-like, caspase-like

* 19S (RP) (Fig: 6 - (B), (C), (D))

- two multisubunit .... lid (蓋部), base (基底部)

lid .... nine "Rpn" subunit
- Rpn 11: de-ubiquitinating activity
- Rpn 10: "Adhesive"

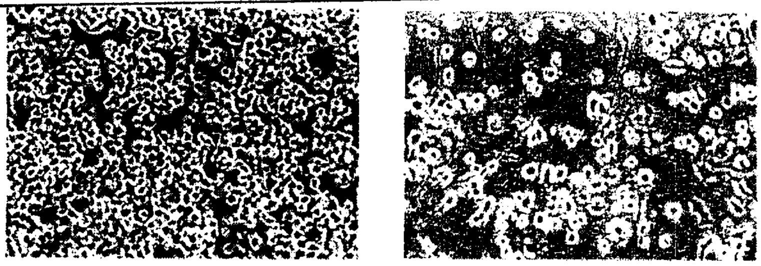
base ..... Rpt 1~6: six ATPase subunit
- Rpn 1, 2: Non-ATPase subunit



(Ref: J. Antibiot. ...
- Yakugaku zasshi, 120(10), 935, (2000),
- J. Med. Chem., 2005, 48, 3684)

⟨B-5⟩ Biological activity of Lactacystin and Omuralide.

Fig 7



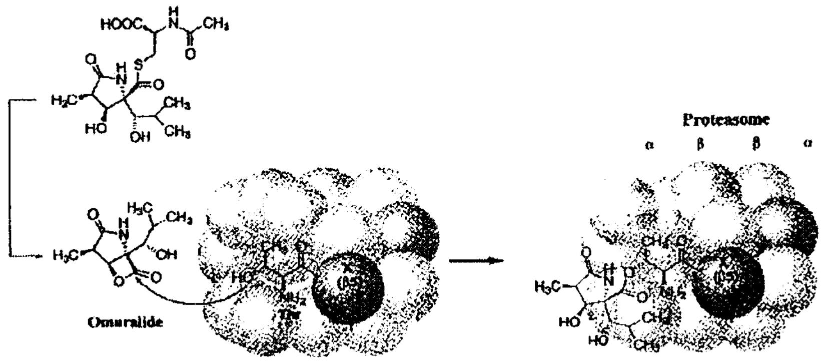
Control (day 4) Lactacystin (1.3 μM, day 4)

Photomicrographs of morphological changes induced by lactacystin in Neuro 2a cells

Bioactivity (PNAS. 1994, 91, 3358)
- induce Neurite outgrowth in Neuro 2A.
- arrest cell cycle at both G_0/G_1 and G_2 phase

⇩

Fig8



Inhibition of Proteasome Activities and Subunit-Specific Amino-Terminal Threonine Modification by lactacystin.

(Science, 1995, vol 268, 726)
(E. J. Corey)

* Lactacystin appears to modify covalently the highly conserved amino-terminal threonine of protease subunit β5.

⇨ Proteasome have NH_2 terminal threonine active site.

= Proteasomes are novel threonine protease.

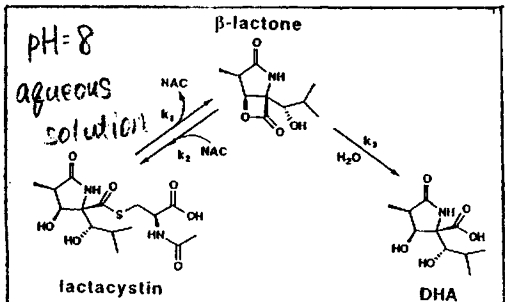


Fig. 9

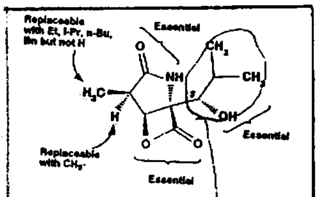


Fig 10

Ph = no biological activity

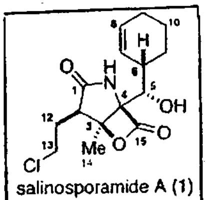


Fig. 11

* Salinosporamide A may intract with proteasome in a manner that is different from Omuralide.

* Application of lactacystin to cell biology

lactacystin: Proteasome inhibitor has been utilized to dissect the proteasome function.

⇨ leading to unexpected finding about importance of Ubiquitin-proteasome pathway and proteasome function.

Ubiquitin-Proteasome } Cell cycle, apoptosis (細胞自滅)
Antigen presentation ... etc

⇩

Ubiqutin-Proteasome system is involved in life-phenomenon.

→ The Nobel Prize in 2004.

Tumorgenesis is deeply related to progression of Cell cycle and apoptosis.

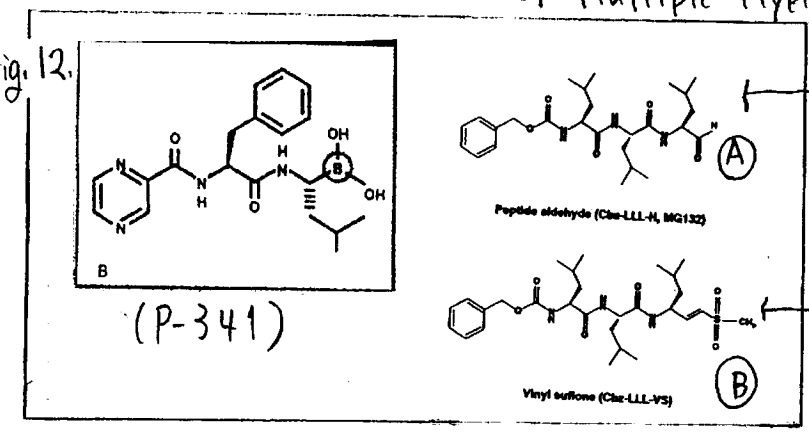
⇩

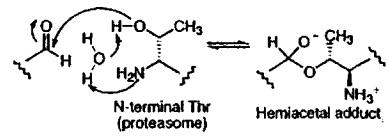
Proteasome inhibitors: Potential therapeutic agents.

〈Ref : http://www.medscape.com/viewarticle/463987_print〉

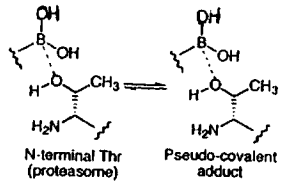
〈B-6〉 Bortezomib (P-341) ; First-in-Class Proteasome inhibitor for the treatment of Multiple Myeloma (MM) (多発性骨髄腫)

Fig. 12.





- Only short-lived proteasome inhibition
- all bind to 20S core particle in irreversible manner.



← dissociate more slowly from the proteasome stable inhibitor.

— Intracellur signaling pathway

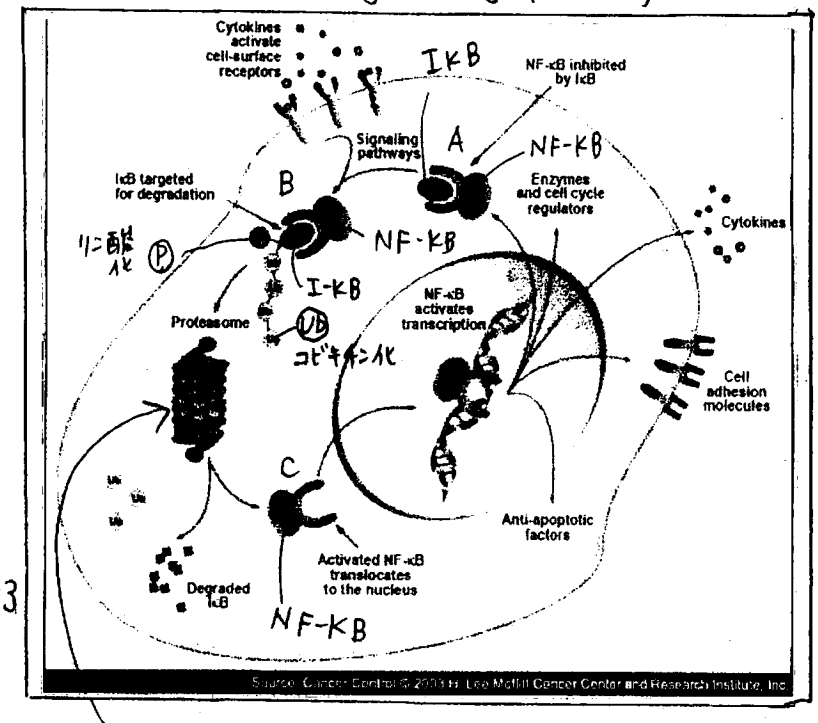


Fig. 13

Proteasome inhibition
↓
stabilize IκB and prevent the activation NF-κB
↓
increased apoptosis in cancer cell.
(細胞死)

In Myeloma cell, Cell growth was related to transcription factor nuclear factor-κB (NF-κB). (転写因子)

NF-κB
- is normally sequestered in the cytoplasm and rendered inactive by its inhibitor protein (IκB) (阻害タンパク質)

numerous stimuli, cellstress
↓
phosphorylation IκB
↓
Ubiquitin-IκB
↓
proteasome : degration IκB and release activated NF-κB
↓
NF-κB translated to the nuclear and initiates the transcription of wide range of genes, including involved in cell survival.