Isolation:	**Hanka, Dietz, Gerpheide, Kuentzel, Martin** **J. Antibiot. 1978, 31, 1211.**
Structure Ident:	**Chidester, Krueger, Mizsak, Duchamp, Martin** **J. Am. Chem. Soc. 1981, 103, 7629.**
Mechanism of Action:	**Hurley, Reynolds, Swenson, Petzold, Scahill** **Science 1984, 226, 843.**
Toxicity:	**McGovren, Clarke, Pratt, DeKoning** **J. Antibiot. 1984, 37, 63.**
SAR and Analogs:	**Kelly, Warpehoski, Wierenga, Bhuyan, Krueger Li, McGovren, Swenson** **Adv. Enz. Reg. 1986, 25, 141.** **Warpehoski, Gebhard, Kelly, Krueger, Li, McGovren, Prairie, Wicnienski, Wierenga** **J. Med. Chem. 1988, 31, 590.**
Reviews:	**Hurley, Needham-Vandevanter** **Acc. Chem. Res. 1986, 19, 230.** **Boger and coworkers** **Nat. Prod. Chem. 1989, 3, 301** **Ad. Het. Nat. Prod. Syn. 1992, 2, 1**

duocarmycin B_1 X = Br
duocarmycin C_1 X = Cl
(pyrindamycin B)

duocarmycin B_2 X = Br
duocarmycin C_2 X = Cl
(pyrindamycin A)

Duocarmycin A - C:
J. Antibiotic 1988, 41, 1915, 1285

Duocarmycin SA: J. Antibiot. 1990, 43, 1037
X-ray of Pyrindamycin A: absolute configuration

DNA cleavage chemistry of Maxam-Gilbert Adenine reaction

5'-d(CTCAATTAGTC):duocarmycin

Binding Directionality: 3' to 5'

bound conformation spans 3.5 base-pairs

Selectivity:

5'-d(AAA)-3' > 5'-d(TTA)-3' >>

5'-d(TAA)-3' > 5'-d(ATA)-3'

5'-d(T/AXXA)-3' >> 5'-d(G/CXXA)-3'

5'-d(XXXAPu)-3' > 5'-d(XXXAPy)-3'

Duocarmycin A = C_2 = C_1

High Affinity Sequences:

5'-d(T/AAAA)-3' and 5'-d(T/ATTAPu)-3'

Intensity:

Duocarmycin A > C_2 > C_1 (4° C)

(+)-CC-1065 > duocarmycin A (1 - 5x)

Adenine N-3 Alkylation of (+)-CC-1065

(+)-CC-1065

Selectivity:

: three base-pair select. = 5'-d(AAA)-3' = 5'-d(TTA)-3' > 5'-d(TAA)-3' > 5'-d(ATA)-3'

: fourth base-pair sensitivity = 5'-d(A/TXXA)-3' >> 5'-d(G/CXXA)-3'

: fifth base-pair sensitivity = 5'-d(A/TXXXA)-3' > 5'-d(G/CXXXA)-3'

: preceding base-pair sensitivity = 5'-d(NNXXAPu)-3' > 5'-d(NNXXAPy)-3'

High Affinity Sequences:

5'-d(A/TA/TTTA)-3', 5'-d(A/TA/TTAA)-3' 5'-d(T/AAAAA)-3' > 5'-d(T/ATAAA)-3'

duocarmycin B_1 X = Br
duocarmycin C_1 X = Cl
(pyrindamycin B)

duocarmycin B_2 X = Br
duocarmycin C_2 X = Cl
(pyrindamycin A)

Duocarmycin A - C:
J. Antibiotic 1988, 41, 1915, 1285
J. Antibiotic 1989, 42, 1299

Duocarmycin SA: J. Antibiot. 1990, 43, 1037
X-ray of Pyrindamycin A: absolute configuration
J. Antibiotic 1988, 41, 1515

J. Antibiotic 1988, 41, 1515

Base-catalyzed Nucleophilic Substitution:

Lewis Acid-Catalyzed Nucleophilic Substitution

NCOPh
OBn
NCOPh
CO2Me
CO2Me
51%
MeO2C
MeO2C
NHCOPh
OBn
NHCOPh
NaBH4, 71%
Me2C(OMe)2
99%
NHCOPh
OBn
NHCOPh
Pb(OAc)4
100%
NCOPh
OBn
NCOPh
61%
MeO
MeO
N
NHCOPh
OBn
NHCOPh
MeO
OMe
O

NHCOPh
OBn
NHCOPh
MeO
O
OMe
HCl-
MeOH
91%
HO
OH
MeO
MeO
NHCOPh
N
COPh
OBn
DEAD-
Ph3P
100%
OH
MeO
MeO
NCOPh
N
COPh
OBn
NH2NH2;
BOC2O
67%
OH
MeO
MeO
NBOC
N
H
OBn
95 - 100%
pH 4 buffer
DMSO-dioxane
MnO2-NaCN
CH2Cl2-MeOH
92%
OH
MeO2C
NBOC
N
H
OBn
10% Pd/C-
HCO2NH4
92%
X
MeO2C
NBOC
N
H
OH
Ph3P-CCl4
88%
X = OH
X = Cl

- reversible DNA alkylation
- deacylation under basic conditions
- stereoelectronic control of solvolysis - alkylation

MM2 model of DSA

- deacylation under basic conditions
- reversible DNA alkylation
- stereoelectronic control

$t_{1/2}$ = 177 h (pH = 3)
stable (pH 5 - 7)

MM2 model of DSA

A STABLE p-QUINONEMETHIDE: VINYLOGOUS AMIDE STABILIZATION

(+)-Duocarmycin SA

- p-quinonemethide isolated and characterized
- partially stable to SiO_2 chromatography
- vinylogous amide stabilzation

Characteristics of Electrophile Nature of DNA Alkylation

+

$\Delta H^0 = +1.9$ to $+4.5$ kcal [MNDO and AM1]

- stereoelectronic control of alkylation
- $t_{1/2} = 177$ h (pH = 3) stable (pH = 5 - 7)
- reversible DNA alkylation
- deacylation under basic conditions

MM2 Model

- Hydrophobic binding-driven-bonding
  - noncovalent binding stabilizes reversible DNA alkylation
  - hydrophobic binding preferentially within the narrower, sterically more accessible A-T rich minor groove

Characteristics of the Reversible DNA Alkylation Reaction

Rate: increases with > pH, temperature, ionic strength

Duocarmycin SA > Duocarmycin A

Rate: decreases with increasing stability of adduct
Rate: decreases with increasing agent reactivity

Duocarmycins > CC-1065

Rate: decreases with increasing noncovalent binding stabilization

OH
NCO2tBu
MeO2C
N
H
OH
86
OAc
HO2C
Ph
EDCI, DMAP
90 %
AcO
Ph
O
O
H
NCO2tBu
MeO2C
N
H
OCOCH(OAc)Ph
Prep. HPLC
LiOMe
84%
resolution
SiO2: 1.31
X
NCO2tBu
MeO2C
N
H
OH
+
X
NCO2tBu
MeO2C
N
H
OH
X = OH
X = Cl
Ph3P, CCl4
X = OH
X = Cl

Cl
NR
MeO2C
N
H
OH
TMI-CO2H
EDCI, DMF
88%
MeO2C
HN
HO
Cl
N
O
N
H
OMe
OMe
OMe
NaH
89%
MeO2C
HN
O
N
O
N
H
OMe
OMe
OMe
(+)-Duocarmycin SA
HCl
EtOAc
R = CO2tBu
R = H-HCl

Cl
NR
MeO2C
N
H
OH
TMI-CO2H
EDCI, DMF
MeO2C
HN
HO
Cl
N
O
N
H
OMe
OMe
OMe
NaH
MeO2C
HN
O
N
O
N
H
OMe
OMe
OMe
(-)-Duocarmycin SA

(+)-N-BOC-DSA

(+)-duocarmycin SA

Comparative In Vitro Cytotoxic Activity

Agent	Configuration	IC_{50} (10^{-11} g/mL, pM)		
(+)-N-BOC-DSA	natural	200	6000	$t_{1/2}$ = 177 h, pH = 3
(-)-N-BOC-DSA	unnatural	2000	60000	
(+)-duocarmycin SA	natural	0.6	10	
(-)-duocarmycin SA	unnatural	6	100	
(+)-CC-1065	natural	1.2	20	
(+)-N-BOC-CPI	natural	10000	330000	$t_{1/2}$ = 37 h, pH = 3
(+)-duocarmycin A	natural	10	200	
(-)-duocarmycin A	unnatural	> 1000	> 20000	

$t_{1/2}$ (pH 3) =	177 h
rel $t_{1/2}$ =	4.8
IC_{50} (L1210) =	6 nM

$t_{1/2}$ (pH = 3) =	133 h	37 h	35 sec
rel $t_{1/2}$ =	3.6	1.0	0.0003
IC_{50} (L1210) =	80 nM	330 nM	18000 nM

DNA cleavage chemistry of Maxam-Gilbert Adenine reaction

Maxam Gilbert Adenine reaction

Selectivity:

40- 45% of all adenines alkylated over 10-fold agent conc. range

(+)-N-BOC-DSA = (-)-N-BOC-DSA

Two base-pair select. =
5'-d(AA)-3' > 5'-d(TA)-3'
80 vs 45% ca 2 : 1

Third base-pair sensitivity =
5'-d(A/TXA)-3' ≥ 5'-d(G/CXA)-3'

Preceding base-pair sensitivity =
5'-d(XAPu)-3' ≥ 5'-d(XAPy)-3'

N-BOC-DSA > N-BOC-CPI but with alkylation of same sites

Affinity:

(+)-duocarmycin SA > (+)-N-BOC-DSA (10000x)

(+)-N-BOC-DSA = (-)-N-BOC-DSA

(+)-N-BOC-DSA > (-)-N-BOC-CPI (5x)

5'-d(GACTAA*TTTTT):N-BOC-DSA

(-)-duocarmycin SA

Binding Directionality: 5' to 3'

bound conformation spans 3.5 base-pairs

Selectivity:

5'-d(AAA)-3' > 5'-d(AAT)-3'

5'-d(TAA)-3' > 5'-d(TAT)-3'

5'-d(XAXA/T)-3' > 5'-d(XAXG/C)-3'

(-)-Duocarmycin SA >> N-BOC-DSA

High Affinity Sequences:

5'-d(AAAA)-3' and 5'-d(AAAT)-3'

Intensity:

(+)-Duocarmycin SA >

Duocarmycin A > C_2 > C_1 (4° C)

(+)-CC-1065 = (+)-Duocarmycin SA

(+)- > (-)-Duocarmycin SA (10x)

(+)-Duocarmycin SA > A (10x)

(-)-Duocarmycin SA > N-BOC-DSA (10^5x)

(+)-Ducarmycin SA

5'-d(GACTAA*TTTTT)

(-)-Duocarmycin SA

5'-d(GACTAA*TTTTT)

(-)-CC-1065 and (-)-CPI-CDPI$_2$

Binding Directionality: 5' to 3'
bound conformation spans 5 base-pairs

Selectivity:

5'-d(AAA)-3' > 5'-d(AAT)-3'
5'-d(TAA)-3' > 5'-d(TAT)-3'
5'-d(XAXA/T)-3' > 5'-d(XAXG/C)-3'
5'-d(XAXXA/T)-3' > 5'-d(XAXXG/C)-3'
(-)-CC-1065 >> N-BOC-CPI

High Affinity Sequences:

5'-d(AAAA/TA/T)-3' and 5'-d(AATA/TA/T)-3'

Intensity:

(+)-CC-1065 ≈ (-)-CC-1065
≈ (+)-CPI-CDPI$_2$ ≈ (-)-CPI-CDPI$_2$

(+)-CC-1065 ≈ (+)-Duocarmycin SA

(-)-CC-1065 >> N-BOC-CPI (10000x)

(+)-CC-1065

5'-d(GACTAA*TTTTT)

(-)-CC-1065

5'-d(GACTAA*TTTTT)

CBI
Me
CPI
HN
CI
N
R
O

-log k
pH = 3
6
5
4
3
2
1
(+)-CBI-CDPI$_n$ (-log k = 5.84)
(+)-CPI-CDPI$_n$ (-log k = 5.28)
(+)-CI-CDPI$_n$ (-log k = 1.70)
R = CDPI$_2$
R = CDPI$_1$
R = O^{t}Bu
-11
-10
-9
-8
-7
-6
-5
-4
log IC$_{50}$ (M, L1210)

● n = 2 o n = 1

Minimum Potent Pharmacophore of the Alkylation Subunit: Cl-TMI

duocarmycin A

Cl-TMI

Common CC-1065/Duocarmycin Pharmacophore: Cl

CBI-TMI: A SIMPLIFIED FUNCTIONAL ANALOG

(+)-Duocarmycin SA

(+)-CBI-TMI

- stereoelectronic control of alkylation
- deacylation under basic conditions

$t_{1/2}$ = 128 h (pH = 3)
stable (pH = 5 - 7)

X-ray ORTEP of CBI

(+)-N-BOC-CBI

(+)-CBI-TMI

Comparative In Vitro Cytotoxic Activity

Agent	Configuration	IC_{50} (10^{-11} g/mL, pM)		
(+)-N-BOC-CBI	natural	2300	77000	$t_{1/2}$ = 128 h, pH = 3
(-)-N-BOC-CBI	unnatural	28000	940000	
(+)-CBI-TMI	natural	1.3	30	
(-)-CBI-TMI	unnatural	130	3000	
(+)-CI-TMI	natural	1000	26000	
(-)-CI-TMI	unnatural	1000	26000	
(+)-N-BOC-DSA	natural	200	6000	$t_{1/2}$ = 177 h, pH = 3
(-)-N-BOC-DSA	unnatural	2000	60000	
(+)-duocarmycin SA	natural	0.6	10	
(-)-duocarmycin SA	unnatural	6	100	
(+)-duocarmycin A	natural	10	200	
(-)-duocarmycin A	unnatural	> 1000	> 20000	

R

MeO2C
DSA
HN
N
R
CBI
MeO2C
Me
DA
HN
N
R
CI
N
R

(+)-DSA-R (-log k = 5.97)
(+)-CBI-R (-log k = 5.84)
(+)-CPI-R (-log k = 5.28)
(+)-DA-R (-log k = 4.75)
(+)-CI-R (-log k = 1.70)
-log k
pH = 3
6
5
4
3
2
1
R = TMI
R = O^tBu
-11
-10
-9
-8
-7
-6
-5
-4
log IC50 (M, L1210)
o R = TMI
+ R = BOC

The N^2 Substituent: Verification of a Direct Relationship Between Chemical Solvolysis Stability and In Vitro Cytotoxic Activity

R	$t_{1/2}$ (hour)	IC_{50} (nM) (L1210)
SO_2Et	383	24
COEt	96	110
CO_2CH_3	59	140
$CONHCH_3$	36	200

CBQ-TMI: AN EXCEPTIONALLY BUT NORMALLY REACTIVE ALKYLATION SUBUNIT

OH
OH
NH3, 130°C
BOC2O, 85%
PhCH2Br, 83%
X
NHCO2tBu
OCH2Ph
NBS
98%
X = H
X = Br
NaH, DMF
BrCH2CH=CH2
98%
Br
X
NCO2tBu
OCH2Ph
9-BBN; H2O2; 75%
(COCl)2-DMSO; 84%
Ph3P=CHOTHP; 86%
X = =CH2
X = -CH2OH
X = -CHO
X = -CH=CHOTHP
Bu3SnH
C6H6, 86%
OTHP
NCO2tBu
OCH2Ph
H+, 92%
Ph3P, CCl4
88%
Cl
NCO2tBu
OR
98%
H2, Pd-C
R = CH2Ph
R = H
NaH, THF
2 h, 0° C
82%
CBQ
NCO2tBu
O
N3-BOC-CBQ

Cl
NR
OH
TMI-CO2H
EDCI, DMF
65%
HO
OMe
OMe
OMe
NaH
75%
(+)-CBQ-TMI
Direct Resolution: Chiralcel OD: 1.2
HCl
EtOAc
R = CO2tBu
R = H-HCl
(-)-CBQ-TMI

(+)-N-BOC-CBQ

$t_{1/2}$ = 2.1 h (pH = 3)

H^+, ROH →

+

1 : 1

- exceptional - but normal - solvolysis reactivity
- loss of stereoelectronic control of solvolysis - alkylation

CBI

CBQ

CBI

CBQ

ORTEP of N-BOC-CBQ X-ray

(-)-N-BOC-CBQ

(-)-CBQ-TMI

Comparative In Vitro Cytotoxic Activity

Agent	Configuration	IC_{50} (pM)	
(-)-N-BOC-CBQ	natural	2000000	$t_{1/2}$ = 2.1 h, pH = 3
(+)-N-BOC-CBQ	unnatural	10000000	
(-)-CBQ-TMI	natural	4000	
(+)-CBQ-TMI	unnatural	35000	
(+)-CBI-TMI	natural	30	
(-)-CBI-TMI	unnatural	3000	
(+)-N-BOC-DSA	natural	6000	
(-)-N-BOC-DSA	unnatural	60000	$t_{1/2}$ = 177 h, pH = 3
(+)-duocarmycin SA	natural	10	
(-)-duocarmycin SA	unnatural	100	
(+)-duocarmycin A	natural	200	

(-) duocarmycin A	unnatural	200
		> 20000

r = 0.924
N-BOC-DSA
N-BOC-CBI
N-BOC-CPI
N-BOC-CBQ
N-BOC-DA
N-BOC-CI
log (1/IC50) (nM)
-log k (sec-1)
0
-2
-4
-6
1
2
3
4
5
6
7
8

r = 0.993
Duocarmycin SA
CBI-TMI
Duocarmycin A
CBQ-TMI
CI-TMI
log (1/IC50) (pM)
-log k (sec-1)
0
-2
-4
-6
1
2
3
4
5
6
7
8

MeO2C
HN
DSA
CBI
Me
CPI
MeO2C
Me
DA
CBQ
CI
R

C_2BI-$CDPI_n$: Achiral DNA Cross-linking Agents

(+)-duocarmycin SA

seco C_2BI-TMI

Proposed Adenine-Adenine Cross-linking Reaction of C_2BI-$CDPI_n$

Adenine N-3 alkylation site

$t_{1/2}$ (pH = 3) =	177 h	133 h	37 h	11 h	2.1 h	0.01 h
rel $t_{1/2}$ =	4.8	3.6	1.0	0.3	0.06	0.0003
IC_{50} (L1210) =	6 nM	80 nM	330 nM	1000 nM	5000 nM	18000 nM

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(+)-N-BOC-CBQ

H^+, ROH →

- exceptional - but normal - solvolysis reactivity
- loss of stereoelectronic control of solvolysis - alkylation

ORTEP of N-BOC-CBQ X-ray