Tuberculosis

--Contents--

1. About tuberculosis

SERIOUSNESS OF TUBERCULOSIS

Tuberculosis(TB) is a disease due to the multiplication of *tubercle bacillus* in various organs.
It is still the greatest single infectius cause of mortality in the world.
Surprisingly, one third of the world's population are infected with TB.

	NEW PATIENT	THE DEAD
WORLD	8 million	2 million
JAPAN	30000	3000

/year

THE RELATIONSHIP TO AIDS

AIDS(=Aquired Immunodeficiency Syndrome 後天性免疫不全症候群).
HIV(=Human Immunodeficiency Virus ヒト免疫不全ウイルス)

infected with HIV → T-cells are destroyed → the immune system declines → susceptible to various microbe. → susceptible to TB especially

5.6 million people have been troubled with complication of TB and AIDS in the world.

THE VARIETY OF TB

Tubercle bacillus is multiplicated not only in lung.
In lymph node, backbone, kidney and brain etc. (7% of TB patient)
TB in brain is most terrible.

THE LAW OF TB PREVENTION

The law of TB prevention in JAPAN was revised in 2005.

	BEFORE	AFTER
The regular medical checkup	The annual obligation for more than 19 years old.	Different by age and place.
Tubercullin reaction	The obligation for under 4 years old.	Abolition

2. About anti-TB drugs

THE BACKGROUND OF ANTI-TB DRUGS

The discovery of SM, INH, RFP

⇩

A rapid decline of TB in many countries
(coupled with generally increasing standard of health care)

⇩

The indifference to the need for fresh drugs

The perception by the pharmacetical industry that would be unlikely to generate a suitable return on investment

⇩

A variety of changes in social, medical and economic factors

⇩

The dramatic increase in immuno-suppressed people.
(AIDS, cancer chemotherapy and organ-transplant practices)

⇩

The resurgence of TB

⇩

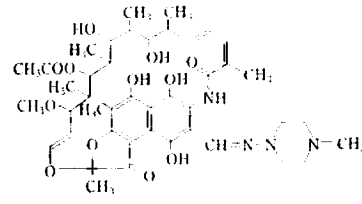
The occurrence of multiple drug resistant disease

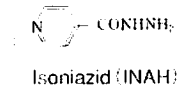
⇩

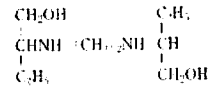
The urgent need of new anti-TB drugs

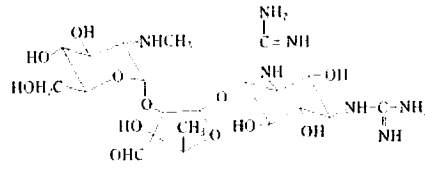
THE VARIETY OF ANTI-TB DRUGS

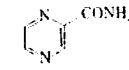
First-line drugs


Rifampicin

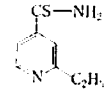

Isoniazid (INAH)

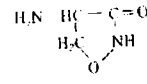

Ethambutol

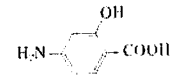

Streptomycin

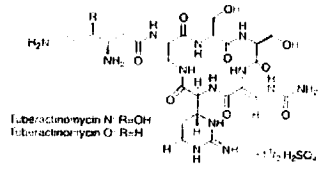

Pyrazinamide

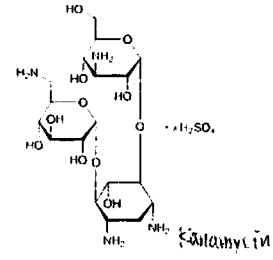
Second-line drugs


Ethionamide


Cycloserine


p-Aminosalicylic acid (PAS)



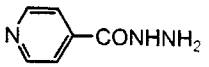
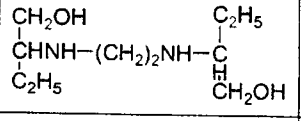
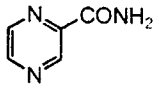


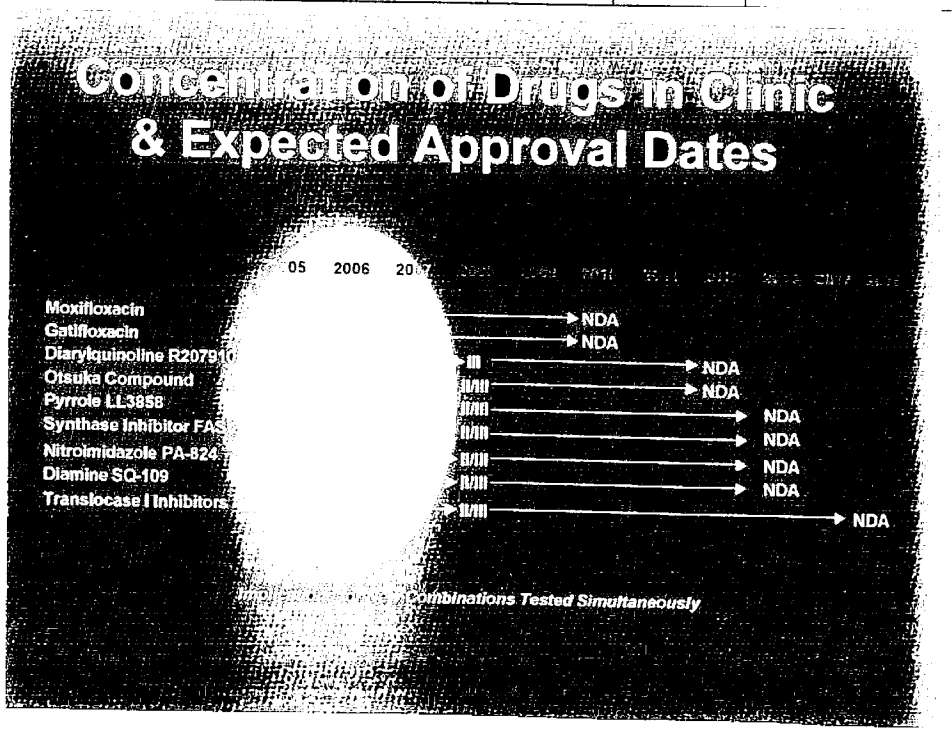
In order to prevent an appearance of the multiple drug resistant tuberculosis, the patient use more than two first-line drugs at the same time.

Treatment of first-line drugs resistant tuberculosis and
Side effects of first-line drugs

⟶ Use several second-line drugs

No development of new anti-TB drugs in the last 40 years

Name	Structure	Year of Introduction	Anti-TB activity	Toxicity	Inhibition site	Side effect	Comment
Isoniazid (Isonicotinic acid hydrazide, INH)		1952	++−+	Low	# Synthesis of mycolic acid # Metabolism of sugar and amino acid # Action of vitamin B_6 family	末梢神経炎 肝障害	# Only active bacteria # No cross reistance
Rifampicin (RFP)	Page 2	1965	−+++	Low	RNA polymerase(β subunit)	血小板減少 肝障害	# Also against against fission pausing cells # Easy to tolerize
Streptomycin (SM)	Page 2	1944	+−+	Medium	Ribosomal proteins	難聴 平衡失調	# First anti-TB drug # Not so strong
Ethambutol (EB)		1968	++	Low	Cell wall polysaccharides	視力障害	# No cross resistance against SM, INH, PAS # Not so strong
Pyrazinamide (PZA)		1970	+−+	Low	Enhancement of INH	肝障害	



3. Current status in the development of the new anti-tuberculosis drugs

THE REQUIREMENTS AS A NEW ANTI-TB DRUG

1. No crossing-resistance to current anti-TB drugs
2. Novel action mechanism
3. Good movement in the body
4. Bactericidal activity against fission pausing cells
5. Unique activituy against only Mycobacteria
6. No inhibition of other drugs activity
7. No excretive activity against other drugs

THE HOPE

1. Shortening the total duration treatment
2. Prevention and treatment of the multiple drug resistant tuberculosis
3. Improvement of the tratment completion ratio
4. Reducing the total medical expenditure

THE DIFFICULTY

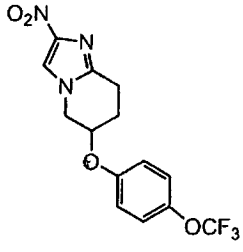
1. The development of new drugs doesn't always bring in good return for pharmaceutical company
2. The existence of side effects

WHY DID MULTIPLE DRUG RESISTANT *M.* TUBERCULOSIS APPEAR?

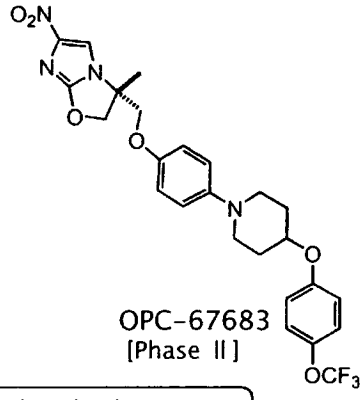
1. Incorrect treatment
2. Irregular taking
3. Stop taking
4. Genetic variation

CANDIDATE DRUGS

1. PA-824 and OPC-67683



PA-824
[Phase I]



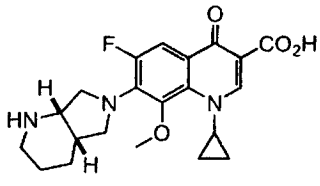
OPC-67683
[Phase II]

Nitroimidazole derivatives

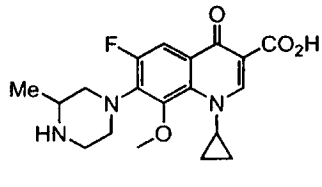
Chiron社（米）　　大塚製薬

Only *M. tuberculosis*
Inhibition both the biosynthesises of protein and mycolic acid
No cross resistance with existing drugs
Bactericidal activity against fission pausing cells
Low toxicity
Concerns about the toxic potential of the nitro group
Possible to same administration with anti-HIV drugs

2. Moxifloxacin (MXFX; Bay12-8039) and Gatifloxacin (GFLX; AM-1155)



Moxifloxacin



Gatifloxacin
[phase III]*

Bayer 社　　杏林製薬

8-Methoxy-fluoroquinolones

MXFX
Same activity as SPFX
Already launched for the treatment of respiratory tract infections
No clinical data for TB indicaton

GFLX
Higher Activity than LVFX
* Phase III-- as anti-respiratory tract infecion drug

Quinolones

Discovery of nalidixic acid

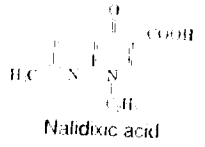
- levofloxacin(LVFX)
- ofloxacin(OFLX)
- ciprofloxacin(CPFX)
- sparfloxacin(SPFX)

} fluoroquinolines

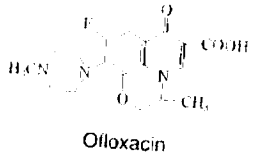
- Moxifloxacin(MKFX)
- Gatifloxacin(GFLX)

} 8-Methoxy-fluoroquinolones

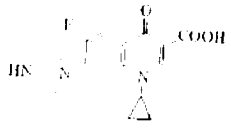
No cross resistance with existing drugs
Effective against the multiple drug resistance *M.* tuberculosis
No inhibition of other drugs activity
Few side-effect
The activity is weak comparatively
Cross resistance between Quinolines
Quick acquisition of resistance



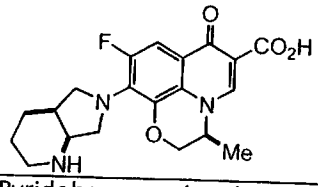
Nalidixic acid



Ofloxacin

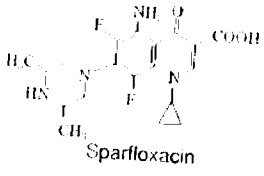


Ciprofloxacin

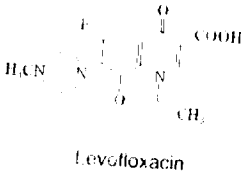


Pyridobenzoxazine derivative
第一製薬

High activity than LVFX and GFLX

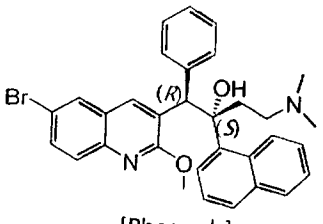


Sparfloxacin



Levofloxacin

3. R207910



[Phase I]
Johnson&Johnson 社

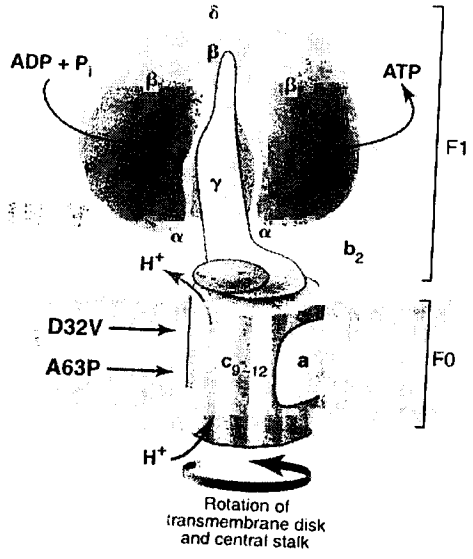
Diarylquinolines

Novel action mechanism
Higher activity than INH, RFP
Grately shorten the duration of therapy alone (less than 3 month from 6 month)
Compatible with existing anti-TB drugs
No potential activation sites

Planar hydrophobic moieties
Hydrogen-bonding acceptor and donor groups
} perfect drug-like features

They inhibit the biosynthesis of ...

Current drugs		R207910
Cell wall Protein folic acid Nucleic acid	*Quite difference!!* ⟺	ATP (Adenosine triphosphate)

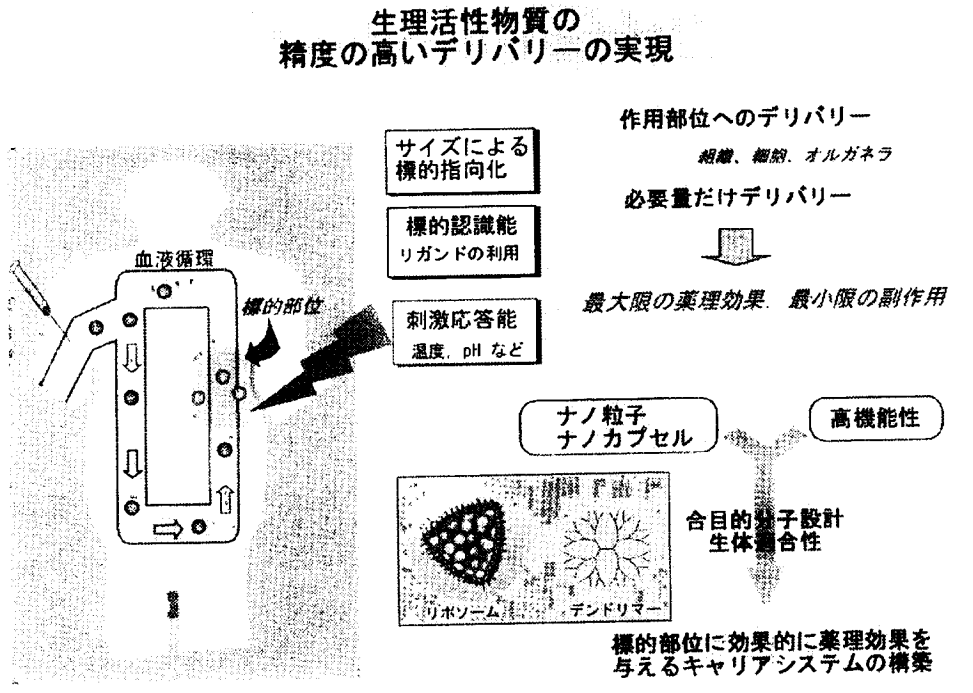


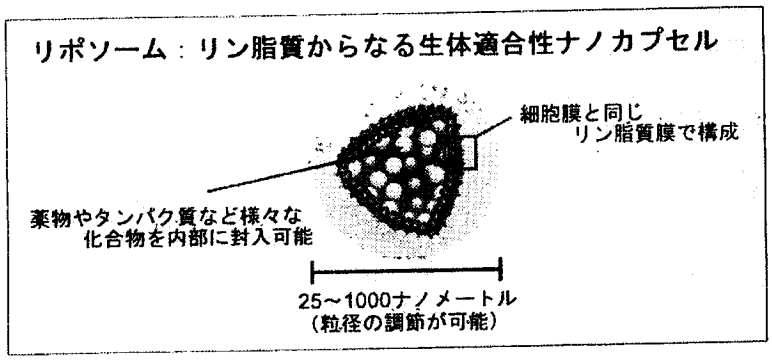
MICs against 29 isolates of *M.ulcerans*

Agent	MIC (μg/ml)	
	MIC_{50}	MIC_{90}
R207910	0.03	0.06
MXF	0.12	0.5
STR	0.25	0.5
RIF	0.5	2.0
AMK	0.5	2.0
LZD	0.5	2.0
PA-824	16	>16

4. Future of anti-tuberculosis drugs

1. DDS (Drug Delivery System)



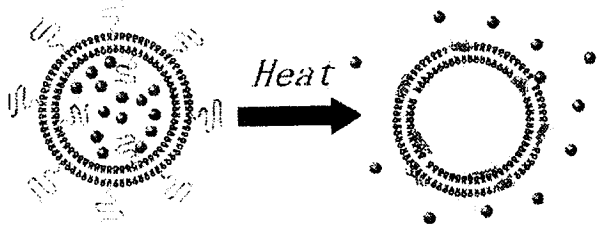


Liposome
Good biodegradability
Good biocompatibility
} Ideal carrier

Liposome technology

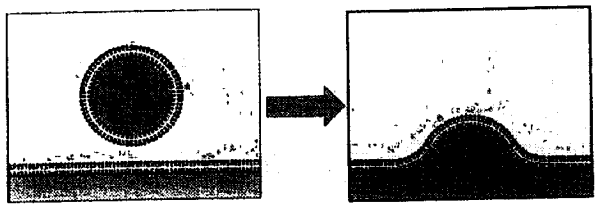
1) Tempeature sensitivity liposome
2) Membrane fusion liposome

1) Tempeature sensitivity liposom

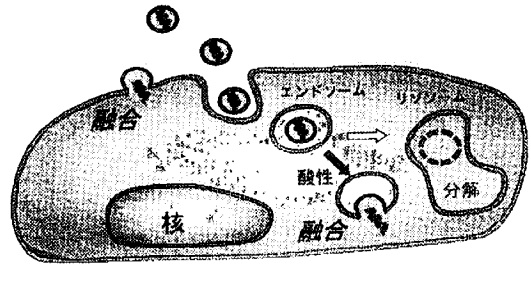


2) Membrane fusion liposome

[Directly fuse with cell-menbrane]



[fuse with endosome]



5. New method of tuberculosis diagnosis ~the alternative to BCG vaccine~

Current

Protein

BCG vaccine
---weakened *M.* tuberculosis

Tuberculin
---only *M.* tuberculosis protein extraction

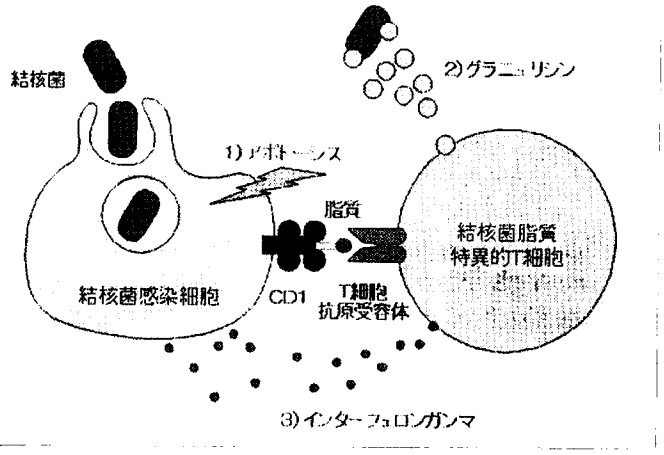
New

Lipid

Pure *M.* tuberculosis lipid vaccine

Lead to protective immunity

脂質抗原特異的T細胞による結核感染制御のメカニズム



A cell was infected with *M.* tuberculosis

CD1 molecule united to lipid generated by *M.* tuberculosis

Lipid-specific T-cell activation

Control over *M.* tuberculosis infection by 3 ways

⇨ *M.* tuberculosis lipid as a novel anti-TB vaccine is very useful !!

Application (New diagnosis)

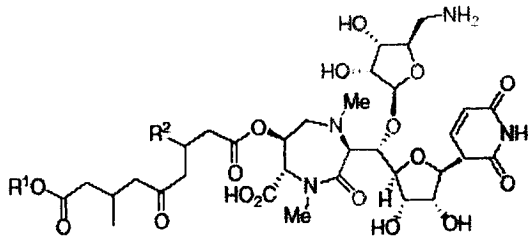
Using *M.* tuberculosis lipid as antigen ----► Antibody is detected in serum **only** Person infected with TB.

[merit]

1) Using **several** *M.* tuberculosis lipids as antigen, TB diagnosis in **very higher sensitivity.**
2) Using lipid **unique** to *M.* tuberculosis as anitigen, the distinction between TB and infectious disease by *Mycobacterium avium-intracellulare* complex (非結核性抗酸菌) is possible.
3) Speedy and inexpensive.

6. Caprazamycin--a new promising anti-tuberculosis drug

6.1 Prospective action mechanism of Caprazamycins ~Based on that of Liposidomycins~



liposidomycins : R^1 = H, R^2 = alkyl or alkenyl chain

caprazamycins : R^1 = fucose derivative, R^2 = alkyl chain

Figure 1. Structure of liposidomycins and caprazamycins, naturally occurring compounds with antibacterial activity.

Novel lipo-nucleoside antibiotics having novel chemical structure

Active against only acid-fast bacteria

MIC= 3.13 μg/ml (*M. tuberculosis* H37Rv)
0.05-0.07 μg/ml (MAC, cf; LPM 1.2-12.5g/ml)

Isolated from *Streptomyces* sp. MK730-62F2

Showed a therapeutic effect in a pulmonary TB model mice.

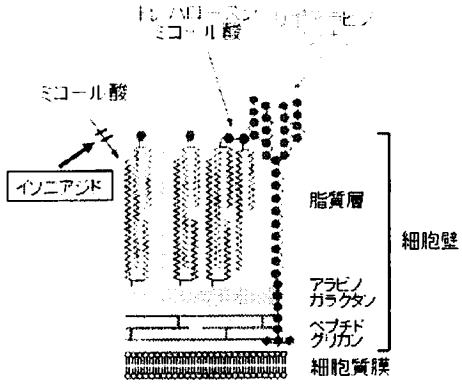
So far, no toxity

Unknown against fission pausing cells

 # Very closely related to Liposidomycins.

It was proved that they **inhibit the biosynthesis of cell wall of acid-fast bacteria.**
Detailed biological study of Caprazamycins is now in progress.
However, The action mechanism of them is similar to that of Liposidomycins prospectively.
So, the action mechanism of Liposidomycin is shown.

結核菌細胞壁の構造



Most bacteria have cell wall.
To withstand the high internal osmotic pressure within cell.
And maintain the form of cell.

Peptidoglycan is an essential component.
It provides much of the strength and rigidity by its mesh structure.

Liposidomycin and Caprazamycin inhibit a part of the biosynthesis of this peptidoglycan.

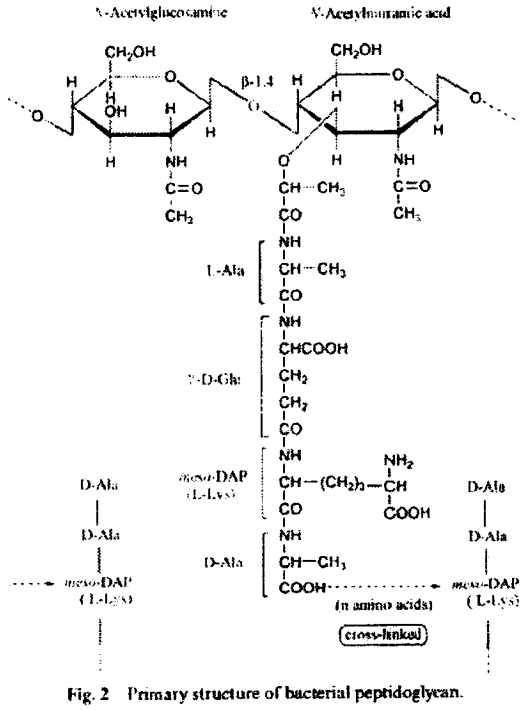
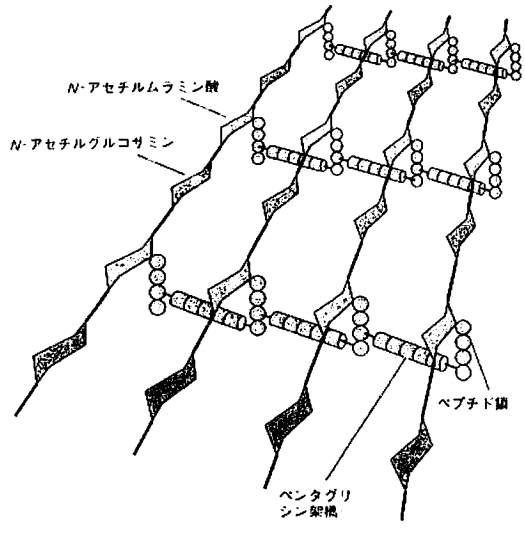


Fig. 2 Primary structure of bacterial peptidoglycan.

β-1,4-linked polysaccharide of alternating *N*-acetyl-glucosamine (Glc*N*Ac) and *N*-acetyl-muramic acid (Mur*N*Ac) sugars.

To the 3-position of muramic acid is attached a pentapeptide sidechain.
(L-Ala, γ-D-Glu, *meso*-DAP or L-Lys, D-Ala, D-Ala)

Cross-linked via amide bindings.
→ Provide the structual rigidity.



The process of biosynthesis of peptidoglycan

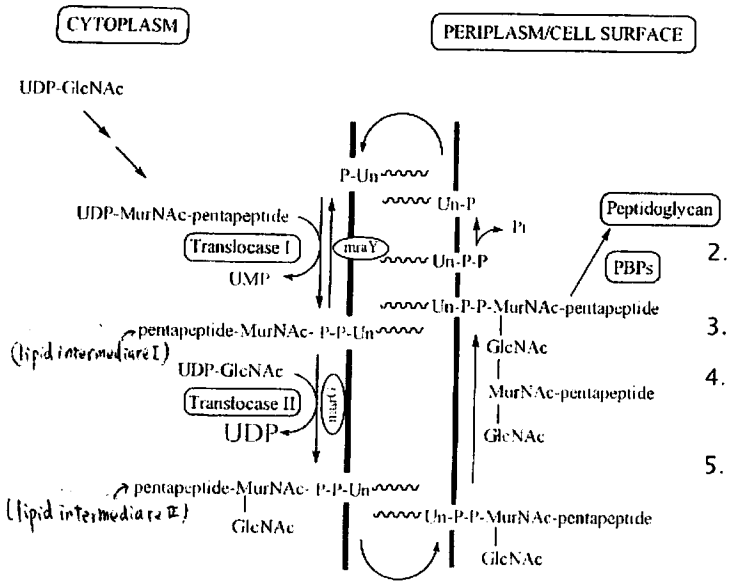
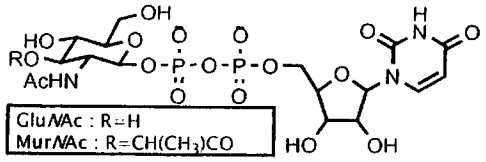
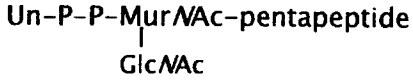


Fig. 3 Scheme of peptidoglycan biosynthesis.

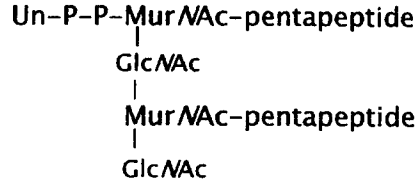
1. The synthesis of the amino sugar nucleosides
   uridine-5'-diphospho-*N*-acetylglucosamine (UDP-Glc*N*Ac)
   uridine-5'-diphospho-*N*-acetylmuramic acid (UDP-Mur*N*Ac)



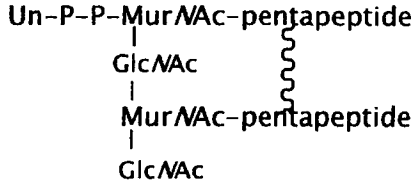
2. L-Ala, γ-D-Glu and *meso*-DAP or L-Lys are added. (by respective ligases)
3. D-Ala-D-Ala are added. **UDP-Mur*N*Ac-pentapeptide**
4. The transfer to undecaprenyl phosphate (lipid carrier) The emission of UMP
   Un-P-P-Mur*N*Ac-pentapeptide
5. Connection of a residue of Glc*N*Ac from UDP-Glc*N*Ac.



6. Translocation across the the cytoplasmic membrane.
7. The lengthener of disaccharide-pentapeptide.



8. The transpeptidation, the synthesis of cross-linking.



Liposidomycin inhibit on the first step of lipid cycle, catalysed by transelocase Mra Y.

There is no commercial antibiotic targeted against translocase I, surprisingly.

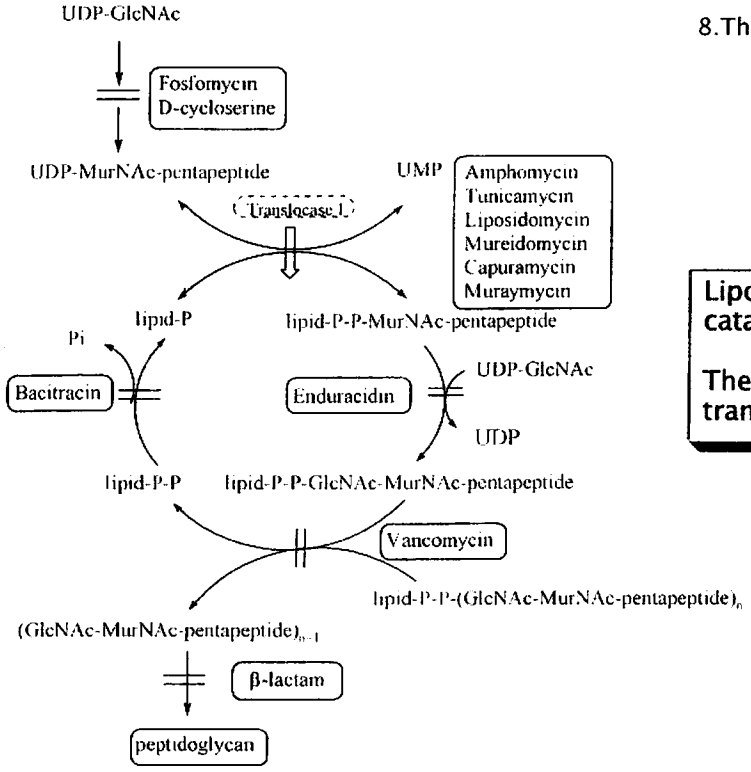
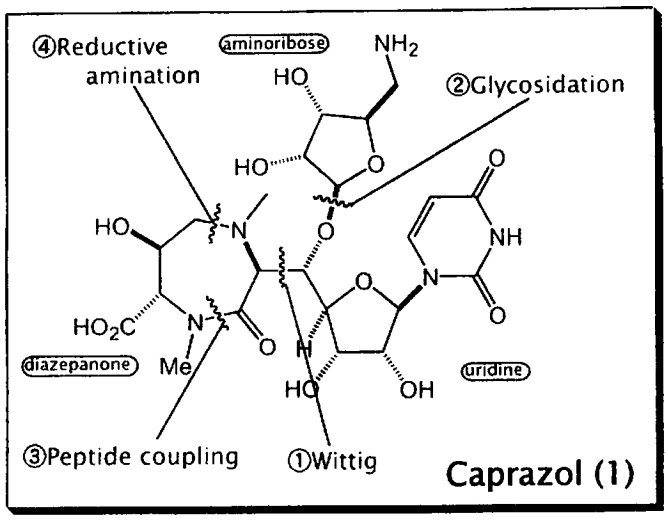


Fig. 4 Lipid cycle of peptidoglycan biosynthesis and inhibitors.

6.2 Total synthesis of Caprazol (1)

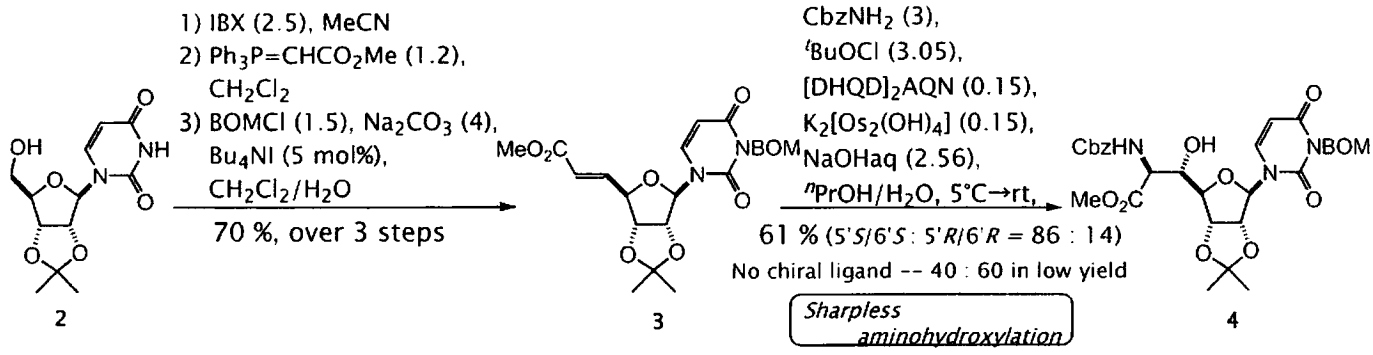


Akira Matsuda et al, *Angew. Chem. Int. Ed.* **2005**, *44*, 1854-1856

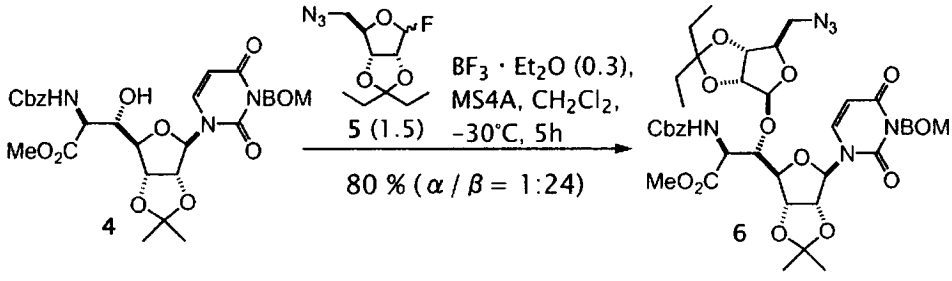
The difficulties posed by the synthesis are...

1. The introduction of 5-aminoribose moiety found in Caprazol after construction of the uridyldiazepanone moiety.
2. β-heterosubstituted caboxyl moiety would be sensitive to basic conditions.
3. The construction of diazepanone.

→ They planned to introduce the aminoribose protected with an acid-libile protecting group at an early stage of the synthesis.

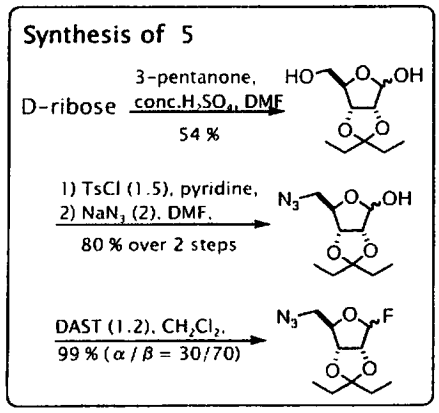


Glycosidation



The control of β-riboside selective by steric hindrance of a group installed on the α face of the ribofuranoside. Without using a ribosyl donor proteced with a 2-*O*-acy group.

The study of acid-labile protecting group



Scheme 1

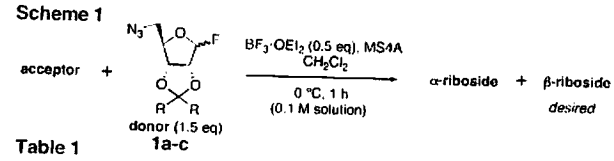
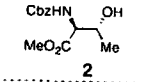
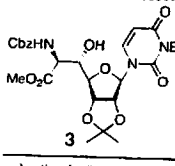


Table 1

acceptor	protective group of donor	yield of ribosides (%)	$\alpha : \beta$ [a]
 2	**1a**; isopropylidene (R = -Me)	86	1 : 4.8
	1b; cyclopentylidene (R = -$(CH_2)_4$-)	86	1 : 3.5
	1c; 3-pentylidene (R = -Et)	95	1 : 37
 3	**1a**; isopropylidene (R = -Me)	72	1 : 2.8
	1b; cyclopentylidene (R = -$(CH_2)_4$-)	75	1 : 2.4
	1c; 3-pentylidene (R = -Et)	80	1 : 24

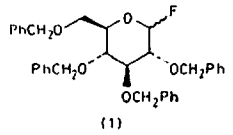
a) ratio of α/β was determined by ^{1}H NMR spectra

A 3-pentylidene group which is more sterically hindered than an isopropylidene group gave in good β selectivity.

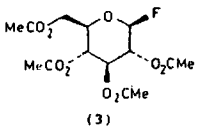
The study of Lewis acid

- $BF_3 \cdot Et_2O$ is an effective catalyst for the coupling reaction.

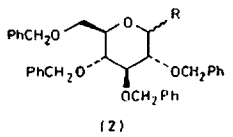
Ref. K. C. Nicolaou et al, *J. Chem. Soc. Chem. Commun.* **1984**. 1155-1158.



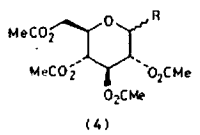
(1)



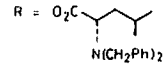
(3)



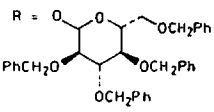
(2)

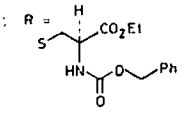


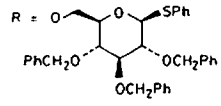
(4)

a: R = 

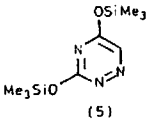
d: R = 

b: R = 

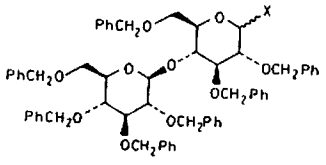
e: R = 

c: R = 

f: R = 



(5)



(6) X = F
(7) X = R(OCOMe)

Table 1. Synthesis of O-, S-, and *N*-glycosides.

Entry	Reagents (equiv.) and conditions	R	Yield ratio α:β[f]
Substrate (1)[a,b,c] → product (2).[b]			
1	$MeCO_2H$ (4), $BF_3 \cdot Et_2O$ (0.5), CH_2Cl_2, 4Å MS,[d] 0→25 °C	Me	97 (*ca.* 3 : 2)
2	RH (2), $BF_3 \cdot Et_2O$ (0.3), CH_2Cl_2, 4Å MS, 0 °C	(a)	76 (10 : 1)
3	RH (1), $BF_3 \cdot Et_2O$ (0.3), CH_2Cl_2, 0→25 °C	(b)	65[e]
4	$(PhCH_2O)_2P(O)OSnBu^n_3$ (2), $BF_3 \cdot Et_2O$ (2), Et_2O, 0→25 °C	$O(O)P(OCH_2Ph)_2$	50 (10 : 1)
5	RH (1), $BF_3 \cdot Et_2O$ (0.5), 4Å MS, −15→0 °C	(c)	51 (2 : 1)
6	Bu^tO_2H (2), $BF_3 \cdot Et_2O$, (0.2), CH_2Cl_2, 4Å MS, −15→0 °C	O_2Bu^t	91 (3 : 1)
7	Me_3SiN_3 (2.5), $BF_3 \cdot Et_2O$ (0.5), CH_2Cl_2, 0→25 °C	N_3	90 (10 : 1)
8	RH (1.5), $MgBr_2 \cdot Et_2O$ (5), CH_2Cl_2, 25 °C	(d)	90 (1 : 10)
9	$H_2NCH_2CH{=}CH_2$ (1), $AlMe_3$ (1), CH_2Cl_2, 25 °C	$NHCH_2CH{=}CH_2$	95 (2 : 1)
10	H_2NPh (2), $AlMe_3$ (2), CH_2Cl_2, 25 °C	NHPh	65 (1 : 1)
Substrate (3)[a,b] → product (4).[b]			
11	RH (1.5), $BF_3 \cdot Et_2O$ (0.1), CH_2Cl_2, 25 °C	(e)	80 (1 : 20)
12	PhSH (2), $BF_3 \cdot Et_2O$ (0.1), CH_2Cl_2, 25 °C	SPh	95 (1 : 20)
13	(5) (1.5), $SnCl_4$ (0.2), CH_2Cl_2, 25 °C	(f)	76 (1 : 20)
Substrate (6)[a,b,c] → product (7).[b]			
14	$MeCO_2H$ (4), $BF_3 \cdot Et_2O$ (0.5), CH_2Cl_2, 4Å MS, 0→25 °C	OCOMe	83 (3 : 2)

[a] Prepared from the corresponding phenylthioglycoside and *N*-bromosuccinimide–diethylamino sulphur trifluoride (ref. 2); [b] structure determined by spectroscopic methods; [c] α:β mixture *ca.* 1:1; [d] MS = molecular sieve; [e] ratio not determined; [f] ratio was determined

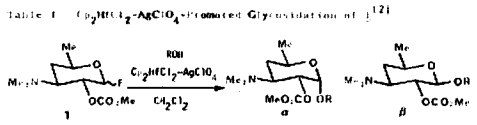
· Glycosyl ester ... 1, 2, 14 · Glycosyl azide ... 7
· Amino glycoside ... 3 Aminoglycoside ... 8-10
· Glycosyl bromide ... Thioglycoside ...

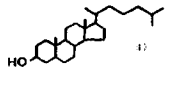
· 6-azauridine (8)
$SnCl_4$ ... mild Lewis acid

× Amino glycoside (8-10)
$AlMe_3$ or $MgBr_2 \cdot Et_2O$
↳ the more active glycosyl bromide

· AgOTf, Cp_2HfCl_2 --- α / β = 11:89 loss of stereoselectivity Ref. K. Suzuki et al, *Tetrahedron Lett.* **1988**, *29*, 3571-3574.

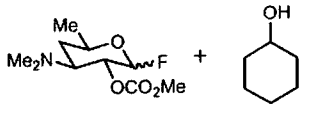
Table 1. $(Cp_2HfCl_2$-$AgClO_4$-Promoted Glycosidation of 1[12]



		Yield (%)	α : β[a]
1		91 (95)	β (β)[b]
2		92 (99)	β (β)[b]
3		93 (76)	1 : 26 (β)[b]
4		91 (64)	1 : 50 (1 : 4)[b]

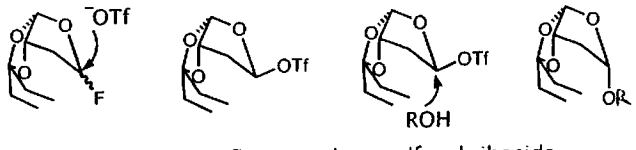
[a] Stereochemistry: 1–ROH–Cp_2HfCl_2–$AgClO_4$ = 1:2:5:5; [b] Determined by ^1H NMR (400 MHz) and isolation of β by $SnCl_2$–$AgClO_4$ method (ref. 7a); [c] 3,6-Diol was used.

The study of metal source

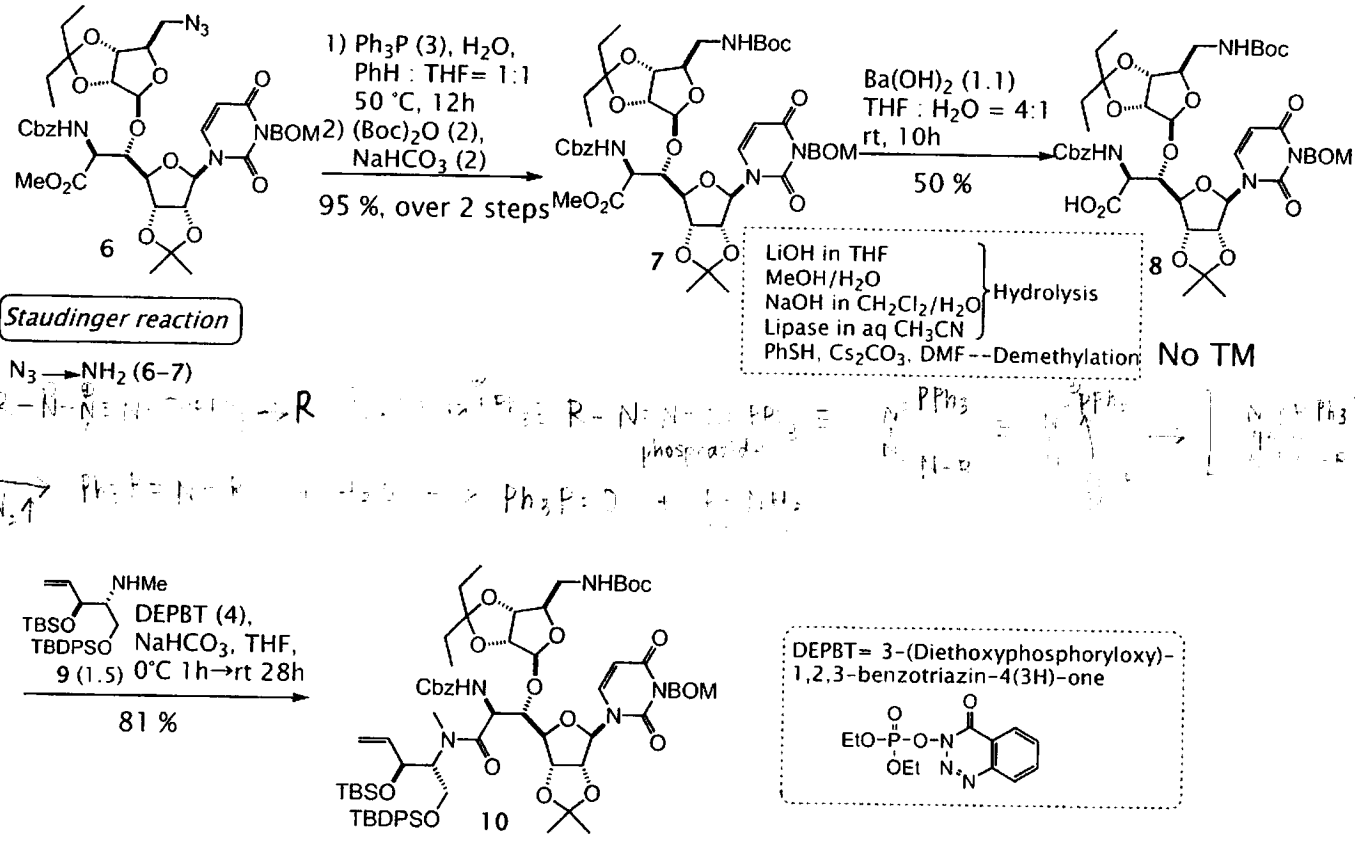


Cp_2ZrCl_2-$AgClO_4$: 12 % yield
Cp_2HfCl_2-$AgClO_4$: 91 % yield
TMSOTf : 52 % yield
$SnCl_2$-$AgClO_4$: 95 % yield

$SnCl_2$-$AgClO_4$ is effective so far as the simple *sec*-alcohols(1,2) are concerned.
In the case of the bulkier glycosyl acceptors, in low yield (3) or low selectivity (4)



The β-*O*-trifluoromethanesulfonyl riboside intermediate might be formed.
And S_N2 attack of the alcohol would give the undesired α - riboside.

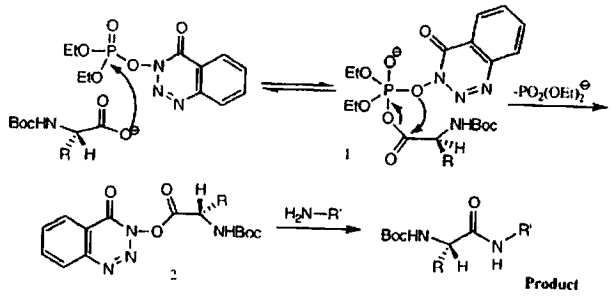


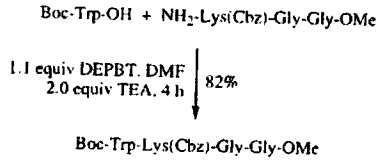
DEPBT--A new coupling reagent

- a remarkable resistance to racemization.
- easy preparation.
- exceedingly stable.
- can be used under normal peptide coupling conditions.

Ref. Murray Goodman et al, *Org Lett.* 1999, *1*, 91-93.

Scheme 1. Proposed Mechanism for DEPBT-Mediated Coupling





Without protecting the hydroxyl of the amino component.

(17) Synthesis of DEPBT: To a solution of HOOBt (14.3 g, 0.088 mmol) and TEA (8.92 g, 0.088 mmol) in CH_2Cl_2 (100 mL) at 0 °C is added diethylphosphorochloridate (17.3 g, 0.1 mmol) in CH_2Cl_2 (50 mL) dropwise. The reaction mixture is stirred for 3 h. After the resulting triethylamine hydrochloride salt is removed by filtration, the solvent is removed. The residue is dissolved in EtOAc (150 mL); washed with 0.1 N HCl, water, and brine; dried over $MgSO_4$; and taken to dryness. The crude product is recrystallized from EtOAc/petroleum ether to give colorless crystals of DEPBT (21.5 g, 82%), mp 72−74 °C. MS (EI): 299 M^+. Anal. Calcd for C, 44.15; H, 4.72; N, 14.05. Found C, 44.20; H, 4.71; N, 14.30.

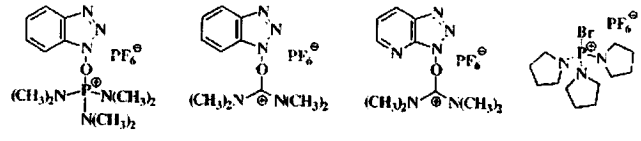
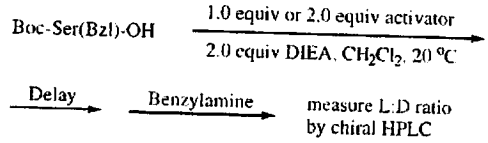


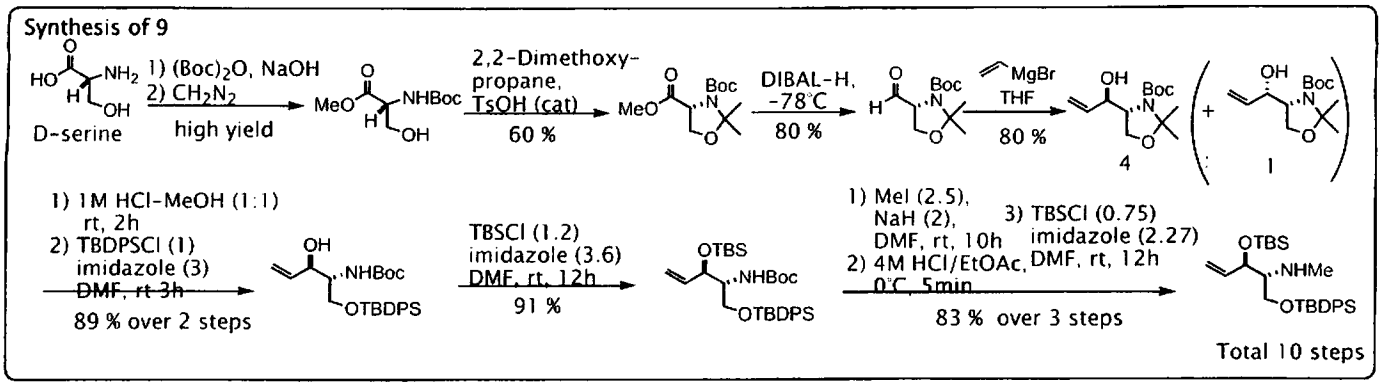
Figure 1. Structures of activating reagents utilized in this study.

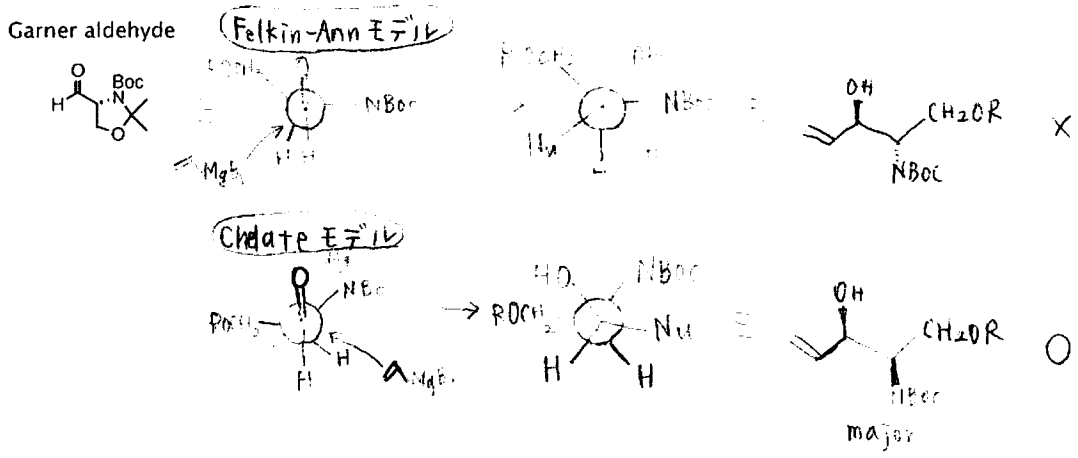
Table 1. Comparative Studies of Racemization during in Situ Activation

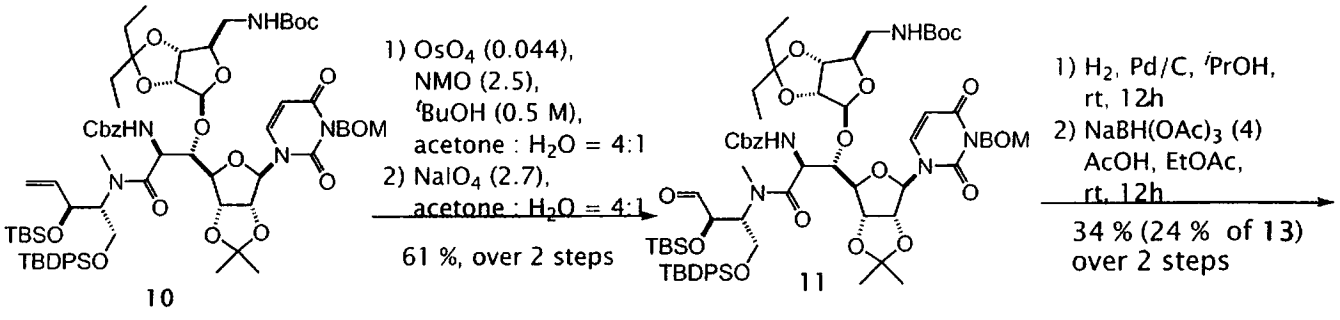


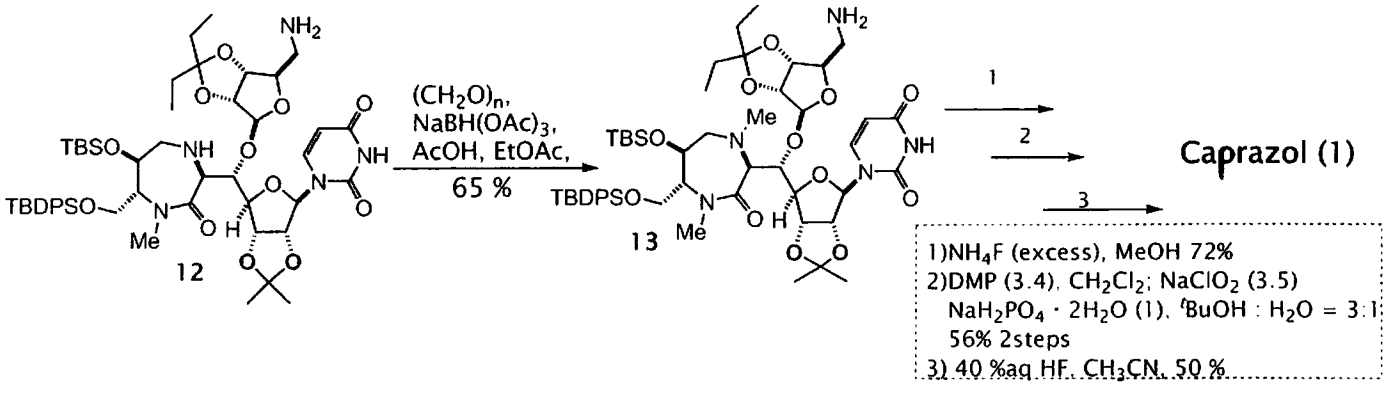
activation	delay time, min	L:D ratio	yield, %
PyBrop (1.0 equiv)	4	65:35	81
HATU (1.0 equiv)	4	84:16	91
HBTU (1.0 equiv)	15	79:21	>99
BOP (1.0 equiv)	15	85:15	95
DEPBT (1.0 equiv)	60[a]	95:5	70
DEPBT (2.0 equiv)	60[a]	96:4	>99

[a] Even with such long delay time, very little racemization is observed.









Synthesis of 7-membered Diazepanone (11→12)

The authers tried several methods as a sysnthesis of Diazepanone.

First trial

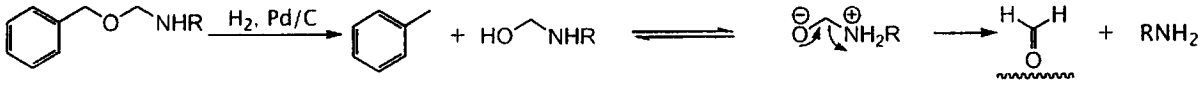
Both deprotection of the Cbz, BOM and reductive amination of aldehyde promoted by catalytic hyderogenetion with Pd/C were successful.
Because of the difficulty in hydrogenating the cyclic imine to give 12.

Second trial

Additional forcing conditions under medium pressure gave the 5,6-dihydrouridine derivertive owing to overreduction.

Along with *N*-methylated 14

When BOM proup was deprotected, formaldehyde was generated.
It was the metyl source.



Reductive amination was abandoned in favor oh hydride reduction with $NaBH(OAc)_3$.

Total 18 steps

All anti-TB drugs are not synthesized
using catalytic enantioselective reaction yet.