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[54] HALOMACROLIDES AND DERIVATIVES HAVING IMMUNOSUPPRESSIVE ACTIVITY

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Related U.S. Application Data

[63] Continuation of Ser. No. 596,177, Oct. 11, 1990, abandoned.

[51] **Int. Cl.**[5] **A61K 31/695**; A61K 31/395; C07D 498/16

[52] **U.S. Cl.** **514/291**; 514/183; 514/411; 540/456

[58] **Field of Search** 540/456; 514/183, 411; 314/29

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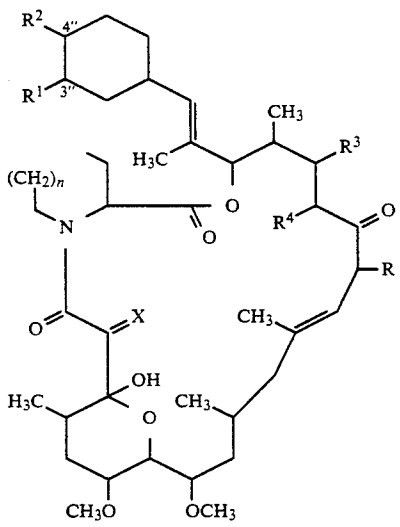
[57] **ABSTRACT**

Novel C-3'' and C-4'' halogen-substituted macrolides of FK-506 type structural Formula I:



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are described. These macrolide immunosuppressants are useful in a mammalian host for the treatment of autoimmune diseases (such as juvenile-onset diabetes melitus, multiple sclerosis and rheumatoid arthritis), infectious diseases and/or the prevention of rejection of foreign organ transplants, e.g. bone marrow and heart transplants. In addition, these macrolide immunosuppressants are useful in the topical treatment of inflammatory and hyperproliferative skin diseases and cutaneous manifestations of immunologically-mediated illnesses such as: psoriasis, atopical dermatitiis, contact dermatitis and further eczematous dermatitises, seborrhoeic dermatitis, Lichen planus, Pemphigus, bullous Pemphigoid, Epidermolysis bullosa, urticaria, angioedemas, vasulitides erythemas, cutaneous eosinophilias, Lupus erythematosus or Alopecia areata.

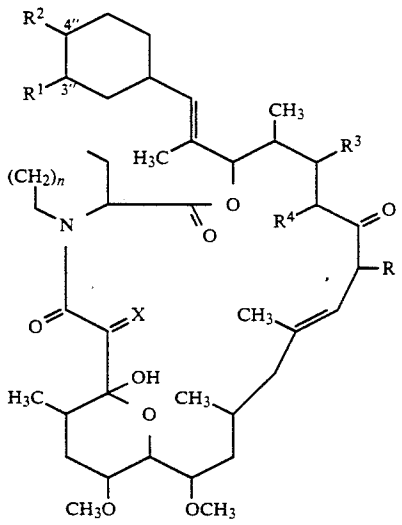
9 Claims, No Drawings

HALOMACROLIDES AND DERIVATIVES HAVING IMMUNOSUPPRESSIVE ACTIVITY

This is a continuation of application Ser. No. 07/596,177, filed on Oct. 11, 1990 now abandoned.

The present invention is related to compounds which are useful in a mammalian host for the treatment of autoimmune diseases (such as juvenile-onset diabetes melitus, multiple sclerosis and rheumatoid arthritis), infectious diseases and/or the prevention of rejection of foreign organ transplants, e.g. bone marrow and heart transplants and are also useful in the topical treatment of inflammatory and hyperproliferative skin diseases and cutaneous manifestations of immunologically-mediated illnesses.

More particularly, this invention relates to the introduction of a halogen substituent at C-3'' or C-4'' of the cyclohexyl ring in compounds of the general structural Formula I:



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wherein R is methyl, ethyl, propyl or allyl; R^1 and R^2 are independently halo, hydroxy, and C_1–C_8 alkoxy, with the proviso that at least one R^1 or R^2 is halogen; R^3 is hydrogen or hydroxy and R_4 is hydrogen; or R^3 and R^4 can be taken together to form a double bond; X=O or (HO, H) and n is 1 or 2. It also relates to pharmaceutical compositions containing the compounds and to a method of use of the present compounds and other agents for the treatment of autoimmune diseases, infectious diseases, the rejection of foreign organ transplants, inflammatory and hyperproliferative skin diseases and/or cutaneous manifestations of immunologically-mediated illnesses.

BRIEF DESCRIPTION OF DISCLOSURES IN THE ART

Fujisawa European and Japanese and U.S. patents (*EPO Publication* No. 0,184,162 and *PBJ Disclosure* 63-17884 U.S. Pat. No. 4,894,366) and publications (*J. Am. Chem. Soc.*, 1987, 109, 5031 and *J. Antibiotics* 1987, 40, 1249) disclose 17-allyl-1,14-dihydroxy-12-[2'-(4''-hydroxy-3''-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo-[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone (FR-900506), 17-ethyl-1,14-dihydroxy-12-[2'-(4''-hydroxy-3''-methoxycyclohexyl)-1'-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone (FR-900520) and related compounds which are the starting materials for the preparation of the compounds described. The synthetic preparation of the aforementioned starting material (FR-900506) has recently been reported (*J. Am. Chem. Soc.*, 1989, 111, 1157).

BACKGROUND OF THE INVENTION

Immunoregulatory abnormalities have been shown to exist in a wide variety of "autoimmune" and chronic inflammatory diseases, including systemic lupus erythematosis, chronic rheumatoid arthritis, type 1 diabetes mellitus, inflammatory bowel disease, biliary cirrhosis, uveitis, multiple sclerosis and other disorders such as Chrons disease, ulcerative colitis, bullous pemphigoid, sarcoidosis, psoriasis, ichthyosis, and Graves ophthalmopathy. Although the underlying pathogenesis of each of these conditions may be quite different, they have in common the appearance of a variety of autoantibodies and self-reactive lymphocytes. Such self-reactivity may be due, in part, to a loss of the homeostatic controls under which the normal immune system operates.

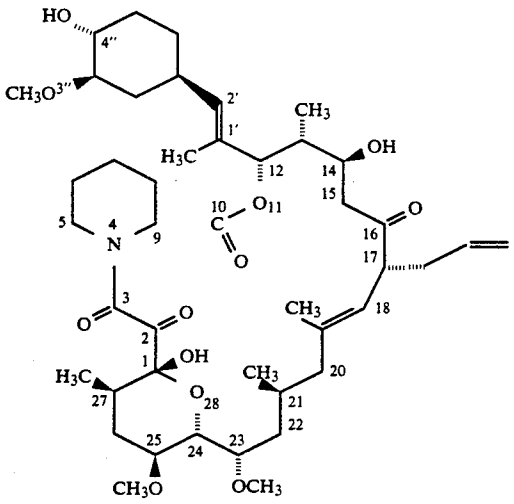
Similarly, following a bone-marrow or an organ transplantation, the host lymphocytes recognize the foreign tissue antigens and begin to produce antibodies which lead to graft rejection.

One end result of an autoimmune or a rejection process is tissue destruction caused by inflammatory cells and the mediators they release. Antiflammatory agents such as NSAID's and corticosteroids act principally by blocking the effect or secretion of these mediators but do nothing to modify the immunologic basis of the disease. On the other hand, cytotoxic agents such as cyclophosphamide, act in such a nonspecific fashion that both the normal and autoimmune responses are shut off. Indeed, patients treated with such nonspecific immuno-suppressive agents are as likely to succumb from infection as they are from their autoimmune disease.

Cyclosporin A which was approved by the U.S. FDA in 1983 is currently the leading drug used to prevent rejection of transplanted organs. The drug acts by inhibiting the body's immune system from mobilizing its vast arsenal of natural protecting agents to reject the transplant's foreign protein. Though cyclosporin A is effective in fighting transplant rejection, it is nephrotoxic and is known to cause several undesirable side effects including kidney failure, abnormal liver function and gastro-intestinal discomfort.

Newer, safer drugs exhibiting less side effects are constantly being searched for in the field.

The 23-membered tricyclo-macrolide immunosuppressant, FR-900506,



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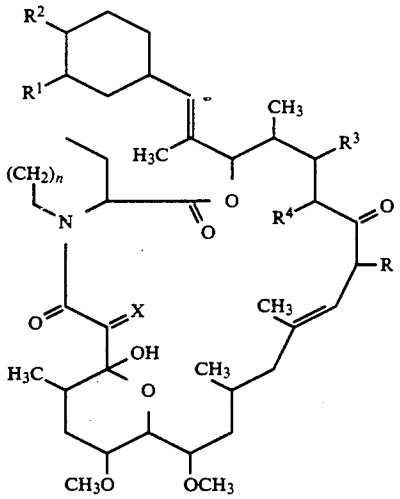

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and related compounds which were isolated and characterized by Tanaka, Kuroda, and co-workers at Fujisawa Pharmaceutical Co. in Japan, see *J. Am. Chem. Soc.*, 1987, 109, 5031, and *EPO Pub. No.* 0,184,162 have been shown to possess exceptional immunosuppressive activity. The compound FR-900506 has been reported to be 100 times more effective than cyclosporin in the suppression of in vitro immune systems (*J. Antibiotics* 1987, 40, 1256). In addition, these compounds are reputed to possess topical activity in the treatment of inflammatory and hyperproliferative skin diseases and cutaneous manifestations of immunologically-mediated illnesses (*EPO Pub. No.* 0,315,978).

Accordingly, an object of the present invention is to provide new analogs of these tricyclomacrolides which will (1) restore the balance of the help-and-suppression mechanism of the immune system by acting at an earlier point than the anti-inflammatory agents and (2) induce specific long-term transplantation tolerance through a suppressor cell circuit without increasing the body's susceptibility to infection.

Another object of the present invention is to provide analogs of these tricyclo-macrolides which possess topical activity in the treatment of inflammatory and hyperproliferative skin diseases and cutaneous manifestations of immunologically-mediated illnesses.

An additional object of the present invention is to provide pharmaceutical compositions for administering to a patient in need of the treatment one or more of the active immunosuppressive agents of the present invention.

Still a further object of this invention is to provide a method of controlling graft rejection, autoimmune and chronic inflammatory dieases by administering a sufficient amount of one or more of the novel immunosuppressive agents in a mammalian species in need of such treatment.

Finally, it is the object of this invention to provide processes for the preparation of the active compounds of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

A. Scope of the Invention

This invention relates to compounds of the general Formula I:

wherein:

R is methyl, ethyl, propyl or allyl; R^1 and R^2 are independently halo, hydroxy, C_1–C_8 alkoxy, with the proviso that at least one R^1 or R^2 is halogen; R^3 is hydrogen or hydroxy and R_4 is hydrogen; or R^3 and

R^4 can be taken together to form a double bond; X═O or (HO, H) and n is 1 or 2;

In the present invention, compounds with asymmetric centers may occur as racemates, racemic mixtures and as individual diastereomers, with all isomeric forms of the compounds being included in the present invention.

In addition compounds with carbon-carbon double bonds may occur in Z— and E— forms with all isomeric forms of the compounds being included in the present invention.

When any variable (e.g., alkyl, R, R^1, R^2, R^3, R^4, etc.) occurs more than one time in any variable or in Formula I, its definition on each ocurrence is independent of its definition at every other occurrence.

As used herein, "alkyl" is intended to include both branched, straight chain and cycloalkyl saturated aliphatic hydrocarbon groups having the specified number of 1–8 carbon atoms, representative examples being methyl, ethyl, isopropyl, tert-butyl, sec-butyl, isopentyl, n-hexyl, n-heptyl, n-octyl, iso-octyl, and the like; "halo", as used herein, means fluoro, chloro, bromo or iodo.

In the present invention it is preferred that in compounds of Formula I:

R is ethyl, propyl or allyl;

R^1 is fluoro, chloro, bromo, or iodo and R^2 is hydroxy or methoxy; or R^2 is fluoro, chloro, bromo or iodo and R^1 is hydroxy or methoxy;

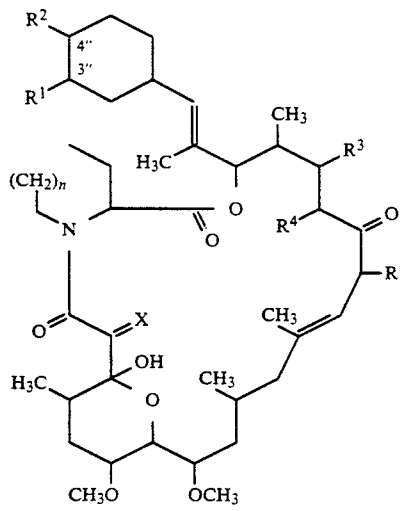
R^3 is hydrogen or hydroxy;

R^4 is hydrogen;

n is 1 or 2.

B. Preparation of Compounds Within the Scope of the Present Invention

The starting materials for the preparation of the 3''-halo and 4''-halo compounds of this invention are represented by Formula II:



II

wherein R is methyl, ethyl, propyl or allyl; R^1 and R^2 are, independently, hydroxy or methoxy; R^3 is hydrogen or hydroxy; R^4 is hydrogen or R^3 and R^4 can be taken together to form a double bond. X is 0; and n is 1 or 2.

The production and characterization of compounds of Formula II is well known in the literature (see EPO Publication No. 0,323,042, EPO Publication No. 0,184,162, PBJ Disclosure 63-17884, *J. Am. Chem. Soc.*, 1987, 109, 5031 and *J. Antibiotics*, 1987, 40, 1249). Both biological fermentation and synthetic processes may be found. A synthetic route to compounds of Formula II can involve modifications of a route described in *J. Am. Chem. Soc.*, 1989, 111, 1157.

Biological fermentation followed by synthetic modification is presently favored in the art as the method to produce compounds of Formula II. Organisms belonging to the genus *Streptomyces* such as *Streptomyces tsukubaensis*, No. 9993 placed in an aqueous nutrient medium will produce desired compounds in isolable amounts. The nutrient medium contains sources of assimilable carbon and nitrogen, preferably under aerobic conditions. Produced in fermentation are four compounds of Formula II, (A) where R is allyl, R^1 is methoxy, R^2 and R^3 are hydroxyl, R^4 is hydrogen, X is 0 and n is 2; (B) where R is ethyl, R^1 is methoxy, R^2 and R^3 are hydroxyl, R^4 is hydrogen, X is 0 and n is 2; (C) where R is methyl, R^1 is methoxy, R^2 and R^3 are hydroxyl, R^4 is hydrogen, X is 0 and n is 2; and (D) where R is allyl, R^1 is methoxy, R^2 and R^3 are hydroxyl, R^4 is hydrogen, X is 0 and n is 1.

A lyophilized sample of the isolated *Streptomyces tsukubaensis*, No. 9993 was deposited with the Fermentation Research Institute, Agency of Industrial Science and Technology (No. 1-3, Higashi 1-chome, Yatabemachi Tsukuba-gun, Ibaraki Prefecture, Japan) under the deposit number of FERM P-7886 (deposit date: Oct. 5th, 1984), and then converted to Budapest Treaty route of the same depository on Oct. 19, 1985 under the new deposit number of FERM BP-927.

Using the four compounds produced in fermentation above, the remaining compounds of Formula II may be easily produced. The allyl of R may be conveniently reduced to propyl by well known methods. The R^2 or R^3 hydroxyls may be protected by well known methods, for example as disclosed in EPO Publication 0,323,042. In addition, the hydroxy of R^3 may be reduced to a hydrogen or eliminated to form a double bond with R^4 (by methods similar to those disclosed in EPO Publication 0,323,042). The carbonyl of X may be reduced to a hydroxy by methods disclosed in EPO Publication 0,323,042 also U.S. Ser. No. 07/486,700 filed Mar. 1, 1990. The methoxy of R^1 as produced may be replaced with hydroxy or demethylated and subsequently protected as desired, if necessary. This demethylation of R^1 may be carried out in a fermentation reaction using the compounds of Formula II as a feedstock. For instance, compound B named under Formula II above may be demethylated at R^1 above by using the microorganism Actinomycetales ATCC No. 53771 (as taught in U.S. Ser. No. 213,025 filed Jun. 29, 1988 and hereby incorporated by reference) or produced directly by a mutant organism (as taught in U.S. Ser. No. 323,653 filed Mar. 15, 1989 and hereby incorporated by reference). Similarly, compound A may be demethylated (as taught in U.S. Ser. No. 213,063 also filed Jun. 29, 1988).

Suitable protecting groups for hydroxyl include those groups well known in the art which are: 1-(lower alkylthio) (lower)alkyl, wherein "lower alkyl" indicates a straight, cyclic or branched chain of one to six carbon atoms, such as lower alkylthiomethyl (e.g. methylthiomethyl, ethylthiomethyl, propylthiomethyl, isopropylthiomethyl, butylthiomethyl, isobutylthiomethyl, hexylthiomethyl, etc.), and the like, in which the preferred one may be C_1–C_4 alkylthiomethyl and the most preferred one may be methylthiomethyl; trisubstituted silyl such as tri(lower)alkylsilyl (e.g. trimethylsilyl, triethylsilyl, tributysilyl, tri-isopropylsilyl (TIPS), t-butyldimethylsilyl, (TBDMS) tri-t-butylsilyl, etc.), lower alkyldiarylsilyl (e.g. methyl-diphenylsilyl, ethyl-diphenylsilyl, propyl-diphenylsilyl, t-butyldiphenylsilyl, etc.), and the like, in which the preferred one may be tri(C_1–C_4-)alkylsilyl and C_1–C_4 alkyldiphenylsilyl, and the most preferred one may be tert-butyl-dimethylsilyl, tri-i-propylsilyl and tert-butyldiphenylsilyl; acyl such as aliphatic acyl, aromatic acyl and aliphatic acyl substituted with aromatic group, which are derived from carboxylic acids; and the like.

Compounds A, B, C and D of Formula II, organisms to produce the same, conditions of fermentation, separation techniques, and chemical modification of the products are fully described in *EPO Publication No.* 0,184,162. This document is hereby incorporated by reference.

The compounds of the present invention which are represented by Formula I are prepared by the methods shown in the following Reaction Schemes wherein R, R_1, R_2, R_3, R_4, X and n are as defined above unless otherwise indicated. It will be readily apparent to one of ordinary skill in the art reviewing the synthetic route depicted below that other compounds within Formula I can be synthesized by substitution of appropriate reactants and agents in the synthesis shown below.

As shown in Reaction Scheme A for preparing the 4''-halogen derivatives, the C-14-oxygen-protected macrolide (V protected with TIPS or TBDMS) is prepared from the 4'',14-dihydroxy macrolide (III) by treating III with silylating agents TIPSX or TBDMSX (where X=chloride or trifluoromethane sulfonate) to form IV and hydrolyzing off the C-4'' protecting group to give V, and reacted with o-nitrobenzene sulfonyl chloride to form VI, which is then reacted with LiX, where X=Cl, Br, I, in an aprotic solvent, e.g., N,N-

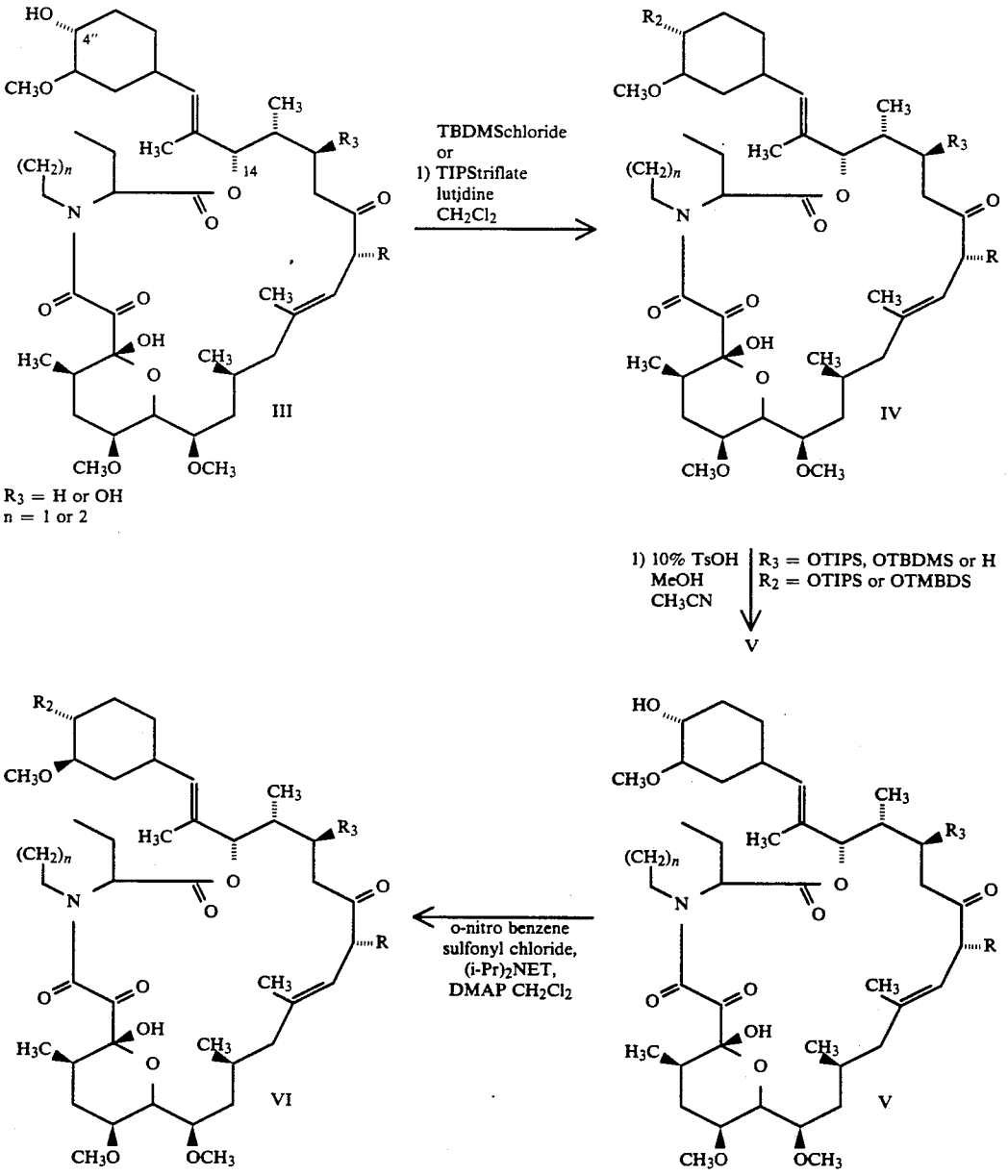
dimethylformamide, to introduce the halo substituent at the C-4″ position to produce VIII. The protecting group at C-14 is then removed to produce IX. Compound V can be treated with DAST at 0° C. in CH_2Cl_2 and after the removal of protecting groups, if any, to give compound IX where R_2=F.

A route to C-3″ halo substituted compounds is shown in Reaction Scheme B. The procedure is analogous to that for forming the C-4″ halo derivatives.

The macrolide III is treated with the demethylating microorganism ATCC No. 53771 or ATCC No. 53828 to produce the C-4″ and C-3″ dihydroxy macrolide X. This is treated with one molar equivalent of protecting group reagent, such as tert-butyldimethylsilyl chloride or tri-isopropylsilyl trifluoromethanesulfonate, in CH_2Cl_2 with imidazole or 2,6-lutidine as an acid scavanger to produce a mixture of C-4″ and C-3″ monooxygen-protected derivatives. The C-4″ Oxygen-protected derivative (XI) is separated and purified by chromatography.

Following the analogous procedure for the C-4″ halo derivatives, the C-3″ hydroxy is reacted with orthonitrobenzene-sulfonyl chloride to form XII, which is then reacted with LiX (where X=Cl, Br, I) to form the C-3″ halo derivative XIII. In addition, C-3″ and C-4″ halo substituted compounds can be alternatively prepared as shown in Scheme C. Treatment of the demethylated natural product (R=$CH_2CH=CH_2$, $CH_2CH_2CH_3$, CH_2CH_3, or CH_3; R_1=OH; R_2=OH; R_3=OH, OTBDMS, OTIPS or H; R_4=H) with o-nitrobenzenesulfonyl chloride followed by base gives a mixture of epoxides which can be separated (XIV). Nucleophilic ring opening with LiX or $TiCl_4/TMSN_3$ affords the C-3″ or C-4″ halo derivative (XIII).

REACTION SCHEME A



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