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Eribulin Mesilate

Prop INNM

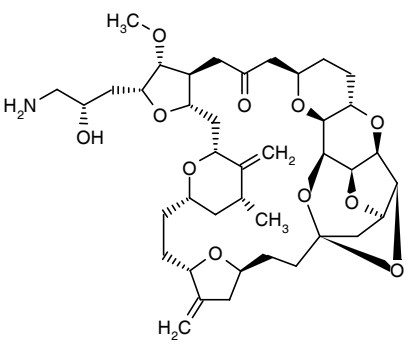
Antimitotic Drug
Tubulin Polymerization Inhibitor
Oncolytic

E-7389
ER-086526 (former code name)
NSC-707389
Eribulin mesylate (USAN)

(2*R*,3*R*,3a*S*,7*R*,8a*S*,9*S*,10a*R*,11*S*,12*R*,13a*R*,13b*S*,15*S*,18*S*,21*S*,24*S*,26*R*,28*R*,29a*S*)-2-[3-Amino-2(*S*)-hydroxypropyl]-3-methoxy-26-methyl-20,27-bis(methylene)-11,15:18,21:24,28-triepoxy-7,9-ethano-12,15-methanoperhydro-9*H*,15*H*-furo[3,2-*i*]furo[2',3':5,6]pyrano[4,3-*b*][1,4]dioxacyclopentacosin-5-one methanesulfonate

(1*S*,3*S*,6*S*,9*S*,12*S*,14*R*,16*R*,18*S*,20*R*,21*R*,22*S*,26*R*,29*S*,31*R*,32*S*,35*R*,36*S*)-20-[3-Amino-2(*S*)-hydroxypropyl]-21-methoxy-14-methyl-8,15-dimethylene-2,19,30,34,37,39,40,41-octaoxanonacyclo[24.9.2.1(3,32).1(3,33).1(6,9).1(12,16).0(18,22).0(29,36).0(31,35)]hentetracontan-24-one methanesulfonate

InChI=1/C40H59NO11.CH4O3S/c1-19-11-24-5-7-28-20(2)12-26(45-28)9-10-40-17-33-36(51-40)37-38(50-33)39(52-40)35-29(49-37)8-6-25(47-35)13-22(42)14-27-31(16-30(46-24)21(19)3)48-32(34(27)44-4)15-23(43)18-41;1-5(2,3)4/h19,23-39,43H,2-3,5-18,41H2,1,4H3;1H3,(H,2,3,4)/t19-,23+,24+,25-,26+,27+,28+,29+,30-,31+,32-,33-,34-,35+,36+,37+,38-,39+,40+;/m1./s1



.CH_3SO_3H

$C_{41}H_{63}NO_{14}S$

Mol wt: 826.0033

CAS: 441045-17-6

CAS: 253128-41-5 (free base)

EN: 287199

Abstract

The natural compound halichondrin B demonstrated promising anticancer activity *in vitro* and *in vivo*, but insufficient natural sources of halichondrin B limited its therapeutic application. Eribulin mesilate (E-7389), a structurally optimized synthetic analogue of halichondrin B, retained the natural compound's subnanomolar anticancer activity *in vitro*. The agent also induced marked tumor regression in a variety of human tumor xenograft models *in vivo*. Preclinical studies demonstrated that eribulin exerted its anticancer activity via a tubulin-based mechanism leading to disruption of the mitotic spindle, mitotic arrest and cancer cell apoptosis. Eribulin exhibits rapid and extensive tissue distribution and a long terminal half-life in animals (rats and dogs) and in patients with refractory or advanced solid tumors. Eribulin showed promising anticancer efficacy in patients with refractory or advanced tumors who had previously received chemotherapy. It has also demonstrated synergistic effects when combined with gemcitabine, cisplatin, epirubicin, trastuzumab, docetaxel and vinorelbine.

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Synthesis

Eribulin can be synthesized as follows. Intramolecular cyclization in the open-chain iodovinyl aldehyde precursor (I) by means of $NiCl_2/CrCl_2$ under Nozaki-Hiyama-Kishi conditions gives the macrocyclic alcohol (II). Subsequent oxidation of (II) to the corresponding ketone either under Swern conditions or by treatment with Dess-Martin periodinane, followed by full desilylation with tetrabutylammonium fluoride and imidazole hydrochloride, gives the ketone (III). Then, cyclization between hydroxyl groups and the unsaturated ketone of (III) in the presence of pyridinium tosylate gives the polycyclic compound (IV). Selective sulfonylation of the primary hydroxyl group of (IV) with either methanesulfonyl chloride or *p*-toluenesulfonic anhydride in the presence of collidine produces the respective sulfonates (Va) and (Vb) (1-3). Displacement of mesylate (Va) with tetrabutylammonium azide in hot DMF yields the alkyl azide (VI), which is finally reduced to the title amine by treatment with trimethylphosphine in moist THF (1). Alternatively, eribulin is obtained by displacement of tosylate (Vb) with ammonium hydroxide in MeOH (2, 3). Scheme 1.

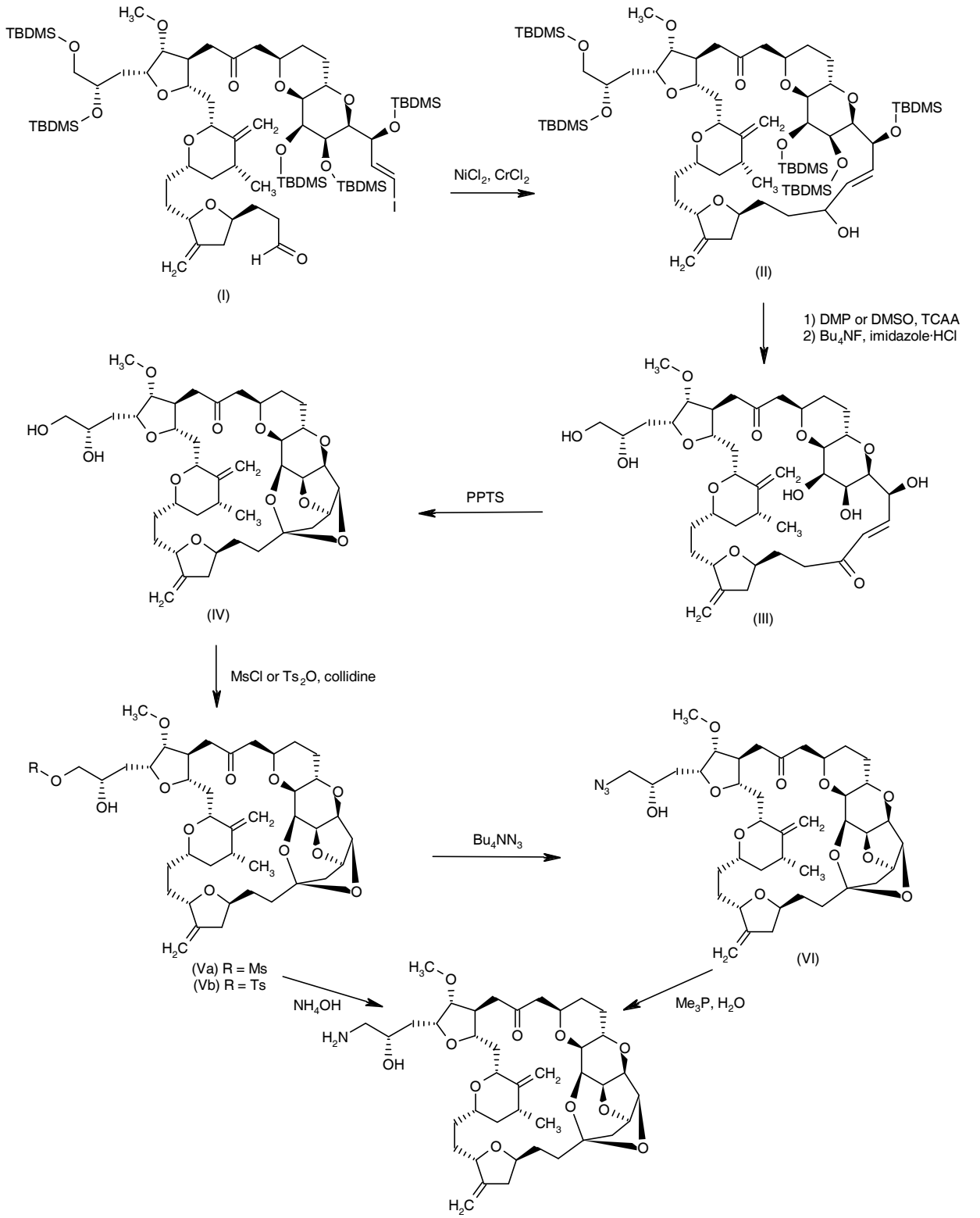
The precursor iodovinyl aldehyde (I) can be prepared by two related methods starting from alkyl iodide (VII) or sulfone (VIII). Lithiation of alkyl iodide (VII) by means of *tert*-butyllithium and subsequent addition of the aldehyde building block (IX) gives the carbinol adduct (X). The methoxytrityl protecting group of (X) is then removed by acidic treatment, yielding diol (XI), which is oxidized to the target keto aldehyde (I) by treatment with Dess-Martin periodinane in CH_2Cl_2 (1). An alternative, more convenient method of preparation of intermediate (I) from sulfone (VIII) has also been reported. The lithium anion generated from sulfone (VIII) and *n*-BuLi is condensed with aldehyde (IX) to produce carbinol (XII), which is subjected to Dess-Martin oxidation of both alcohol functions, and subsequent reductive cleavage of the phenylsulfonyl group by means of an *in situ*-generated THF solution of SmI_2 to furnish (I) (1-3). Scheme 2.

The intermediates (VII) and (VIII) can be prepared by the following common route. Treatment of L-arabinose (XIII) with *tert*-butyldiphenylsilyl chloride and imidazole followed by acetylation of the resulting monosilylated furanose affords (XIV). Introduction of an allyl group into (XIV) by reaction with allyl trimethylsilane (XV) and boron trifluoride etherate produces the allyl derivative (XVI) as a diastereomeric mixture. After basic hydrolysis of the acetate esters of (XVI), the required diastereoisomer (XVII) is isolated employing flash chromatography. Regioselective silylation of diol (XVII) with *tert*-butyldiphenylsilyl chloride followed by methylation of the remaining free hydroxyl group gives the methyl ether (XVIII), which is then desilylated to diol (XIX) with methanolic HCl. After protection of the primary alcohol of (XIX) by acylation with pivaloyl chloride in pyridine, the secondary alcohol group is converted to the benzyl ether (XX) with benzyl bromide under phase-transfer conditions. Subsequent Sharpless asymmetric dihydroxylation of the allyl moiety of (XX) with OsO_4 and $K_3Fe(CN)_6$ in the presence of $(DHQ)_2PYR$ produces glycol (XXI). After protection of diol (XXI) as the silyl ether (XXII), the *O*-benzyl group is removed by hydrogenolysis in the presence of Pearlman's catalyst, yielding alcohol (XXIII), which is oxidized to the corresponding ketone (XXIV) employing *N*-methylmorpholine *N*-oxide in the presence of a catalytic amount of tetrapropylammonium perruthenate (TPAP). Ketone (XXIV) is then converted to the methylene derivative (XXV) by treatment with Tebbe's reagent, generated from bis(cyclopentadienyl)titanium and trimethylaluminum. Olefin (XXV) hydroboration with 9-borabicyclononane followed by oxidative work-up with sodium perborate provides the primary alcohol (XXVI) as the undesired diastereoisomer. The configuration of alcohol (XXVI) is inverted via Swern oxidation to the corresponding aldehyde, which is epimerized to (XXVII) under basic conditions. Then, aldehyde (XXVII) reduction with $NaBH_4$ at 0 °C generates the desired alcohol (XXVIII) (1). Scheme 3.

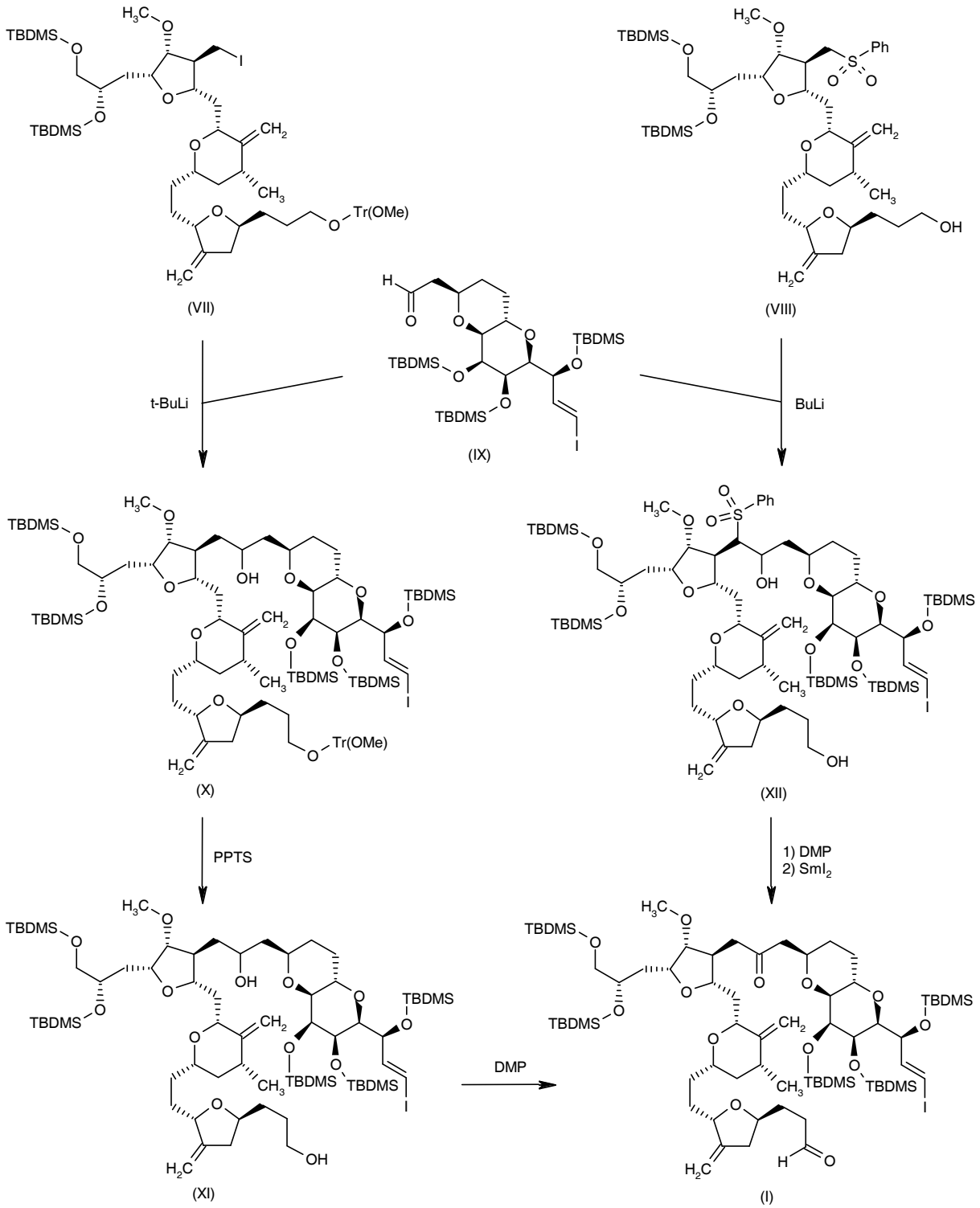
Alcohol (XXVIII) is protected as the 4-methoxybenzyl ether (XXIX) by treatment with *p*-methoxybenzyl trichloroacetimidate and $BF_3.Et_2O$. Subsequent reductive cleavage of the pivaloyl ester (XXIX) with $LiAlH_4$ followed by Swern oxidation of the deprotected alcohol yields aldehyde (XXX). Homologation of aldehyde (XXX) is effected by Wittig reaction with methylene triphenylphosphorane to give the vinyl derivative (XXXI), which undergoes hydroboration with 9-BBN followed by oxidative work-up to furnish alcohol (XXXII). Further Swern oxidation of (XXXII) leads to the homologous aldehyde (XXXIII). Coupling of aldehyde (XXXIII) with the known vinyl iodide (XXXIV) produces a mixture of the pyran derivative (XXXV) and some uncyclized intermediate, which undergoes complete cyclization to (XXXV) by treatment of the reaction mixture with potassium hexamethyldisilazide. Oxidative cleavage of the *p*-methoxybenzyl protecting group of (XXXV) by treatment with DDQ followed by chromatographic separation of the diastereomeric mixture provides the desired isomer (XXXVI) (1, 2). After conversion of (XXXVI) to the corresponding tosylate (XXXVIIa) employing *p*-toluenesulfonyl chloride and pyridine, the target intermediate (VII) is obtained by replacement of the pivaloyl protecting group for a mono-methoxytrityl group, followed by tosylate group displacement with NaI in refluxing acetone (1). Sulfone (VIII) is in turn prepared by conversion of alcohol (XXXVI) to either triflate (XXXVIIb) or mesylate (XXXVIIc), followed by condensation with thiophenol, oxidation to sulfone and then reductive cleavage of the pivaloyl protecting group (1, 2). Scheme 4.

An alternative procedure has been reported for the intermediate sulfone (VIII). After protection of D-glucurono-6,3-lactone (XXXVIII) as the 1,2-acetonide (XXXIX) by means of acetone and H_2SO_4, the 5-hydroxyl group is removed via chlorination with SO_2Cl_2 followed by hydrogenolytic dehalogenation of the resulting chloride (XL) in the presence of Pd/C to yield (XLI). Subsequent reduction of lactone (XLI) with DIBAL in cold toluene affords lactol (XLII), which is condensed with (trimethylsi-

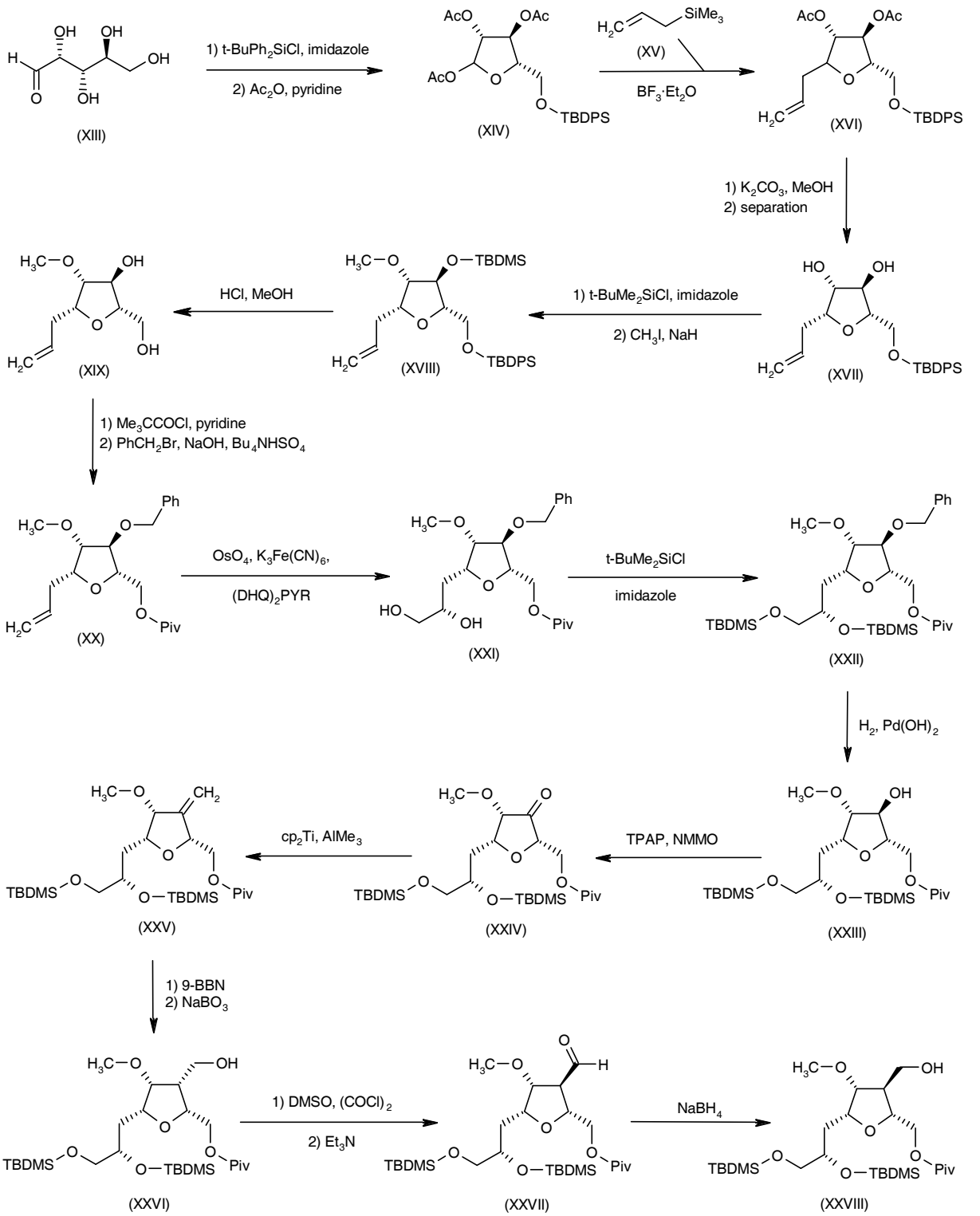
Scheme 1: Synthesis of Eribulin



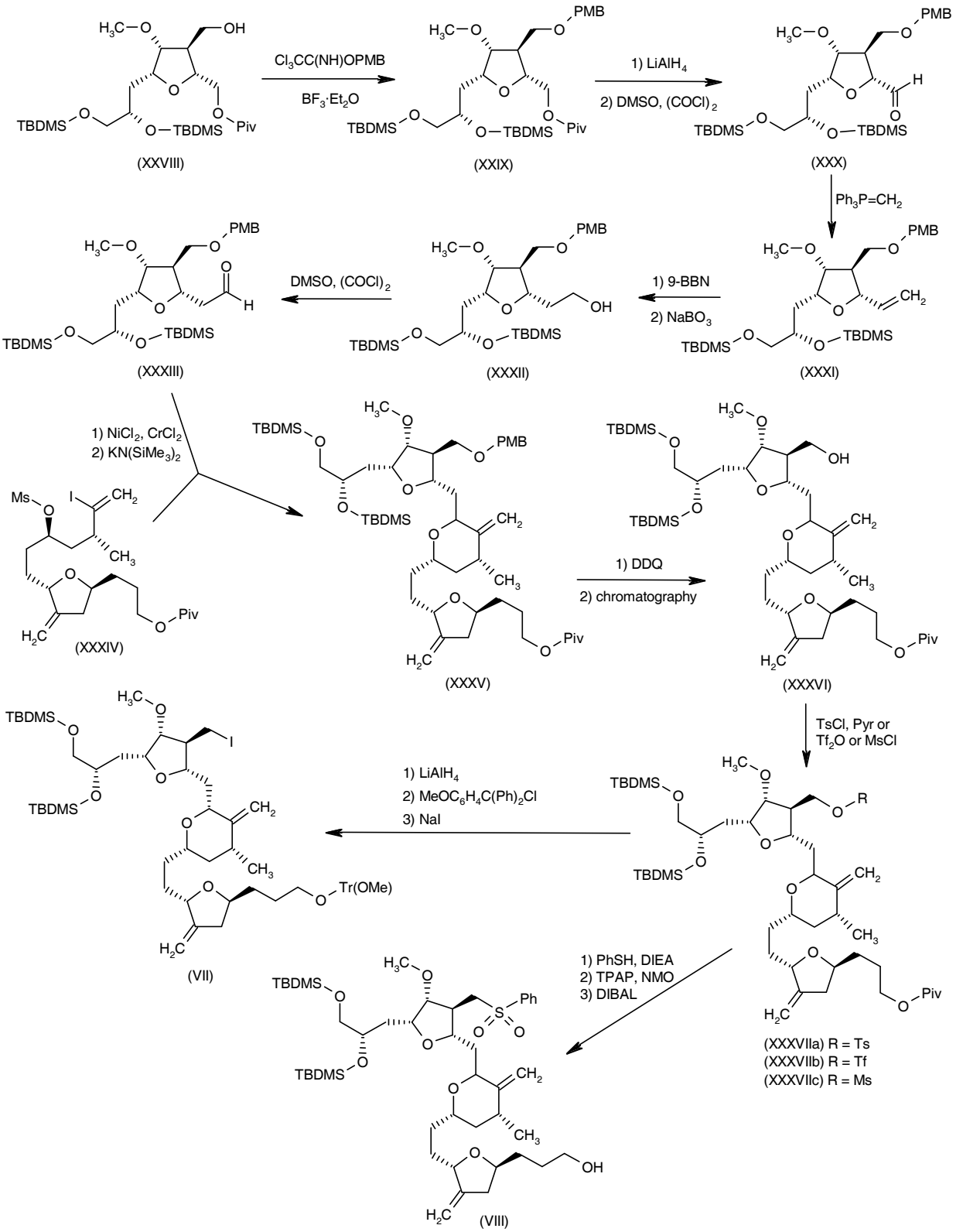
Scheme 2: Synthesis of Intermediate (I)



Scheme 3: Synthesis of Intermediates (VII) and (VIII) (continued in Scheme 4)



Scheme 4: Synthesis of Intermediates (VII) and (VIII) (continuation of Scheme 3)

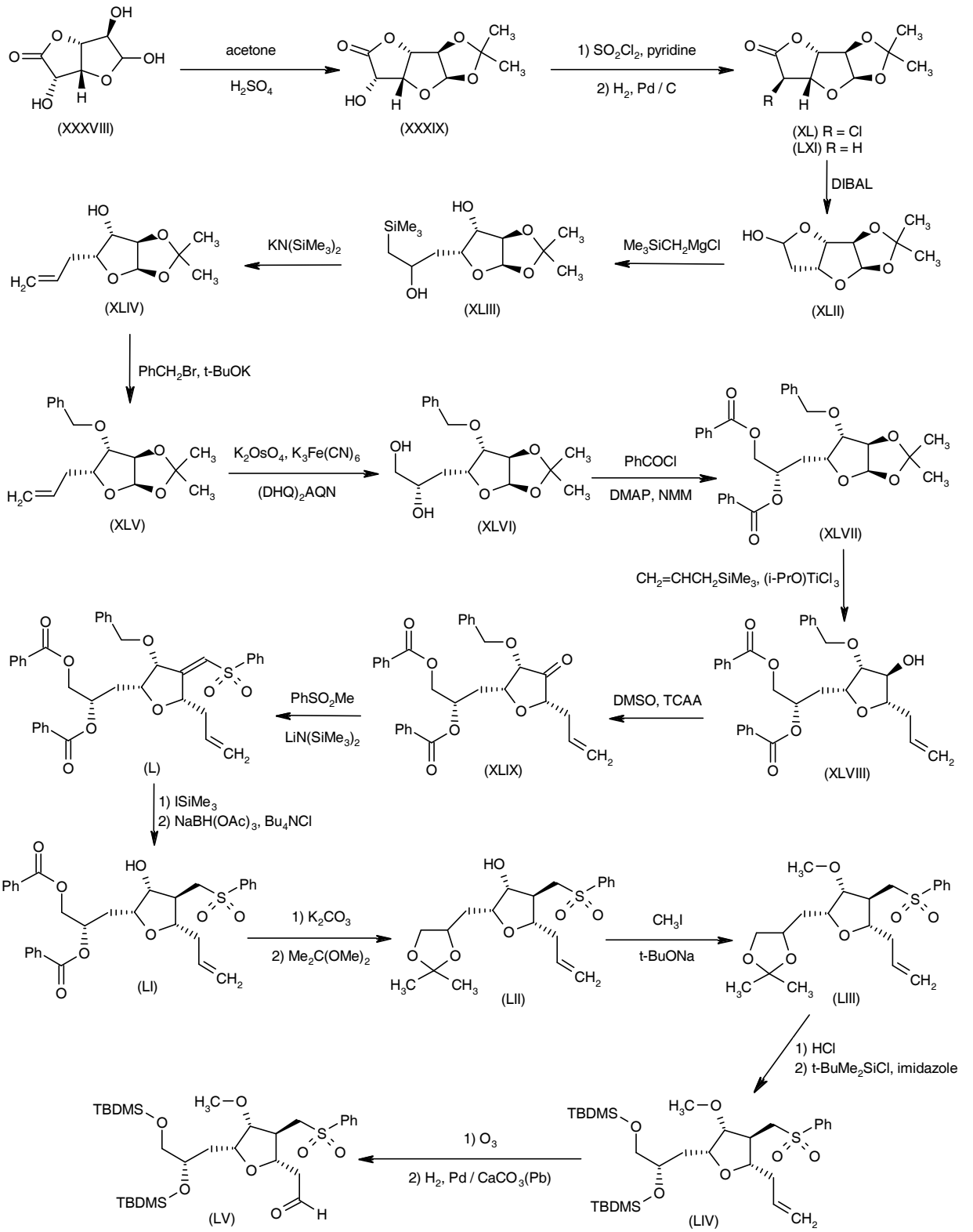


lyl)methylmagnesium chloride to give the α-silyl alcohol (XLIII). This undergoes elimination in the presence of KHMDS in THF to furnish the allyl derivative (XLIV), which is protected at the free hydroxyl group with benzyl bromide and *t*-BuOK, giving the benzyl ether (XLV). Asymmetric dihydroxylation of the allyl group utilizing $K_2OsO_4/K_3Fe(CN)_6$ in the presence of $(DHQ)_2AQN$ leads to the diol (XLVI) as the major isomer, which is further converted to the dibenzoate ester (XLVII) with benzoyl chloride and DMAP. Addition of allyl trimethylsilane to the acetal (XLVII) in the presence of (*i*-PrO)$TiCl_3$ gives the allyl tetrahydrofuranol (XLVIII), which is oxidized to the corresponding ketone (XLIX) under modified Swern conditions utilizing DMSO and trichloroacetic anhydride. Then, condensation of ketone (XLIX) with the lithium derivative of methyl phenyl sulfone provides the α,β-unsaturated sulfone (L). Debenzylation of (L) with iodotrimethylsilane followed by reduction of the unsaturated sulfone with $NaBH(OAc)_3$ and Bu_4NCl affords (LI). Subsequent hydrolysis of dibenzoate (LI) with K_2CO_3 in MeOH followed by reprotection of the obtained diol with 2,2-dimethoxypropane and H_2SO_4 leads to the acetonide (LII). The remaining hydroxyl group in (LII) is then methylated to ether (LIII) by treatment with iodomethane and *t*-BuONa. Replacement of the acetonide protecting group of (LIII) with the bis-silyl ether (LIV) is effected by acidic ketal hydrolysis and then treatment with TBDMSCl and imidazole. Subsequent ozonolysis of olefin (LIV) followed by reductive treatment with H_2 and Lindlar catalyst provides the aldehyde building block (LV) (3). Scheme 5.

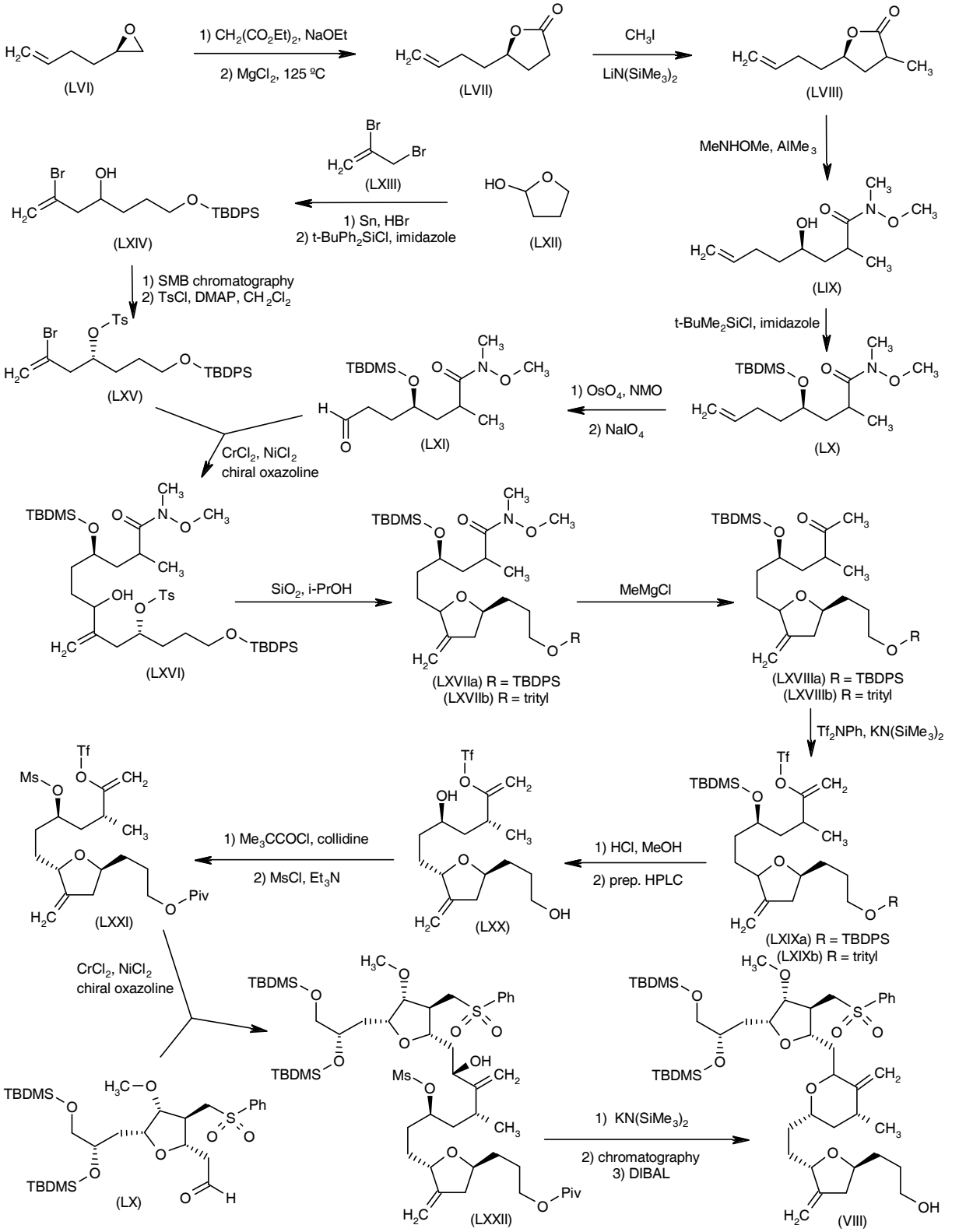
Condensatin of 2(*R*)-(3-butenyl)oxirane (LVI) with diethyl malonate followed by decarboxylation with $MgCl_2$ in hot DMF affords the 4(*R*)-butenyl butyrolactone (LVII), which is methylated to (LVIII) employing iodomethane and LHMDS in cold THF. Ring opening of lactone (LVIII) with *N,O*-dimethylhydroxylamine in the presence of $AlMe_3$ gives the Weinreb amide (LIX), which is protected at the alcoholic hydroxyl group by silylation with TBDMSCl and imidazole, giving (LX). The terminal olefin of (LX) is then converted to aldehyde (LXI) via dihydroxylation with OsO_4/NMO, followed by oxidative cleavage with $NaIO_4$. Tetrahydro-2-furanol (LXII) (obtained by hydration of 2,3-dihydrofuran with an aqueous suspension of Amberlyst 15 resin) is reacted with 2,3-dibromopropene (LXIII) and tin powder in the presence of catalytic HBr to afford 6-bromohept-6-ene-1,4-diol, which is selectively silylated at the primary hydroxyl group with TBDPSCl and imidazole, yielding (LXIV). The racemic monosilylated diol (LXIV) is resolved utilizing simulated moving bed (SMB) chromatography, and the desired 4(*R*)-alcohol is further converted to the corresponding tosylate (LXV) by means of tosyl chloride and DMAP. Coupling between vinyl bromide (LXV) and aldehyde (LXI) in the presence of $CrCl_2$, $NiCl_2$ and the chiral catalyst 4(*R*)-isopropyl-2-(2-methylsulfonamido-3-methylphenyl)-2-oxazoline produces the allyl alcohol adduct (LXVI), which undergoes cyclization to the tetrahydrofuran (LXVIIa) upon treatment with silica gel in isopropanol. Condensation of Weinreb amide (LXVIIa) or its trityl-protected analogue (LXVIIb) (preparation shown below) with methylmagnesium chloride affords the respective methylketones (LXVIIIa/b), which are converted to vinyl triflates (LXIXa/b) by treatment with *N*-phenyltrifluoromethanesulfonimide and KHMDS. Subsequent desilylaton of (LXIXa) with methanolic HCl followed by preparative HPLC separation of the diastereoisomers provides diol (LXX). Similarly, diol (LXX) is obtained by acidic cleavage of the trityl protecting group of (LXIXb). After protection of the primary alcohol group of (LXX) as the pivalate ester with pivaloyl chloride and 2,4,6-collidine, the secondary hydroxyl is converted to the corresponding mesylate (LXXI) employing methanesulfonyl chloride and Et_3N. Vinyl triflate (LXXI) is then coupled with the sulfonyl aldehyde (LX) by means of $CrCl_2$, $NiCl_2$ and 4(*S*)-isopropyl-2-(2-methylsulfonamido-3-methylphenyl)-2-oxazoline as the chiral catalyst to furnish the allylic alcohol adduct (LXXII). Subsequent cyclization of the hydroxyl mesylate (LXXII) in the presence of KHMDS in cold THF followed by chromatographic separation of the diastereoisomers and the reductive cleavage of the pivalate ester group with DIBAL in cold CH_2Cl_2 yields intermediate (VIII) (3). Scheme 6.

The previously mentioned trityl-protected intermediate (LXVIIb) is prepared as follows. Treatment of quinic acid (LXXIII) with cyclohexanone and H_2SO_4 produces a lactone ketal, which is subsequently silylated at the free hydroxyl group with $ClSiMe_3$ and imidazole, giving (LXXIV). Lactone (LXXIV) reduction with DIBAL in cold THF affords the lactol (LXXV), which, after desilylation with aqueous AcOH, is acylated with Ac_2O and DMAP, producing the diacetate (LXXVI). Coupling of acylal (LXXVI) with methyl 3-(trimethylsilyl)pent-4-enoate (LXXVII) in the presence of $BF_3.Et_2O$ and TFAA affords the pentenoate adduct (LXXVIII), which upon cyclization in methanolic NaOMe gives the fused pyranoacetate (LXXIX). Acidic ketal (LXXIX) hydrolysis provides the deprotected diol, which is converted to epoxide (LXXX) by reaction with 2-acetoxy-2-methylpropionyl bromide, followed by elimination of the intermediate bromo ester with methanolic NaOMe. Reduction of the ester function of (LXXX) with $NaBH_4$ and subsequent protection of the obtained alcohol with TBDPSCl and imidazole affords (LXXXI). Subsequent rearrangement of epoxide (LXXXI) in the presence of LDA leads to the allyl alcohol (LXXXII). Ozonolysis of the cyclohexenol ring of (LXXXII) followed by reductive work-up with $NaBH_4$ produces the triol (LXXXIII), which undergoes oxidative cleavage with $NaIO_4$ to give the cyclic hemiacetal (LXXXIV). Then, Wittig condensation of (LXXXIV) with carbomethoxymethylene triphenylphosphorane provides the unsaturated ester (LXXXV). After reduction of (LXXXV) to the saturated analogue by catalytic hydrogenation over Pd/C, ester group reduction with $LiAlH_4$ yields the primary alcohol (LXXXVI). Selective protection of the newly formed hydroxyl of (LXXXVI) with chlorotriphenylmethane and imidazole affords the *O*-trityl compound, which is then desilylated to (LXXXVII) employing TBAF in THF. After iodination of diol (LXXXVII) with NIS and PPh_3, the ethyl iodide group in the resulting diiodo compound is selec-

Scheme 5: Synthesis of Intermediate (VIII) (continued in Scheme 6)



Scheme 6: Synthesis of Intermediate (VIII) (continuation of Scheme 5)



tively displaced with KCN to give nitrile (LXXXVIII). Reductive elimination of the remaining iodide in (LXXXVIII) with concomitant cyclization of the generated hydroxy nitrile by means of Zn dust in boiling EtOH produces the methylene lactone (LXXXIX). Lactone (LXXXIX) is then alkylated with iodomethane and LDA to furnish (XC). Ring opening of the α-methyl butyrolactone (XC) with *N,O*-dimethylhydroxylamine in the presence of $AlMe_3$ furnishes the target Weinreb amide (LXVIIb) (3). Scheme 7.

Intermediate (LXX) can alternatively be prepared from compound (LXXIII) by two additional methods. The polycyclic ester (LXXIX), obtained from (LXXIII) as in Scheme 7, is reduced to alcohol (XCI) employing $LiAlH_4$. After conversion of (XCI) to the corresponding mesylate by means of methanesulfonyl chloride and Et_3N, displacement with KCN in aqueous EtOH and subsequent acidic ketal hydrolysis provides the dihydroxy nitrile (XCII). After conversion of diol (XCII) to the corresponding bromo ester with 2-acetoxy-2-methylpropionyl bromide, elimination of HBr in the presence of DBU affords the allylic acetate (XCIII). Ozonolysis of the cyclohexene ring of (XCIII) followed by reductive treatment with $NaBH_4$ and K_2CO_3, and then oxidative cleavage of the resulting triol with $NaIO_4$, produces the cyclic hemiacetal (XCIV). Horner-Emmons condensation of (XCIV) with trimethyl phosphonoacetate leads to the unsaturated ester (XCV), which is converted to the iodo alcohol (XCVI) by double bond hydrogenation over Pd/C, alcohol iodination with NIS and PPh_3, and ester group reduction with $NaBH_4$. Reductive cleavage of iodide (XCVI) with Zn in EtOH and subsequent silylation of the free hydroxyl group with TBDPSCl and DMAP gives the lactone olefin (XCVII), which can be processed to (LXX) by methods analogous to those in Scheme 7. Scheme 8.

The synthetic precursor (LXX) can also be obtained as follows. Quinic acid (LXXIII) is treated with H_2SO_4 and the resulting lactone acetonide is esterified with bromoacetyl bromide in the presence of pyridine, yielding the bromoacetate ester (XCVIII). Treatment of bromoester (XCVIII) with PPh_3 and DBU produces an intermediate phosphonium salt, which undergoes *in situ* intramolecular Wittig condensation to the furanone derivative (XCIX). After catalytic hydrogenation of the furanone (XCIX) double bond and $LiAlH_4$ reduction of the saturated lactone, the obtained diol is converted to the iodo alcohol (C) by tosylation and subsequent displacement with NaI. Alkyl iodide (C) is then coupled with silylated 4-hydroxy-3-methylpentanal (CI) to furnish the carbinol adduct (CII). Oxidation of (CII) to the corresponding ketone employing Dess-Martin periodinane proceeds with concomitant cyclization to the polycyclic hemiketal (CIII). This is then reduced with triethylsilane and $BF_3.Et_2O$ to the tetrahydropyran derivative (CIV), which can be converted to the synthetic precursor (LXX) following conventional methods (3). Scheme 8

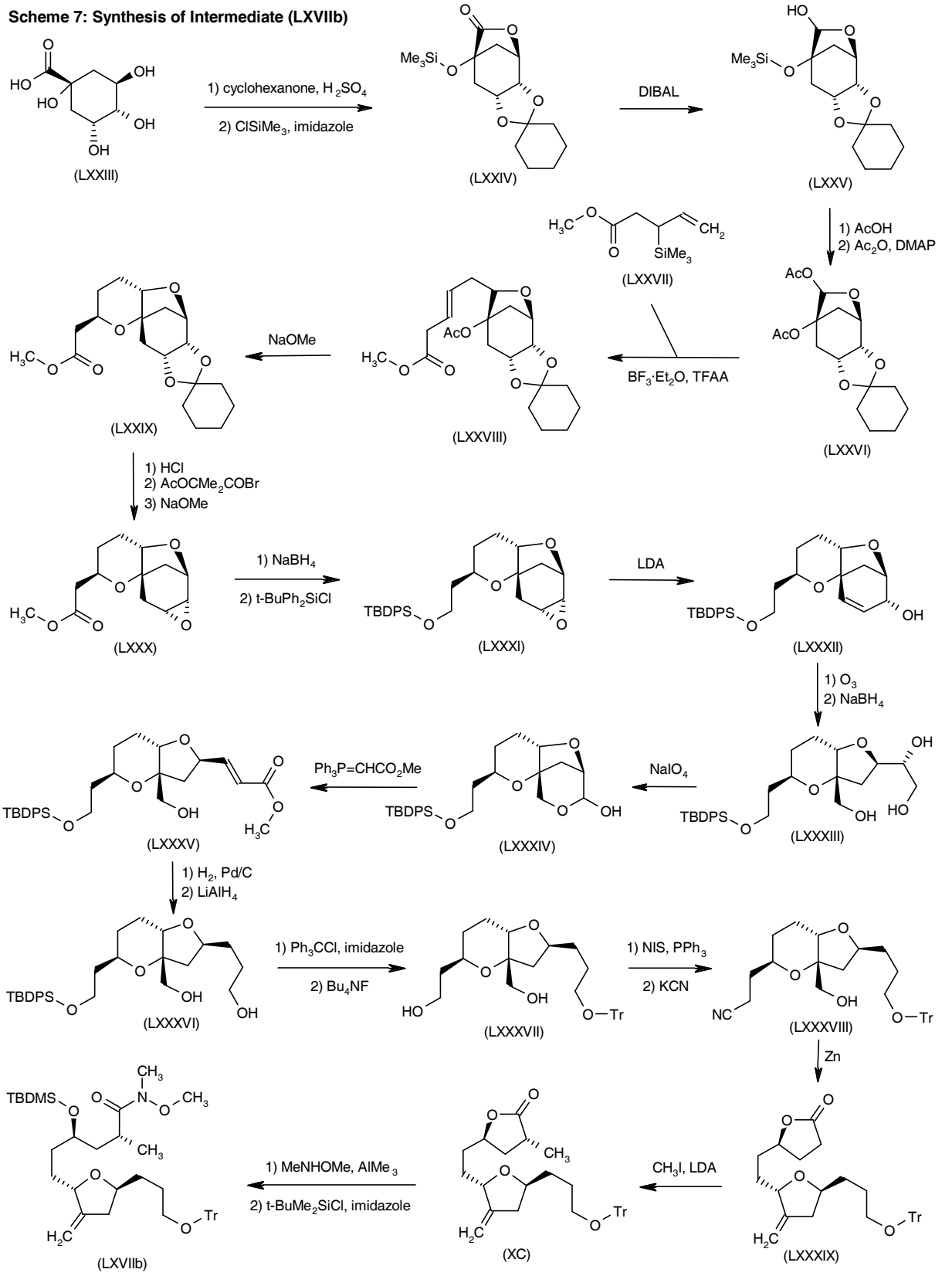
One further method has been reported for the synthesis of the intermediate furylacetaldehyde (XXXIII). Regioselective ring opening of epoxide (CV) with the lithium acetylide of (CVI) in the presence of $BF_3.Et_2O$ furnishes the alkynol adduct (CVII) as the major isomer. Partial hydrogenation of acetylene (CVII) using Lindlar's catalyst followed by acetylation yields the *cis*-olefin (CVIII). Alkene (CVIII) dihydroxylation with OsO_4 affords a mixture of diastereomeric diols which, after conversion to the corresponding dimesylates, are separated by column chromatography to provide (CIX). The acetoxy dimesylate (CIX) is then subjected to cyclization in the presence of Triton B to give the tetrahydrofuran derivative (CX). Methylmagnesium bromide-mediated desulfonylation of (CX) followed by methylation of the resulting alcohol leads to the methyl ether (CXI). The ketal protecting group of (CXI) is then replaced by a bis-silyl ether (CXII) via acidic hydrolysis, followed by treatment with TBDMSCl and imidazole. Then, selective cleavage of the benzyl ether (CXII) by hydrogenation over Raney nickel and subsequent Swern oxidation provides the target aldehyde (XXXIII) (2). Scheme 9.

The vinyl iodide building block (IX) can be obtained as follows. Reaction of L-mannonic acid γ-lactone (CXIII) with cyclohexanone and H_2SO_4 in toluene gives the bis-cyclohexylidene ketal (CXIV), which is reduced with DIBAL in dichloromethane, yielding lactol (CXV). The condensation of (CXV) with methoxymethylene triphenylphosphorane in refluxing THF affords the vinyl ether (CXVI), which is dihydroxylated to (CXVII) with OsO_4 and NMMO in the presence of dihydroquinidine-4-chlorobenzoate as the chiral ligand. Subsequent acylation of (CXVII) with acetic anhydride in the presence of pyridine or $ZnCl_2$ yields the diacetate (CXVIIIa) and the tetraacetate (CXVIIIb), respectively. Condensation of (CXVIIIa/b) with the functionalized allyl silane (LXXVII) by means of $BF_3.Et_2O$ in acetonitrile gives the corresponding adducts (CXIXa) and (CXIXb). Then, cyclization of (CXIXa) by means of Triton B(OMe) in THF/methyl acetate yields the perhydropyrano[3,2-*b*]pyran derivative (CXX). Alternatively, (CXX) is obtained by base-catalyzed cyclization of (CXIXb), followed by selective ketal hydrolysis in hot aqueous HOAc. Oxidative cleavage of diol (CXX) with $NaIO_4$ in THF affords aldehyde (CXXI), which is coupled with the silylated vinyl iodide (CXXII) in the presence of $NiCl_2$ and $CrCl_2$ in DMSO to give the silylated allyl alcohol (CXXIII). Hydrolysis of the cyclohexylidene ketal (CXXIII) by means of HOAc/TFA yields the trihydroxy compound (CXXIV), which is protected as the tris-silyl ether (CXXV) by means of TBDMSOTf and lutidine in dichloromethane. Iododesilylation of (CXXV) with *N*-iodosuccinimide in acetonitrile/chloroacetonitrile provides the vinyl iodide (CXXVI), which is then subjected to ester group reduction with DIBAL to furnish aldehyde (IX) (4). Scheme 10.

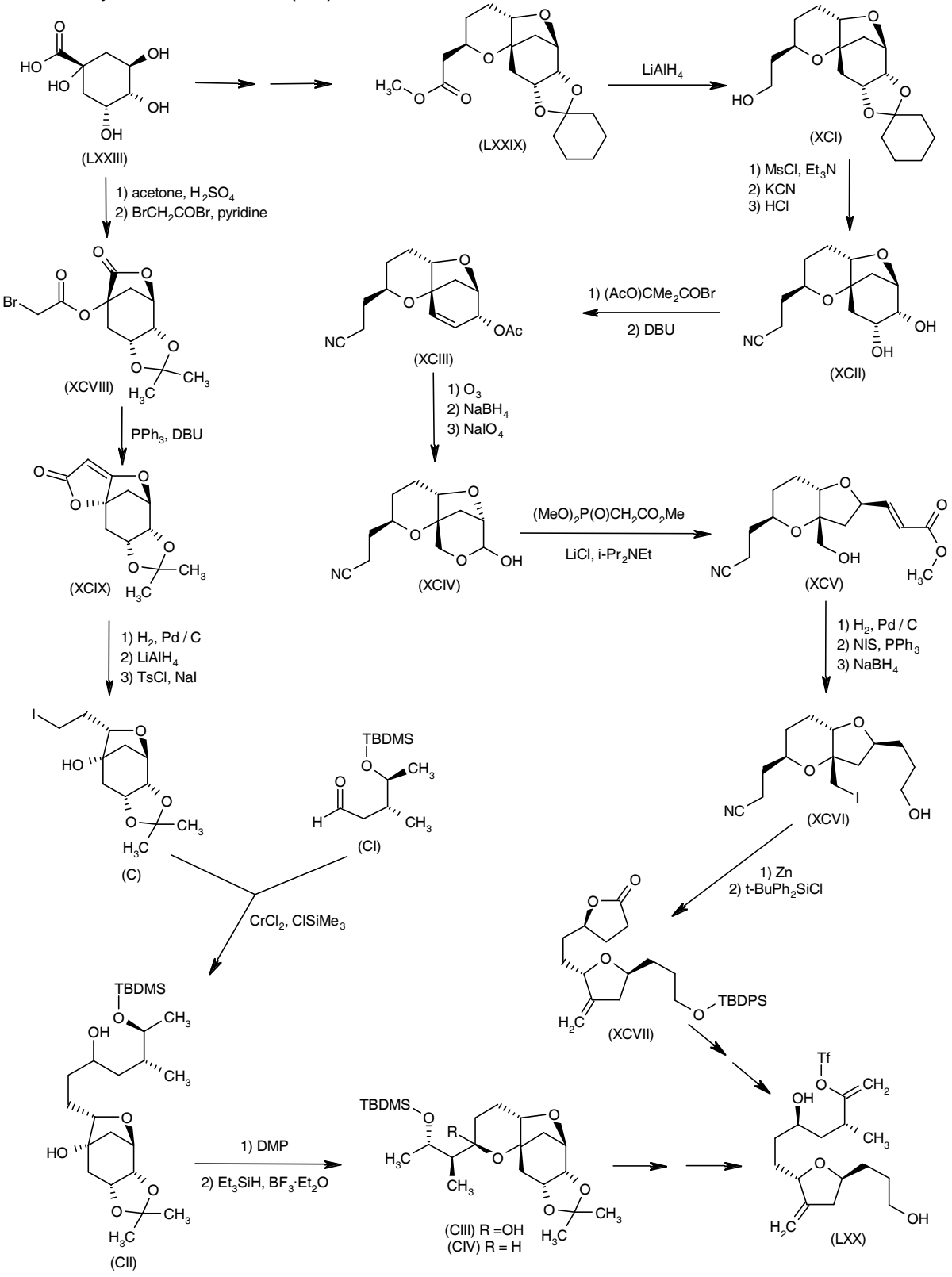
Background

Halichondrin B, a large polyether macrolide produced by marine sponges, demonstrated high potency in inhibiting the proliferation of tumor cells. Studies indicated that halichondrin B inhibited the proliferation of tumor cells via a unique antimitotic mechanism that is different from

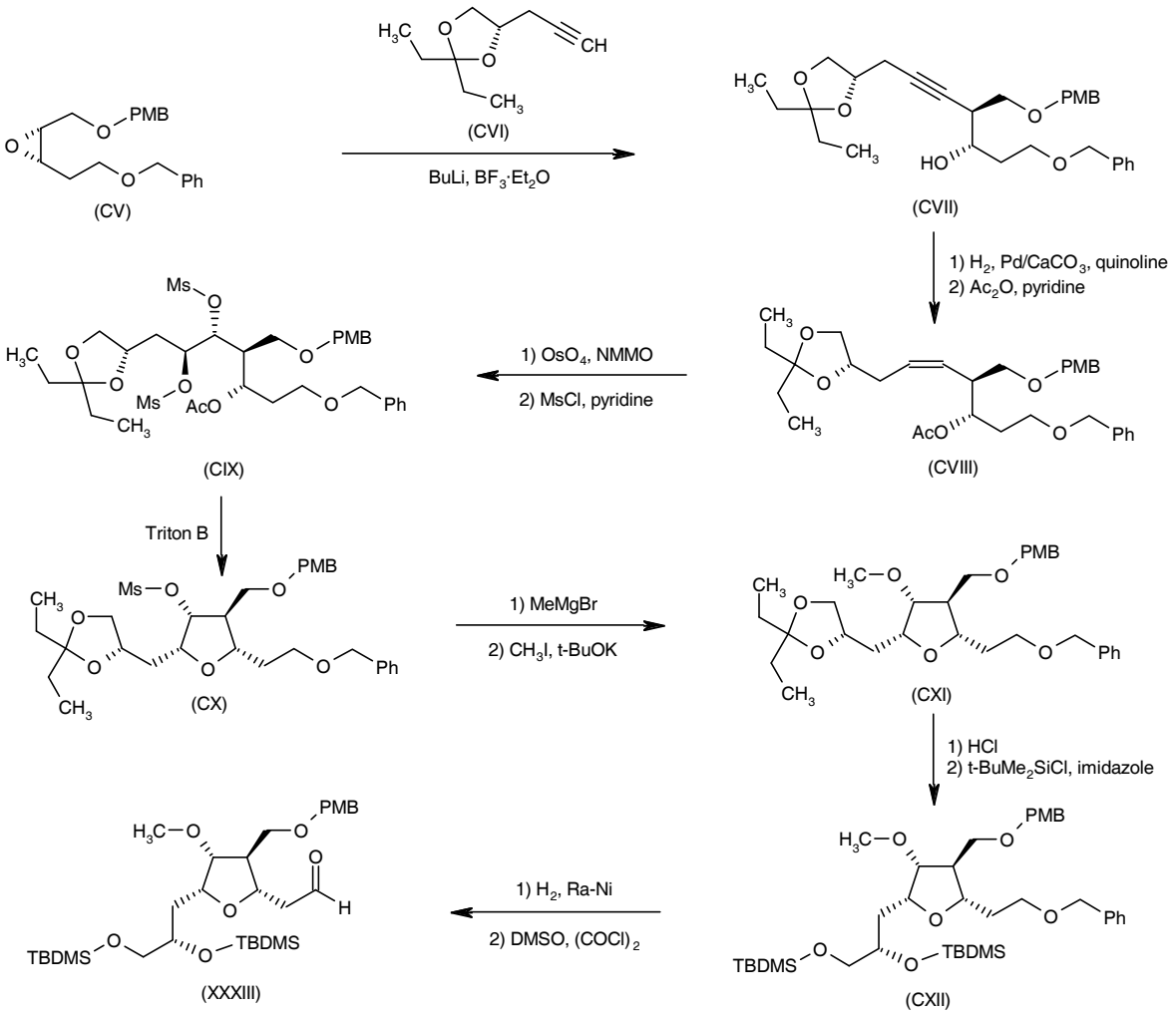
Scheme 7: Synthesis of Intermediate (LXVIIb)



Scheme 8: Synthesis of Intermediate (LXX)



Scheme 9: Synthesis of Intermediate (XXXIII)

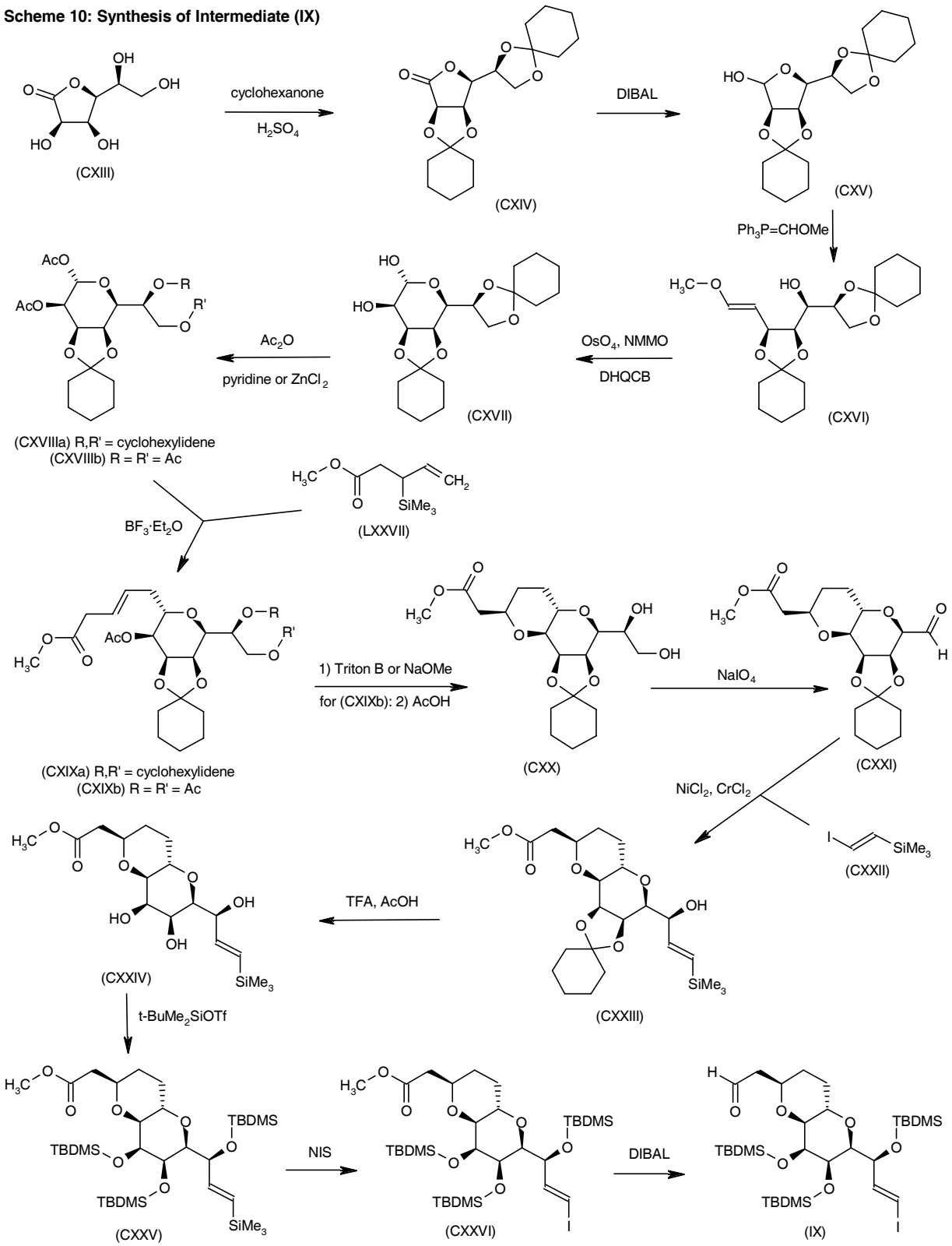


other tubulin-based anticancer agents, such as *Vinca* alkaloids, dolastatins and cryptophycin (5, 6). However, natural supplies of halichondrin B were limited and synthetic compound and analogues were required for drug development. In 1992, a group of scientists at Harvard University successfully synthesized halichondrin B, making further evaluation of the potential therapeutic application of the agent possible (7). Since then, numerous structurally simplified and chemically stabilized synthetic analogues of halichondrin B have been developed and were found to retain halichondrin B's potent inhibitory activity *in vitro*. Among all the synthetic analogues of natural halichondrin B, eribulin (E-7389, NSC-707389, formerly ER-086526), a macrocyclic ketone analogue of halichondrin B's right half (C1-C38), showed subnanomolar growth-inhibitory activity *in vitro* against many human cancer cell lines and *in vivo* against a number of human tumor xenografts, via a tubulin-based antimitotic mechanism similar to that of halichondrin B. The agent also proved stable and retained complete biological activity after incubation in mouse serum for 6 h at 37 °C. Eribulin was therefore chosen as a clinical candidate (8-15).

Preclinical Pharmacology

The *in vitro* antiproliferative effects of eribulin and a related compound, ER-076349, were compared to those of the antimitotic agents vinblastine and paclitaxel in human cancer cells. Low nanomolar or subnanomolar activity was seen for eribulin against human breast cancer MDA-MB-435, colon cancer COLO 205 and DLD-1, prostate cancer DU 145 and LNCaP, melanoma LOX, leukemia HL-60 and lymphoma U-937 cells, with IC_{50} values ranging from 0.09 to 9.5 nM, whereas no cytotoxicity was seen at up to 1 μM in human fibroblasts. ER-076349 was somewhat more active, and both agents were more

Scheme 10: Synthesis of Intermediate (IX)



active than vinblastine or paclitaxel against these cell lines; the activity of eribulin and ER-076349 was similar to that previously reported for synthetic halichondrin B. Experiments using human histiocytic lymphoma U-937 cells demonstrated that both eribulin and ER-076349 induced G2/M phase block, followed by apoptosis. Like vinblastine and paclitaxel, the compounds induced marked mitotic spindle disruption, and like vinblastine, they were found to inhibit tubulin polymerization with IC_{50} values in the low micromolar range. *In vivo* activity was also demonstrated in several human tumor xenograft models (MDA-MB-435, COLO 205, LOX and ovarian cancer OVCAR-3). Doses of 0.05-1 mg/kg i.v. or i.p. were associated with significant antitumor activity in all models, eribulin having superior efficacy. Eribulin treatment was able to induce tumor regression and was associated with long-term suppression of tumor regrowth in some cases, and it also demonstrated a superior therapeutic window compared to paclitaxel (16).

Experiments in human breast cancer MCF7 and human osteosarcoma U-2 OS cells indicated that the antimitotic mechanism of eribulin is different from other microtubule-targeted agents such as vinblastine, vincristine or paclitaxel, and that it involves alterations in microtubule dynamics; it appeared to predominantly inhibit microtubule growth rather than shortening (17-19).

The excellent *in vivo* efficacy of eribulin has been suggested to involve its ability to induce irreversible complete mitotic block and apoptosis following prolonged mitotic block. In U-937 cells, eribulin and ER-076349 induced complete mitotic block at concentrations of 3 and 11 nM, respectively, following 12-h incubation. However, complete mitotic block was maintained for 10 h after washout at this concentration of eribulin, whereas ER-076349 required higher concentrations (35 nM) for a similar effect. Moreover, the effect on cell viability was correlated with postwashout mitotic block (20). In other experiments in U-937 cells, apoptosis was detected starting at 12 h, following prolonged mitotic blockade, which was associated with phosphorylation of Bcl-2, mitochondrial release of cytochrome *c*, proteolytic activation of caspase-3 and -8 and cleavage of poly(ADP-ribose) polymerase (PARP); similar results were seen in human prostate cancer LNCaP cells (21).

Synergistic combinations of eribulin with conventional drugs used for breast cancer treatment were studied *in vitro* in SK-BR-3 cells. Eribulin demonstrated synergistic effects when combined with gemcitabine, cisplatin, epirubicin, trastuzumab, docetaxel and vinorelbine. The agent showed additive effects when combined with carboplatin and antagonistic effects when combined with 5'-DFUR (22). A synergistic effect for the combination of eribulin with gemcitabine was also observed in non-small cell lung cancer (NSCLC) NCI-H522 xenografts in mice, whereas combination with doxorubicin wws not synergistic in the MDA-MB-435 xenograft model (23).

The schedule dependency of eribulin was tested in the MDA-MB-435 breast cancer model. Among the four i.v. schedules (q1dx5, q2dx3[x3], q4dx3 and q7dx3) tested, the two intermittent i.v. schedules, q2dx3(x3) and q4dx3, demonstrated the best antitumor efficacy with the least toxicity. The anticancer activity of eribulin monotherapy was further studied in fibrosarcoma HT-1080 and pancreatic cancer PANC-1 xenograft models using a q4dx3 i.v. dosing schedule. In the HT-1080 model, eribulin produced long-lasting tumor regression at all doses (1.3-4.0 mg/kg); when treated with eribulin at 1.7 mg/kg (maximum tolerated dose, or MTD) and 1.3 mg/kg dose levels, 10 of 10 and 9 of 10 mice were tumor-free by day 38 and 42, respectively. In PANC-1, eribulin at doses ranging from 0.4 to 4 mg/kg also produced long-lasting tumor regression (23).

The anticancer activity of eribulin was further studied in human tumor xenograft models, including lung cancer NCI-H522 and breast cancer MDA-MB-435. Intermittent i.v. treatment regimens (0.375-1.5 mg/kg/dose q4dx3) led to complete tumor regressions in 14 of the 15 animals with MDA-MB-435 xenografts and 14 of the 15 animals with NCI-H522 xenografts were tumor-free. In the MDA-MB-435 model, the average duration of remissions was 24-41 days, and in the NCI-H522 model, animals remained tumor-free with no signs of tumor regrowth for at least 37 days after cessation of treatment (24).

Pharmacokinetics and Metabolism

The preclinical pharmacokinetics of eribulin were evaluated in rats and dogs. After a single i.v. dose (9.0 mg/m^2), eribulin showed triexponential plasma distribution in rats, with short α- (0.03 h) and β-phase (0.36 h) half-lives, followed by a long γ-phase half-life (11.4 h). The agent achieved a steady-state volume of distribution of 109.7 l/m^2, indicating extensive tissue distribution. In dogs administered a single i.v. dose of 0.6 or 1.5 mg/m^2 as a 1-h infusion, the AUC and C_{max} increased proportionally to dose; the volume of distribution was 38.2 and 82 l/m^2, respectively, for the low and high doses of eribulin. In dogs administered multiple doses of 0.08, 0.6 and 0.8 mg/m^2/day via 1-h infusion on a q4dx3 schedule, eribulin again exhibited rapid and extensive tissue distribution, with a long half-life (25).

The first clinical trial of eribulin was conducted in patients with refractory or advanced solid tumors and the pharmacokinetics of the drug administered as a weekly bolus x 3 every 4 weeks were evaluated. Eribulin (starting dose of 0.125 mg/m^2/week) demonstrated a triphasic elimination process, with a prolonged terminal half-life ranging from 36 to 48 h. At the MTD (1.4 mg/m^2/week), plasma concentrations exceeded the concentration required for *in vitro* cytotoxicity for over a week (26). Clearance was independent of dose and a mean of 10% of dose was recovered in urine in 48 h (27).

Another phase I study was conducted in 26 patients with advanced solid tumors who received eribulin as a 1-h i.v. infusion on days 1, 8 and 15 of a 28-day cycle at doses of 0.5-2.8 mg/m^2. Following administration, eribulin demonstrated a rapid distribution phase (mean $t_{1/2}$ = 0.45 h), followed by a slow elimination phase (mean $t_{1/2}$ = 36

h). The mean systemic clearance (CL) and volume of distribution at steady state (Vd_{ss}) were 1.7 ± 0.9 l/h/m^2 and 55 ± 25 l/m^2, respectively. A small portion (6-7%) of the dose was excreted as unchanged drug in the urine within 72 h after administration (28). Eighteen patients were enrolled in another phase I study assessing the pharmacokinetics of eribulin on day 1 every 21 days at doses of 0.25-4 mg/m^2. Pharmacokinetics were linear and the drug once again showed rapid distribution (mean $t_{1/2}$ = 0.40 h) and slow elimination (mean $t_{1/2}$ = 41 h), extensive distribution and slow to moderate clearance (28, 29).

Safety

Target organ toxicity of eribulin was evaluated in preclinical studies in dogs and rats. Dogs received multiple doses (0.004, 0.03 and 0.04 mg/kg/day) by 1-h i.v. infusion, and rats received multiple doses (0.013, 0.13 and 0.20 mg/kg/day) by i.v. bolus injection. Drug-related hematological effects were reported in both species. Administration of eribulin at 0.03 and 0.04 mg/kg/day decreased white blood cells and neutrophils in dogs, and doses of 0.13 or 0.2 mg/kg/day in rats affected white and red blood cell parameters starting on day 12. The agent also decreased alkaline phosphatase and increased aspartate aminotransferase in rats at doses of 0.13 and 0.20 mg/kg/day. Histopathological lesions, bone marrow atrophy, thymic atrophy and testicular degeneration were also observed in rats at 0.13 and 0.20 mg/kg/day. Several rats in the highest dose group experienced skeletal muscle degeneration. By day 35, full recovery, except for testicular degeneration, was observed in rats. In dogs, only lymphoid atrophy and splenic lymphoid depletion were seem and were fully reversible by day 35 (30).

The toxicity of eribulin and halichondrin B was compared in rats and dogs administered the agents i.v. every 4 days x 3. In rats, the total lethal dose of eribulin was 13.3 mg/m^2 (4.3 mg/m^2/dose) compared to 0.81 mg/m^2 (0.27 mg/m^2/dose) of halichondrin B. Eribulin caused reversible toxicity at a total dose of 2.4 mg/m^2. *In vitro*, eribulin proved less toxic to granulocyte-macrophage colony-forming units (CPU-GM) than halichondrin B (24).

In the initial phase I study in patients with refractory or advanced solid tumors, neutropenia was the dose-limiting toxicity (DLT) and the MTD was 1.4 mg/m^2 every 3 weeks on a 4-week schedule. Other serious nonhematological toxicities included hypoglycemia, hypophosphatemia and fatigue (26).

In the above-mentioned trial in 18 patients with nonhematological cancer administered eribulin at doses of 0.25-4 mg/m^2 every 21 days, febrile neutropenia was seen in 3 of 3 patients at 4 mg/m^2 and 2 of 3 at 2.8 mg/m^2, but only in 1 of 3 at 2.0 mg/m^2. Other adverse events were minor (29).

Patients with advanced solid tumors were administered eribulin (0.25-1.4 mg/m^2) on days 1, 8 and 15 by 1-h i.v. infusion every 28 days in a phase I study. Grade 3/4 neutropenia was dose-limiting at 1.4 mg/m^2 and dose-limiting grade 3 fatigue was seen in a patient at 0.5 mg/m^2; 1 death due to interstitial pneumonia occurred at 1 mg/m^2 (31).

In a phase II clinical study in patients with refractory breast cancer who had previously received treatment including anthracyclines and taxanes (see details below), the most commonly reported drug-related adverse events were neutropenia (75%), fatigue (52%), alopecia (41%), nausea (37%) and anemia (36%). Additionally, 34% of the patients experienced peripheral neuropathy (32-34).

The safety of i.v. bolus administration of eribulin in patients with recurrent NSCLC was evaluated in another phase II study (see details below). The most frequent treatment-related adverse events included neutropenia (23% grade 3, 26% grade 4, 4% febrile), fatigue, nausea and peripheral neuropathy (35, 36).

Clinical Studies

The first clinical study of eribulin was conducted in 40 patients with refractory or advanced solid tumors (see above). Two patients with NSCLC and bladder cancer achieved partial responses and 3 patients with NSCLC, breast and thyroid cancer achieved a minor response. Twelve patients had stable disease for a median of 4 months (2-14 months). Fluorescent IHC analysis of a series of tumor biopsies indicated that eribulin disrupted microtubule structures in the tumors (26).

The efficacy of eribulin was also assessed in the open-label, single-arm phase II trial in previously treated (including anthracylines and taxanes) patients with refractory breast cancer. Patients were divided into two groups, the first group receiving a 2-5-min i.v. bolus administration of eribulin (1.4 mg/m^2) on days 1, 8 and 15 of a 28-day treatment cycle, and the second group receiving the drug on days 1 and 8 of a 21-day treatment cycle. A total of 104 patients were enrolled in the study and 103 patients received eribulin treatment (70 in group 1 and 33 in group 2). The overall partial response (PR) rate was 14.5% and 15.2% in groups 1 and 2, respectively. The objective response rate (ORR) was 14.7% and the median progression-free survival (PFS) was 85 days, with a 6-month PFS rate of 31% (32-34).

The efficacy of eribulin in patients with NSCLC was evaluated in an open-label, single-arm, stratified phase II study. The treatment plan in this study was similar to that conducted in patients with refractory breast cancer. Patients with measurable, recurrent and/or metastatic NSCLC who progressed during or after platinum-based chemotherapy were stratified into two groups. Patients in the first group (n=77) received an i.v. bolus of eribulin on days 1, 8 and 15 of a 28-day cycle and patients in the second group (n=26) received the same dose of eribulin on days 1 and 8 of a 21-day cycle. The PR rate was 9.7% and the overall disease control rate (PR + stable disease) was 55.3%, with a 12-week PFS rate of 53.0%, a median PFS of 102 days, a median overall survival of 287 days and a 1-year survival rate of 46.4% (35, 36).

Eribulin continues to undergo clinical evaluation as monotherapy or in combination with other agents for the

treatment of various types of cancer, including bladder, prostate, gynecological, pancreatic and head and neck cancer, NSCLC and soft tissue sarcoma, and phase III trials are under way in locally recurrent or metastatic breast cancer (37-48).

Source

Eisai Research Institute (US).

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