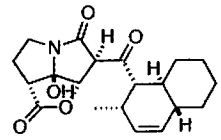
literature seminar

Targeting telomere/telomerase

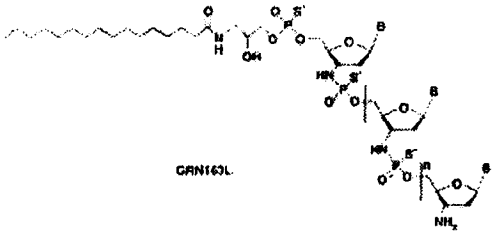
Whether telomere/telomerase find out to be a near universal anticancer target?

31st January, 2007
Noriko Takahashi (B4)

Contents



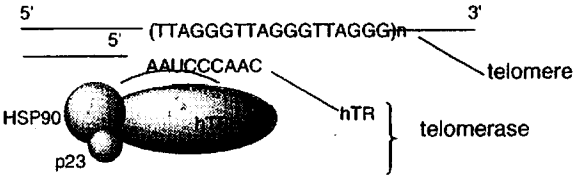
UCS1025A

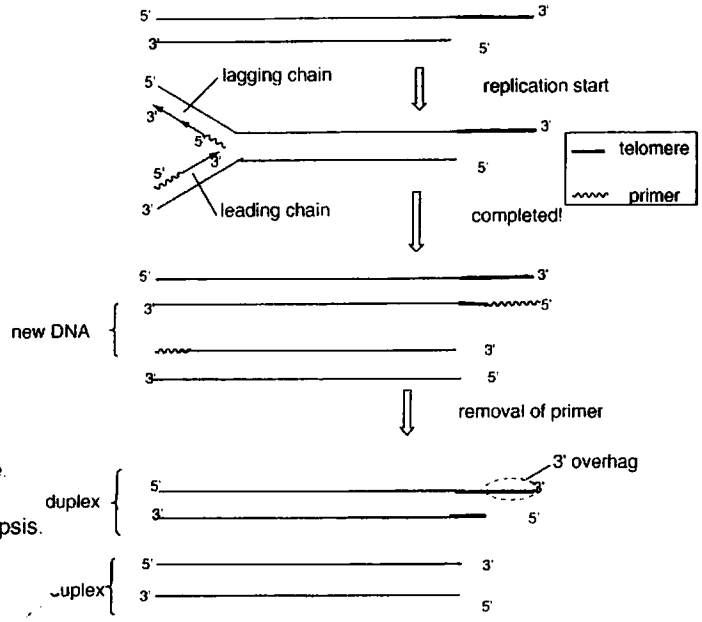


1. What is telomere and telomerase?

Telomere

part of DNA structures (at the end of eukaryotic chromosomes)
15-20 kbp(human)
repeating hexameric TTAGGG
single-strand overhang of the 3'-G-rich strand.





What is telomere for?

When cells divide by mitosis, telomeres get shorter in every replication.
because the DNA polymerases can't copy the very end of each telomere.
(End-Replicatiom problem) (50 to 100 base pairs shorter)
When telomeres get critically short, cells stop dividing and commit apotopsis.
(only 50-100 times replication)

⟹ Telomere serves as molecular clock!?

Telomarase

enzyme that catalyzes the lengthening of telomeres.
a ribonucleoprotein complex.
composed of
(1) the reverse transcriptase protein subunit(hTERT),
one of the major component
a catalytic protein subunit
(2) an endogenous 455-nucleotide RNA subunit(hTR)
closely associated with hTERT.
the second of the major components.
a template for telomere lengthening

A lot of proteins connect with hTERT and hTR, to control their functions.(ex. p23, HSP90)

90% of cancer cells → active
85% of normal cells → inactive

⟹ Tumor cells ; typically **long**, **constant** telomere length.
Normal cells; slowly shortening telomeres.

These difference make cancer cells **more sensitive** to telomerase inhibitors.
One of molecular targeting therapy
Treatment **without significant side effect.**

⟹ Whether telomere/telomerase find out to be a near universal anticancer target?

#Where to target?

Targeting telomere agent(TTAs)

small organic molecules ; inhibit by distorting the duplex ------→ addition of new bases difficult ,
or by overstabilizing the duplex ------→ hinder unwinding, an essential step in the DNA replication process.

Targeting telomerase agent

oligonucleotides(template antagonist); to block specific RNA-protein contacts

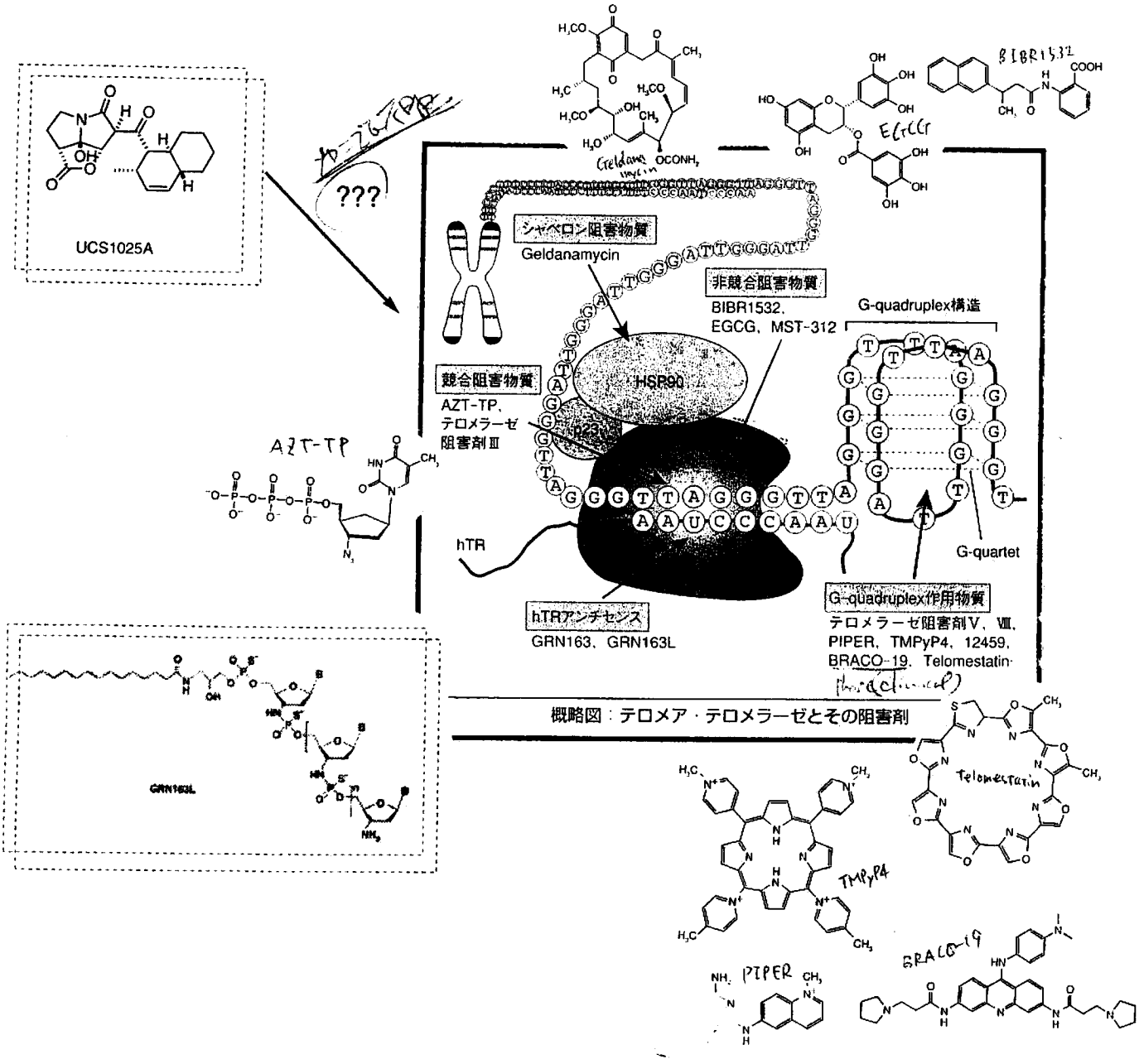
Others

Immunotherapeuic agents; cancer cells only presents telomerase peptide fragments on their cell surface.
↓
antigens.

going to Phase 2 clinical trial

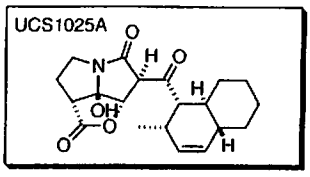
For example

Targeting telomerase agent ; **GRN163L**, BIBR1532, AZT-TP, EGCG
Targeting telomere agent(TTAs); BRACO19, PIPERT, TMPyP4, Telomestatin, 12459

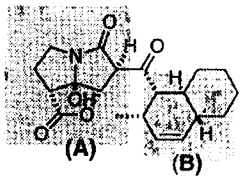


概略図：テロメア・テロメラーゼとその阻害剤

2. UCS1025A -a new antitumor antibiotics

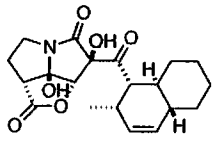


Isolation; from Acremonium sp. KY4917 in1999 by Kyowa Hakko group.
Structure and stereochemistry elucidation
studied by Kyowa Hakko group (*Organic letters* **2002**, *4*, 4387)
a novel natural product
two segments; an unique **tricyclic skeleton(A)** including a pyrrolidine fused with a γ-lactone
a **trans decaline** moiety(**B**).



Bioactivity; Against both Gram-positive and negative bacteria,
antiproliferative activityagainst human tumor cell lines
IC_{50} 21-58μM
relatively

UCS1025B was also isolated
But no biological activity



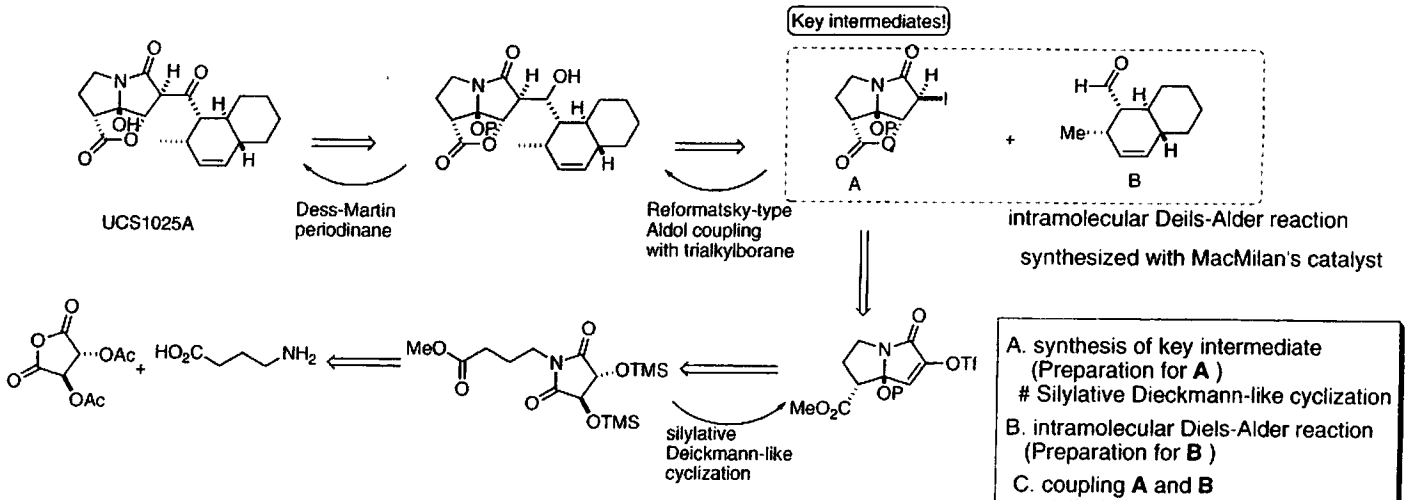
UCS1025B

Total synthesis by Danishefsky (2-1.) Coupling **A + B**
by Dyornikovs (2-2.) Biomimetic

2-1. Total synthesis by Danishefsky (*J. Am. Chem. Soc.* **2006**, *128*, 426)

2006 December 18, C&EN
p17- Chemistry Hightlights 2006
-The first total synthesis of UCS1025A, a promising inhibitor of the enzyme telomerase, was achieved in an remarkably concise manner
an approach that sidestepped problems encountered in earlier efforts-

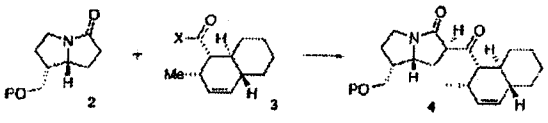
Retrosynthetic analysis



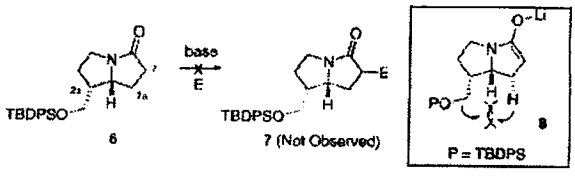
A. synthesis of key intermediate
(Preparation for **A**)
Silylative Dieckmann-like cyclization
B. intramolecular Diels-Alder reaction
(Preparation for **B**)
C. coupling **A** and **B**
#trialkylborane-induced reraction

Unsuccesful attempt

Scheme 1. Original Synthetic Strategy toward UCS1025A

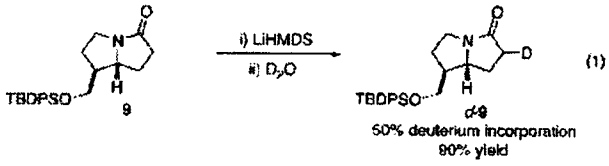


Scheme 2. Attempted Functionalization of Core Fragment 2



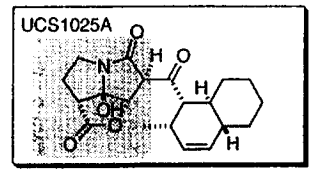
diastereomer is able to enolize.

steric repulision



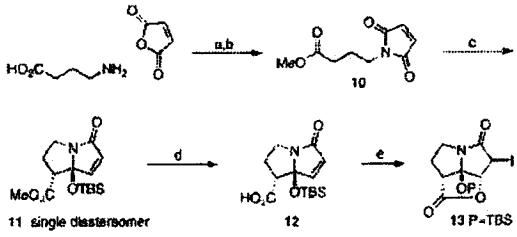
A. synthesis of key intermediate **A**

UCS1025A



Racemic synthesis of target intermediate 13

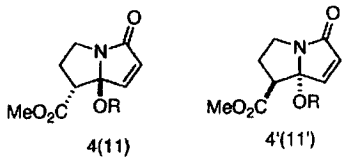
Scheme 3. Synthesis of Iodolactone 13[a]



[a] Key: (a) AcOH, then toluene, Et_3N, reflux, Dean–Stark trap (75%); (b) $SOCl_2$, MeOH (77%); (c) TBSOTf, Et_3N, CH_2Cl_2, r.t. (80%); (d) LiOH·H_2O, 3:1 THF:H_2O (99%); (e) I_2, sat. $NaHCO_3$, Et_2O, THF (84%).

(a) 1,2 addition of amine to carbonyl group and cyclization
(b) methyl esterfication
(c) Silylative Dieckmann-like cyclization and OH protection with TBS
(d) hydrolysis
(e) iodolactonization

11 was obtained in high yield as a single diastereomer, but to get optically pure material, they tried asymmetric synthesis

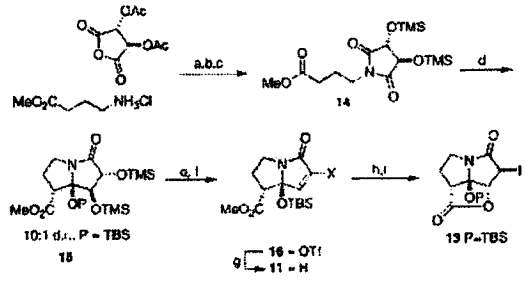


4(11) 4'(11')

to get desire 4 only

Asymmetric synthesis of target intermediate 13

Scheme 4. Asymmetric Synthesis of 13[a]

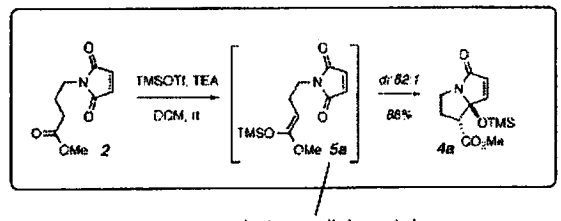


[a] Key: (a) (i) Et_2NH, THF; (ii) AcCl, reflux (51%); (b) AcCl, MeOH (79%); (c) TMSCl, Et_3N, CH_2Cl_2 (85%); (d) TBSOTf, $^iPr_2NEt$, CH_2Cl_2, −78 °C to r.t. (79%); (e) AcOH, 1 N HCl, THF; (f) Tf_2O, pyridine, CH_2Cl_2, −78 °C to r.t., then pyridine (76%, two steps); (g) Bu_3SnH, $Pd(PPh_3)_4$, LiCl, THF (52%); (g)' LiOH, 3:1 THF:H_2O (99%); (h) I_2, sat. $NaHCO_3$, Et_2O, THF (84%).

(a) (i) 1,2 addition of amine to carbonyl group
(ii) then cyclization
(b) deprotection of acetyl groups
(c) OH protection with TMS
(d) Silylative Dieckmann-like cyclization and OH protection with TBS
(e) deprotection of TMS group
(f) conversion of OH to triflate
(g) cross-coupling
(g)' hydrolysis
(h) iodolactonization

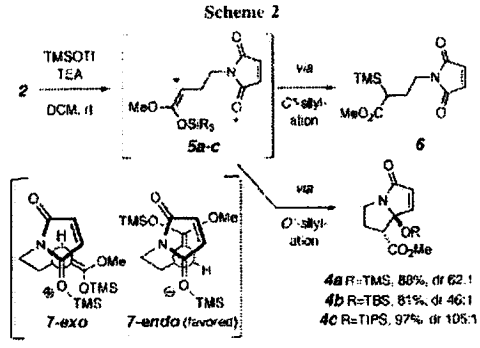
Silylative Dieckmann-like cyclization

14 to 15; Silylative Dieckmann-like cyclization of Ester-Imides (*Organic lettters*, **2006**, *8*, 5191)



ketene silyl acetal

Scheme 2

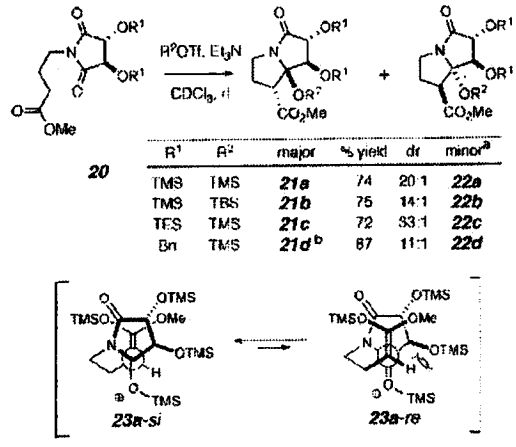


cationic process involving ketene silyl acetal(5a-c), and it serves as nucleophiles

the bicyclic lactam 4a was produced in high diastreomeric ratio.
the endo product was predomonantly formed
O-silylated imide carbonyl prevent nucleophies from appoarching .

6 wasn't obtained.
C*-silylated ester was minor product,
because silylation at the O* atoms is faster tha that of C*

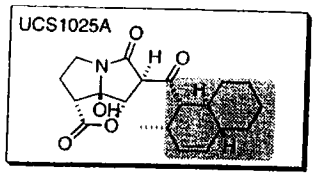
Scheme 6



	R^1	R^2	major	% yield	dr	minor[a]
20	TMS	TMS	***21a***	74	20:1	***22a***
	TMS	TBS	***21b***	75	14:1	***22b***
	TES	TMS	***21c***	72	33:1	***22c***
	Bn	TMS	***21d***[b]	67	11:1	***22d***

lactam 21 were obtained with excellent diastereoselectivity.
approach from the *re* face is disfavored by repulsion between OR_1 and H.

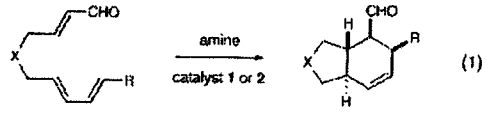
B. Enantioselective Organocatalytic Intermolecular Diels-Alrer reaction
(*J.Am,. Chem.Soc.* **2005,** *127,* 11616)
(*J.Am,. Chem.Soc.* **2000,** *122,* 4243)

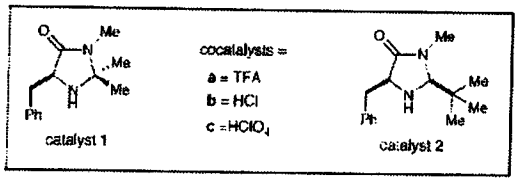


MacMillan's catalyst

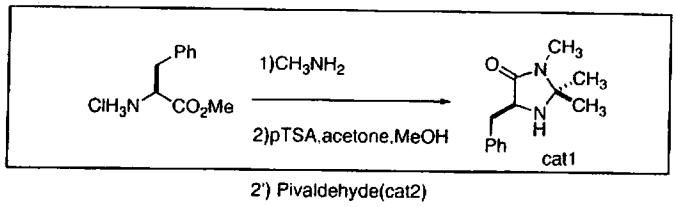
the LUMO-lowering activation of a.b-unsatuated carbonyls via reversible formation of iminium cation.

Organocatalytic Intramolecular Diels–Alder (IMDA)



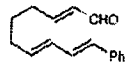
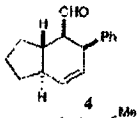
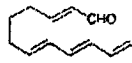
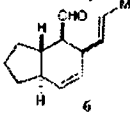
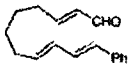
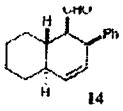


Synthesis of catalyst



2') Pivaldehyde(cat2)

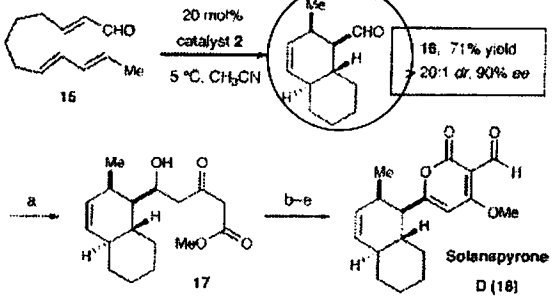
Table 1. Organocatalyzed Intramolecular Diels–Alder Reaction

entry	triene[a]	amine catalyst[b]	product	% yield[c]	*endo*/*exo*	% ee
1		1a		84	>20:1	77
2	3	2a	4	85	>20:1	93
3		1a		47	4:1	87
4	5	2a	6	75	>20:1	94
11		1		<10	–	–
12	13	2c	14	70	>20:1	92

cat 2 showed better result than cat1

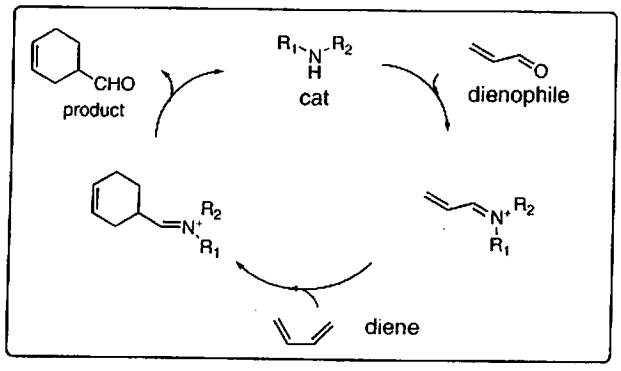
key intermediate

Scheme 1. Catalytic Total Synthesis of Solanapyrone D[a]

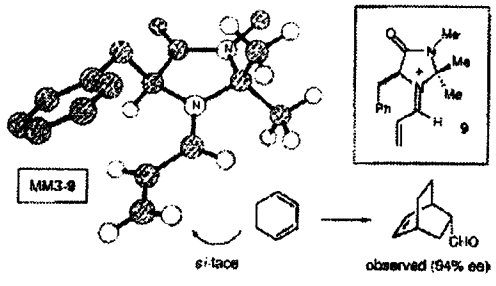


[a] Key: (a) Methyl acetoacetate bis(trimethylsilyl) enol ether, $TiCl_4$, CH_2Cl_2, −78 °C, 75%. (b) Dess–Martin Periodinane, CH_2Cl_2, 71%. (c) DBU, benzene, 60 °C, 87%. (d) Methyl *p*-toluenesulfonate, K_2CO_3, DMF, room temperature, 81%. (e) LDA, THF, −78 °C to 0 °C; methyl formate, −78 °C, 57% (91% based on recovered starting material).

Mechanism

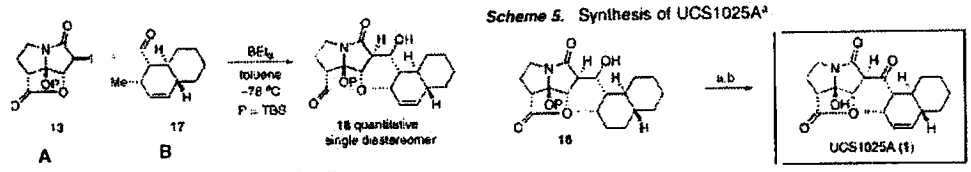


Inspection of structure of catalyst by MM3



the benzyl group effectively shields the *re* face of the dienophile *si* face exposed to cycloaddition.

C.Coupling

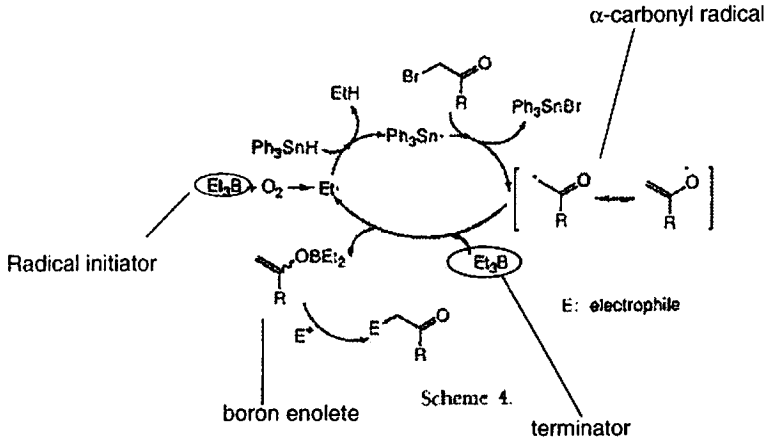


Scheme 5. Synthesis of UCS1025A[a]

[a] Key: (a) TBAF, THF (85%); (b) Dess–Martin periodinane, CH_2Cl_2 (84%).

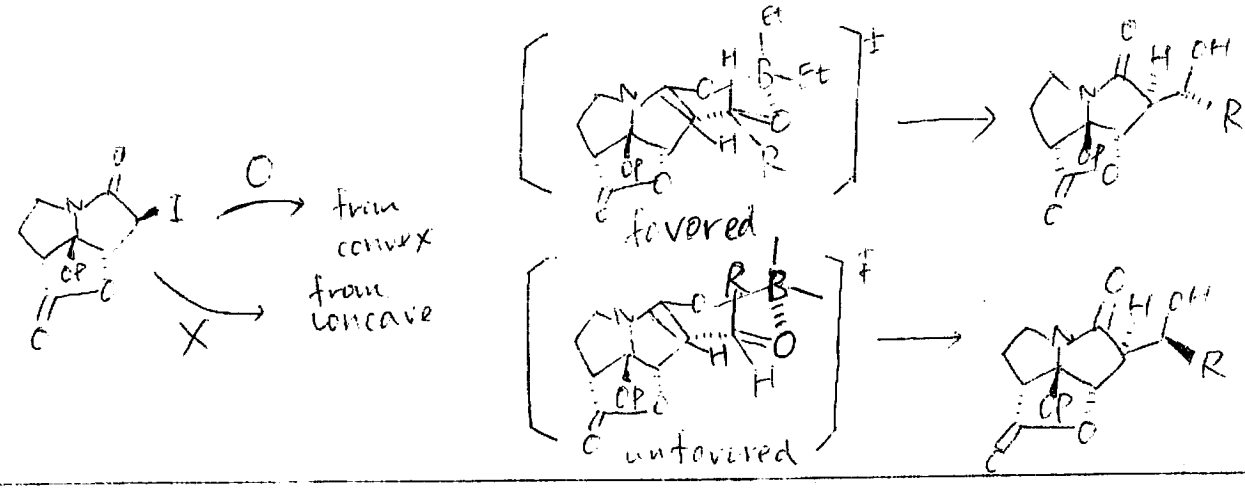
13 to 18
#Trialkylborane as an initiator and terminator of free radical reaction
reduction of a-halo ketenes.
(*Bull.Chem.Soc.Jpn.* **1991**, *64*. 403)

Reformatsky type reaction

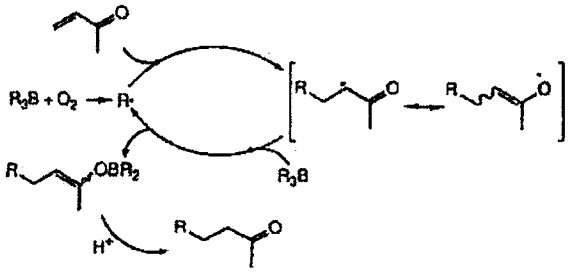


Scheme 4.

trialkylbonrane acts as an initiator
and as a terminator to trap a-carbonyl radical
reaction of α-iodo ketones proceeded <u>without Ph_3SnH</u>



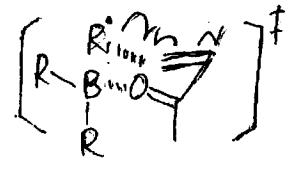
addition to α,β–unsatuated carbonyl compounds



Scheme 1.

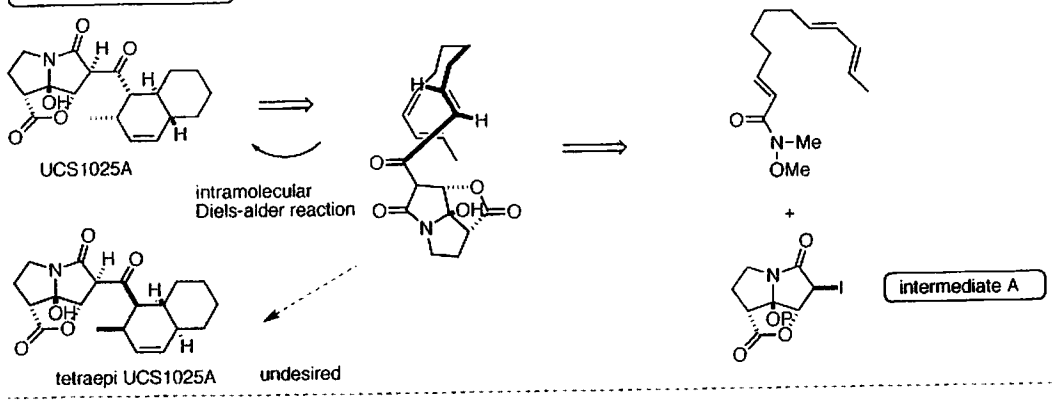
unsatuated bonds
carbonyl compound
trialkylboranes
BR_3
α,β-unsatuated carbonyl compound
α-carbonyl radical

transition state.
six-memberd ring



1-3. Total synthesis by Dvornikovs (Biomimetic Total Synthesis) (*J.Am.Chem.Soc.* **2006**, *128*, 2550)

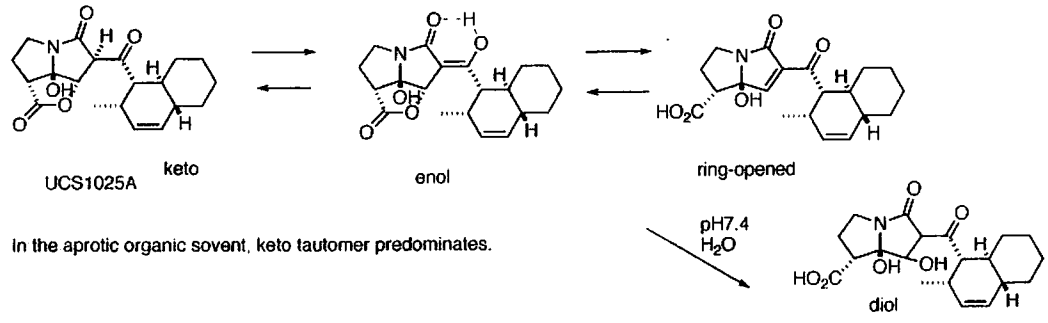
Outline of Snythesis



How trans decalin formation occurs during the biosynthesis ?

under chemical condition { only UCS1025A is formed → the Diels-Alder reaction is thermodynamical reaction.
both UCS1025A and tetraepimer are formed → an intermolecular Diels-Alder(IMDA) cyclization is operative

chemical equilibria- three tautomeric isomers exsist



In the aprotic organic sovent, keto tautomer predominates.

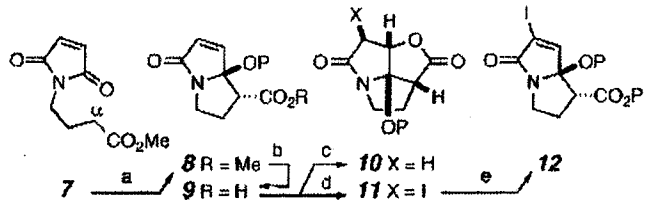
Isomeric species (keto and enol and the ring-opened carboxylate) were observed during the isolation study.

↓

All isomeric spieces possesses dienophilic character. ⟶ Is the reaction rate and diastereoselectivity unique to each of these structures?

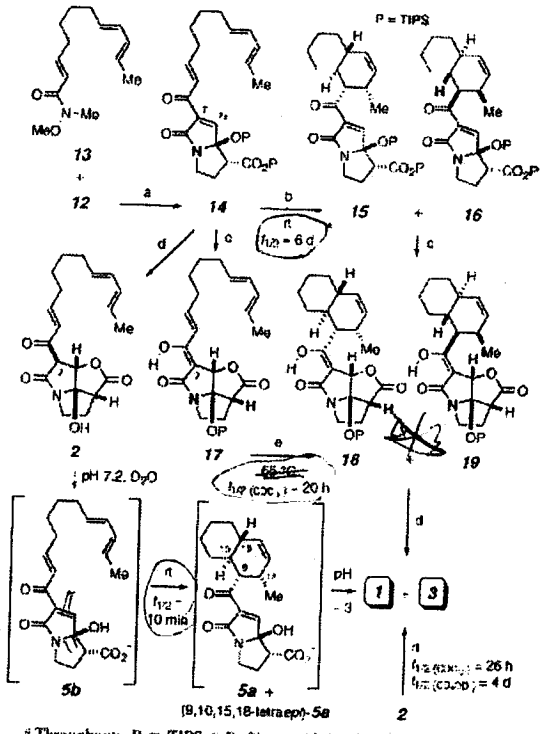
Synthesus of intermediateA

Scheme 2[a]



[a] Throughout: P = TIPS (*i*-Pr_3Si). (a) TIPSOTf, Et_3N, $CHCl_3$, rt, 97%; (b) LiOH, THF, H_2O, rt, 97%; (c) C_6D_6, 80 °C, quantitative (by ^1H NMR); (d) $NaHCO_3$, H_2O, CH_2Cl_2; I_2, rt, 93%; (e) TIPSCl, Et_3N, Et_2O, rt, 82%.

Scheme 3 [a]



[a] Throughout: P = TIPS (*i*-Pr$_3$Si). (a) *i*-PrMgCl, THF/ether, −50 °C, 10 min; then **13**, −50 °C to rt, 35%; (b) $CDCl_3$, 65 °C, 2 days, 69%; (c) KF, MeOH, rt, 5 min, 70–100%; (d) HF•py, MeCN, rt, 2 days (56% to **1** + **3** from **17**; 74% to **2**); (e) $ZnCl_2$•Et_2O, CH_2Cl_2, rt, ca. 18 h, 56% or $CDCl_3$, 65 °C.

14 (OH protected ring-opened) ⟶ 65°C,2days ⟶ 15:16 = 4:3 unseparable — not selective

17 (OH protected enol) ⟶ rt // ⟶ 65°C, 18h. with Lewis acid ⟶ 18:19=1:2 — not selective

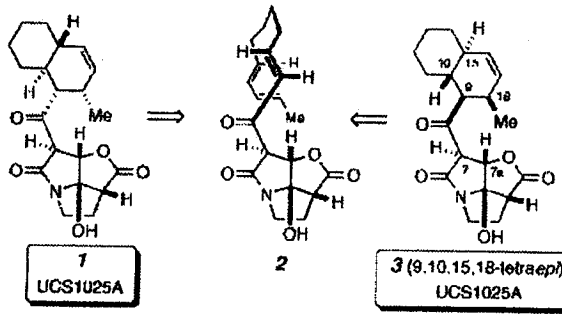
5b (ring opend natural substurate) ⟶ rapid cyclization!! ⟶ rt,>10min ⟶ 1:3=1:1 — not selective

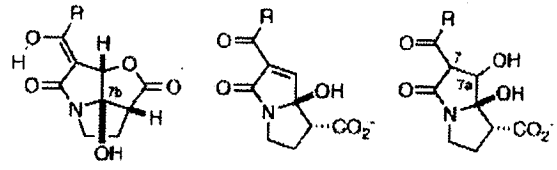
under argument

This IMDA needs some enzyme to occure the reaction(Diels-Alderase??) , or might be an enzyme that breaks the one product.

Experiments in biologically relevant (aqueous, metal-ion mediated) is needed.

Scheme 1



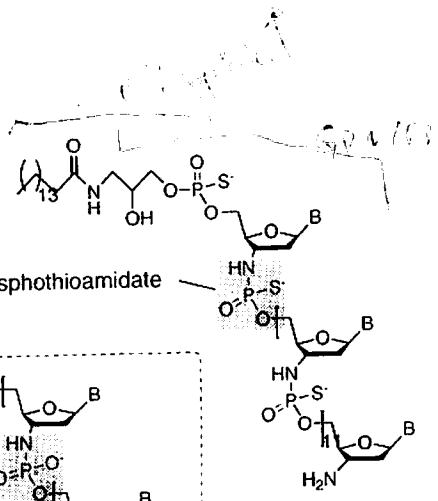


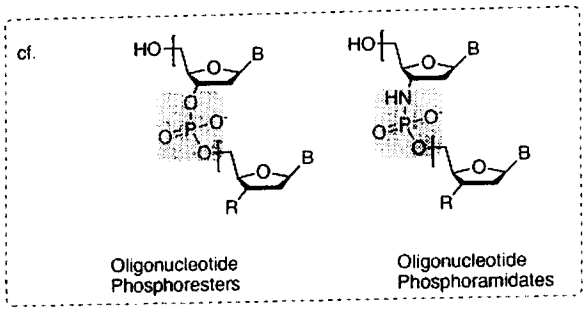
a R = methyloctalinyl (as in ***1***) ***b*** R = undeca-1,7,9-trienyl (as in ***2***)

3. GRN163L - an application to clinic -

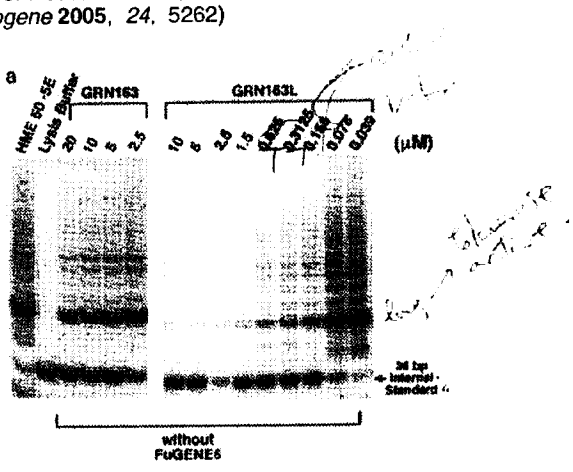
GRN163L

oligonucleotide N3'-P5' phosphothioamidate
negatively charged at neutral pH
resistant to nuclease degradation
high specificity and stability for DNA targets
secondary stabilizing interactions with the regions of hTERT

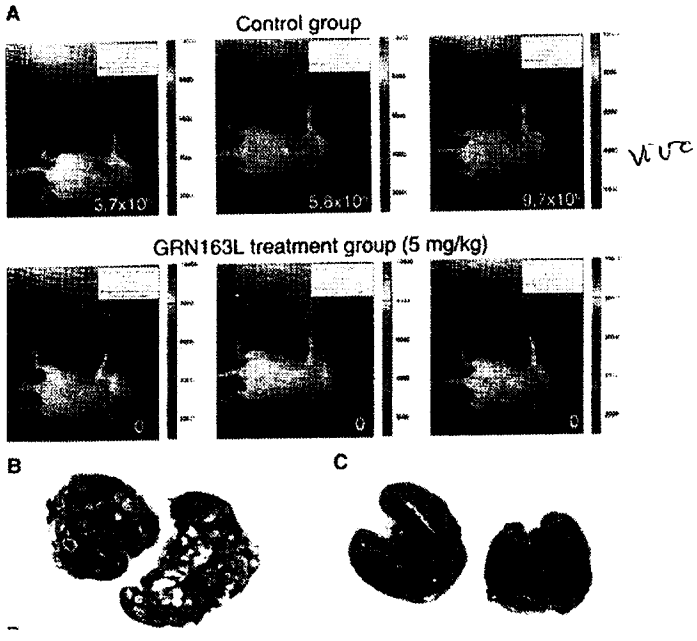




both in vitro and in vivo improvement
(Cancer Research **2005**, *65*, 7866
Oncogene **2005**, *24*, 5262)



comparsion of GRN163 and GRN163L
in TRAP



in vivo

Table 1 Comparison of telomerase activity IC_{50} values between GRN163L and GRN163 in tumor cell lines[a]

Cell type	*Cell line*	*GRN163L: IC_{50} (μM)*	*GRN163: IC_{50} (μM)*	*Fold Δ*
Cervical	HT-3	0.29	1.39	4.8
Glioblastoma	U251	0.17	1.75	10
	U87	0.18	0.8	4.4
Hepatoma	Hep3B	1.35	3.02	2.2
	HepG2	0.48	2.72	5.7
Lung	NCI-H522	0.23	0.75	3.3
Melanoma	M14	0.35	0.69	2.0
	SK-MEL-2	0.19	1.13	5.9
	SK-MEL-5	0.38	0.54	1.4
	SK-MEL-28	0.94	3.29	3.5
Myeloma	RPMI 8226	0.38	2.69	7.1
Ovarian	Ovcar5	0.92	3.03	3.3
Prostate	DU145	0.15	5.8	39

comparsion of GRN163 and GRN163L

1st generation ; GRN163 —lipid modification→ 2nd generation ; GRN163L

sevenfold higher activity
due to increase of up-take

another trial was lipophilic carrier; not easily translate to clinic
(low toxicity and easily administered)

Currently in Phase 1 clinical trials for solid tumors patients
Phase 1/2 for chronic lymphocytic leukemia(CLL)

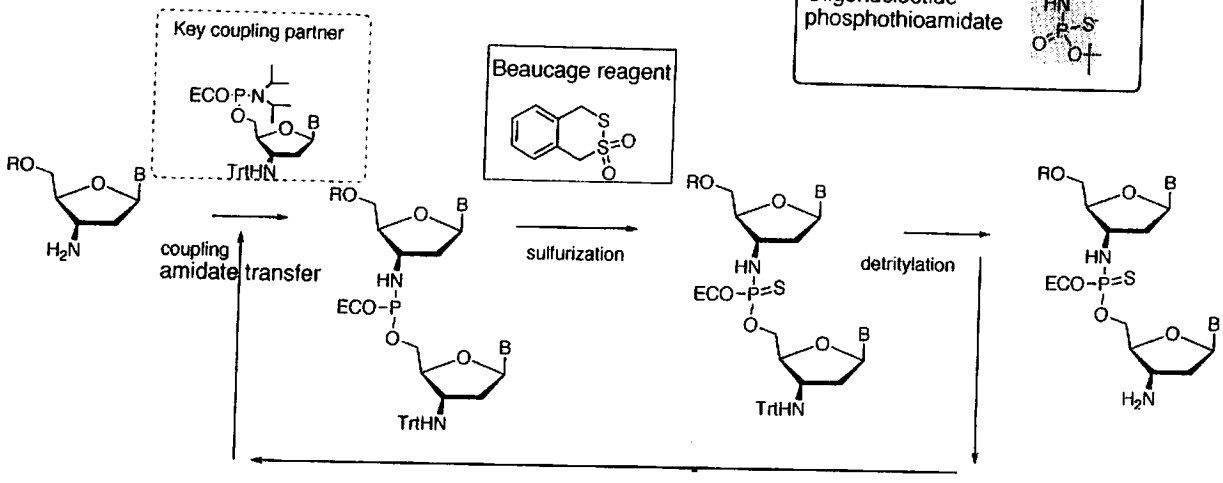
Nov. 10, 2006
Geron reported
the first clinical trial data, which showed the **safety , tolerability and predicted pharmacokinetics**
in low-dose cohorts from a Phase1/2 trial in patients with CLL and a Phase 1 trial in patients with solid tumors.

Synthesis
phosphoramidate transfer methology

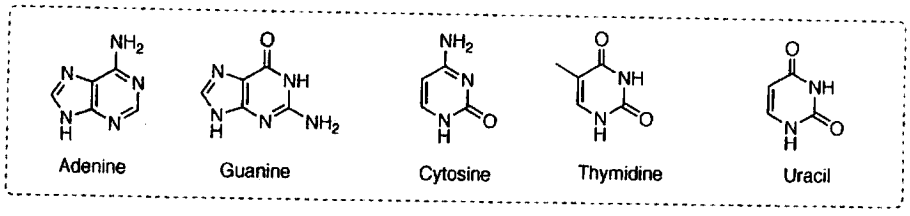
Oligonucleotide N3' to P5' thiophosphoramidates (*Tetrahedron letters* **1999**, *40*, 7661)

How to synthesis?

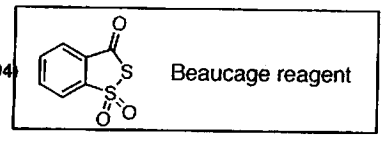
Oligonucleotide phosphothioamidate



Base

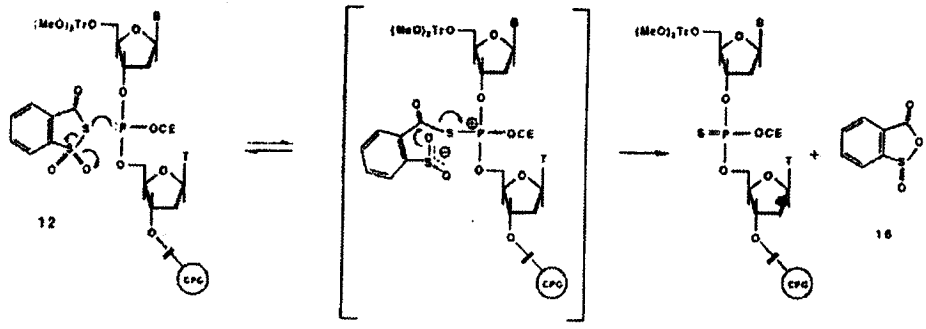


Sulfur-transfer reagent(Beaucage reagent) (*J. Org.Chem.* **1990**, *55*, 4694)



* the most common reagent for oligonucleotide phosphorothioates
* satable in CH_3CN for prolonged period of time without losing signicicant activity.
* poor stability in solution once installed on the DNA synthesizer.

Reaction mechanism

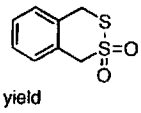


13a: B=Ts1-Thyminyl
b: B=1-(N-4-Benzoylcytosinyl)
c: B=9-(N-6-Benzoyladeninyl)
d: B=9-(N-2-isobutyrylguaninyl)

15a-d were obtained in 99% yield!!

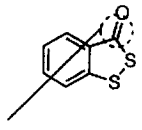
$(MeO)_2Tr$: di(*p*-anisyl)phenylmethyl
CE: 2-cyanoethyl
CPG: Controlled-pore glass

1st generation



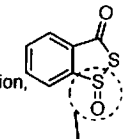
Only 25% yield

2nd generation



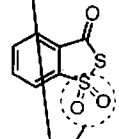
*accelerate the intramolecular nucleophilic attack

H_2O_2, rt, rapid addition, once



H_2O_2, 40-42°C, slow addition three times

3rd generation



Beaucage reagent

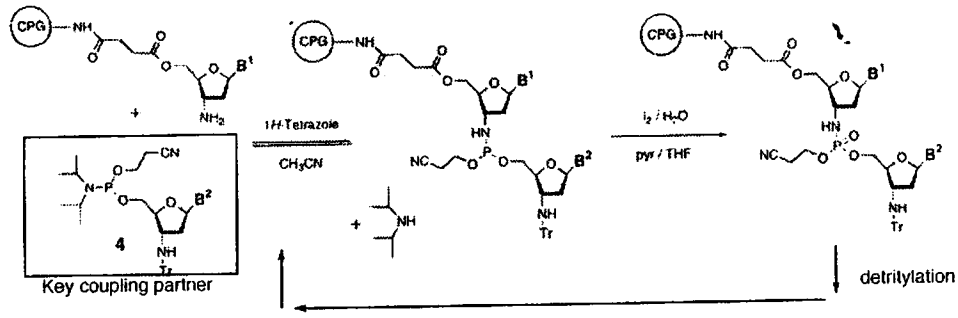
*increase the electrophilicity of sulfonyl group
*destabilize the sulfur-sulfur linkage

N3' to P5' oligonucleotide Phosphoramidates(Phosphoramidate Amine-Exchange reacton)
(J. Org. Chem. **1997**, *62*, 7278)

How about?

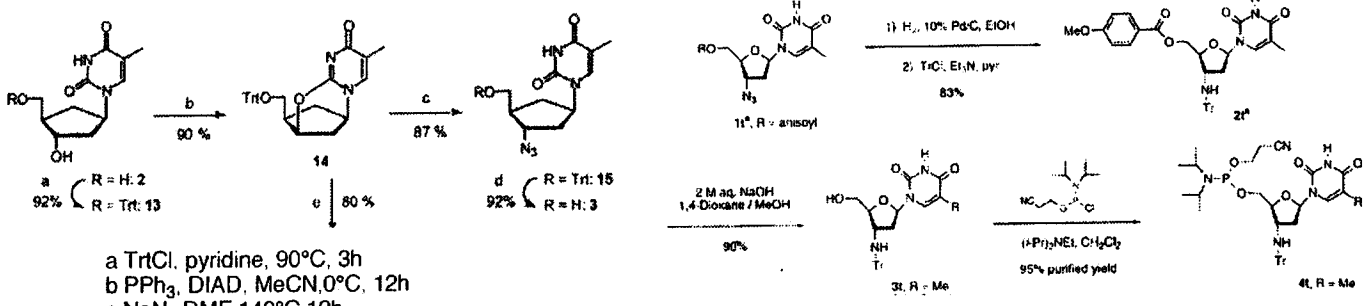
Oligonucleotide Phosphoramidates

Scheme 1



How to prepare key inetermediate 4

Scheme 2



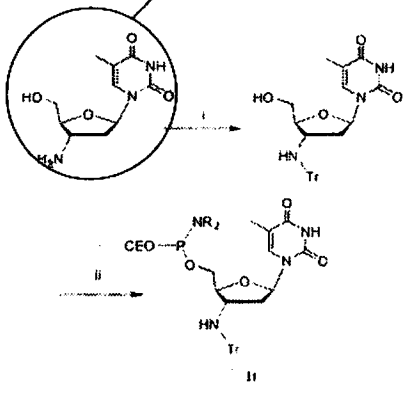
a TrtCl, pyridine, 90°C, 3h
b PPh_3, DIAD, MeCN,0°C, 12h
c NaN_3,DMF,140°C,12h
d TFA,CH_2Cl_2/MeOH,rt,10h

total yield 4-5%(purine) and 15-20%(pyrimidine)
preparation of purine 10steps, pyrimidine 7steps.

New approarch to oligonucleotide N3' to P5'' phosphoramidate building blocks
(Tetlahedron Letters **2006**,*47*, 4495)

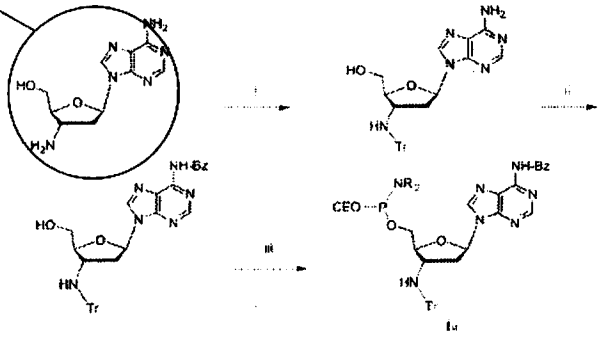
Nowdays 3' amino nucleosides are comercially available!! ⟶ overall yield up to 70%

Preparation of Thymine



Preparation of Adenine

Due to presence of secondary amino group, different tritylation condition is used.



Scheme 1. Synthesis of thymidine phosphoramidite 1t, where (i) TrCl, Py/EtN(*i*Pr)$_2$, (ii) (*i*Pr$_2$N)PtCl)OCE, EtN(*i*Pr)$_2$ R = *i*Pr; CE = 2-cyanoethyl

Scheme 2. Synthesis of adenosine phosphoramidite 1a, where (i) TrCl, Py/DMF/Et$_3$N, (ii) BzCl, Py, (iii) (*i*Pr$_2$N)PCl)OCE, EtN(*i*Pr)$_2$ R = *i*Pr; CE = 2-cyanoethyl.

4. What' going on telomerase inhibitor?

comparsion

	Advantage	Limitation
targeting telomerase	on the way to the first approved telomerase inhibitor!	delay of efficiancy effects dependent on initial telomere length.
TTAs(telomere targeting agents)	significant antitumour activity in vivo within a few days of treatment. broard-spectrum utility (solid tumors and haematological malignancies)	interact with nonteloimeric G-quadruplexes **(non-specific)**

Problems to be addressed

Underlying biology is complicated.

telomere length $\xrightarrow{??}$ telomerase activity $\xrightarrow{??}$ telomere capping

the engagement of an alternative lengthening of telomere mechanism $\longrightarrow$ resistance ???

recombination
participation of other enzyme(DAN replication, DNA repair)

Selection of relavent spieces

Mice have relatively long telomeres and different telomerase biology.
So it's difficult to predict clinical utility of findings from regulatory toxicology and safety pharmacology studies conducted in mice.

$\longrightarrow$ appropriate preclinical human xenografted tumor experiments

Clinic assessment

optimal scheduling, predictive markers,

pre-selection of patients to use measures of telomere length in biopsy material

$\longrightarrow$ need to combine with other established molecular targeting agents