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PREPARATION AND *IN VITRO* ACTIVITY OF ARYL ETHER DERIVATIVES OF THE FK-506 RELATED IMMUNOSUPPRESSIVE MACROLIDES ASCOMYCIN AND L-683,742

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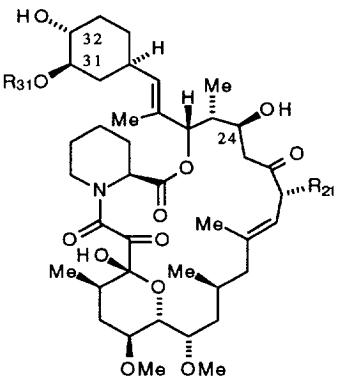
Abstract. The arylation of the immunosuppressive macrolides ascomycin and L-683,742 using pentavalent bismuth reagents is described. The *in vitro* activities of the aryl ether analogs are reported.

The macrolide FK-506 (**1**) is a fungal metabolite first isolated and characterized in 1987.[1] It has been shown to be a powerful immunosuppressant *in vitro* and *in vivo*,[2] being 50 to 100 times more potent than cyclosporin A - the therapy of choice for prevention of graft rejection following organ transplantation. Although structurally dissimilar, FK-506 and cyclosporin have been shown to elicit their immunosuppressive effects through a common mechanism.[3] Specifically, both bind with their cognate receptors (FKBP and cyclophilin, respectively) and those drug-receptor complexes inhibit the Ca^{2+}-dependent protein phosphatase calcineurin. The inhibition of calcineurin interferes with early gene transcription following T-cell activation and results in suppression of lymphocyte proliferation. More importantly, FK-506, like cyclosporin, has been shown to be effective in the prevention of organ graft rejection in the clinic.[4] Unfortunately, there are side effects associated with the clinical use of FK-506: most notably nephrotoxicity, neurotoxicity and gastric toxicity.[4,5] Research in these laboratories has focused on the derivatization of ascomycin (**2**) (L-683,590, FK-520) and its 31-desmethyl congener L-683,742 (**3**), in an effort to identify a less toxic immunosuppressant. This communication is a preliminary account of the synthesis, via novel organobismuth chemistry, and *in vitro* immunosuppressive activity of a new class of 31- and 32-O-aryl ethers.

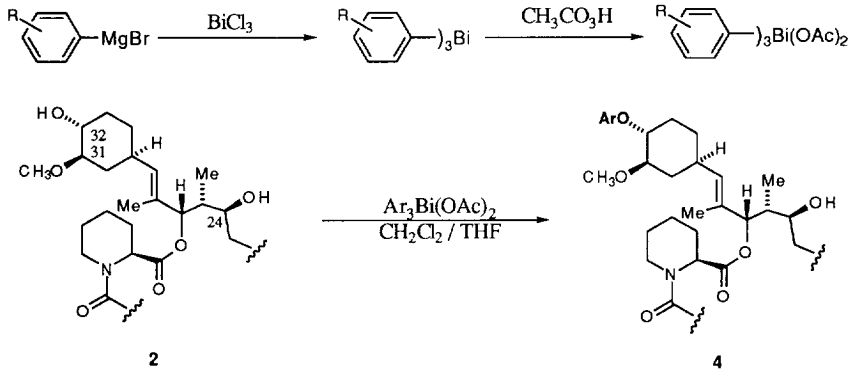


1	FK-506	R_{21} = allyl	$R_{31} = CH_3$
2	Ascomycin	R_{21} = ethyl	$R_{31} = CH_3$
3	L-683,742	R_{21} = ethyl	R_{31} = H

The unique functional array found in FK-506 and related macrolides presents a significant synthetic challenge to chemists. The synthesis and *in vitro* bioactivity of 31- and 32-O-alkyl ether analogs of ascomycin has already been reported.[6] As in that case, only mild conditions can be utilized to effect the arylation of the 31- and 32-hydroxyls since the macrolide is prone to both base and thermal mediated rearrangements.[7] The macrolide will not survive the harsh alkaline reaction conditions ordinarily employed by classical methods of aryl ether formation. However, an alternate route exists that permits the formation of aryl ethers under mild

conditions: the copper mediated arylation of alcohols using triarylbismuth(V)diacetate reagents. Although recent reviews describe the utility of pentavalent bismuth reagents with relatively simple substrates,[8] no examples employing this methodology on substrates of the complexity of FK-506 have been reported.

The arylation of organic compounds at oxygen, nitrogen and carbon using pentavalent organobismuth reagents has been amply demonstrated. Generally, alcohols that are proximal to a second heteroatom can be readily arylated using a triarylbismuth diacetate. These reagents are prepared by treatment of $BiCl_3$ with a Grignard or organolithium reagent and subsequent oxidation of the resultant triarylbismuthines.[9] The oxidation of Bi(III) to Bi(V) can be carried out with a halogen source (such as Cl_2, Br_2 or SO_2Cl_2) to give the triarylbismuthine dihalide.[9] Treatment of the triarylbismuth dihalide with potassium carbonate followed by acetic acid affords the desired reagent. Alternatively, the triarylbismuth dihalide can be reacted with silver acetate to give the bismuth(V) diacetate directly.[9] In these laboratories the preferred procedure entails *in situ* oxidation of the triarylbismuthine to give the bismuth(V) diacetate. This is accomplished by addition of 1-1.2 equivalents of peracetic acid (32% solution in dilute acetic acid) to a solution of the triarylbismuthine in 3:1 CH_2Cl_2:THF. The macrolide (e.g. **2**) and copper(II)acetate are then added. The reactions are generally allowed to proceed overnight at room temperature or, if necessary, at reflux. The arylated compounds were isolated in yields ranging from 14-70% (unoptimized). While small amounts of the 24,32-diaryl compounds were isolated in some cases, arylation takes place preferentially at the 32-hydroxyl thus making protection of the 24-hydroxyl unnecessary. Arylation of **3** (L-683,742, the desmethyl analog of ascomycin) results in formation of both the 31- and 32-O-aryl ethers (**5** and **6**, respectively) in approximately a 1:1 ratio.[10] The preparation of the pentavalent organobismuth reagents and subsequent arylation of the macrolide is compatible with a variety of substituted aromatic groups as illustrated in Tables 1 and 2.



2 **4**

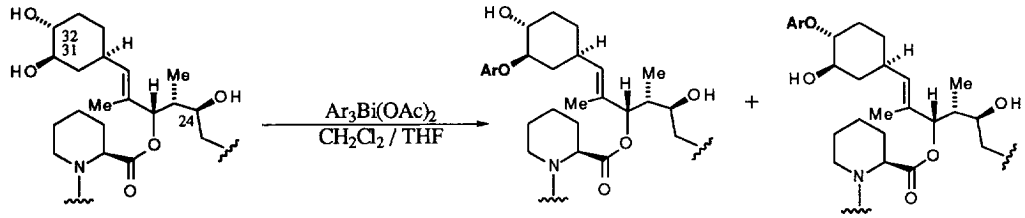
Tables 1 and 2 also list the *in vitro* immunosuppressive activity of the aryl ethers as measured by their ability to inhibit proliferation of PMA and ionomycin stimulated murine T cells.[11] This inhibition of proliferation is reversible by addition of exogenous IL-2. The observed activities indicate that aryl substitution at the 31- and 32-oxygens of the macrolide can be tolerated with maintenance of good activity. The effect of aromatic ring substituents on the *in vitro* activity of these compounds can be dramatic. In general, aryl ether analogs possessing electron donating substituents on the phenyl ring (e.g. **4 j**, **4 l**) showed enhanced bioactivity relative to compounds possessing electron withdrawing groups (e.g. **4 a**,**4 b**). This trend holds for a series of

compounds of approximately the same steric demands (cf. **4 b**, **4 f**, and**4 l**). Additionally, para substitution is slightly preferred over meta substitution.

TABLE 1: *IN VITRO* ACTIVITY OF 32-O-ARYL ETHERS OF ASCOMYCIN

COMPOUND	Ar	Yield[a] (%)	IC_{50}[b] (nM)	COMPOUND	Ar	Yield[a] (%)	IC_{50}[b] (nM)
2	H	--	0.69	**4 g**	4-CH_3S-phenyl	14	2.6
4a	3,5-di-(F_3C)-phenyl	46	607	**4 h**	3-CH_3O-phenyl	62	3.4
4 b	4-CF_3-phenyl	51	35.8	**4 i**	4-CH_3O-phenyl	18	1.4
4 c	4-F-phenyl	35	3.4	**4 j**	4-Me_2N-phenyl	17	1.1
4 d	phenyl	42	5.4	**4 k**	3-HO-phenyl[c]	34	1.7
4 e	3-CH_3-phenyl	42	5.4	**4 l**	4-HO-phenyl[d]	65	0.8
4 f	4-CH_3-phenyl	42	2.7	--	----	--	--

[a]Isolated yield of coupled product from arylation reaction.
[b]IC_{50} for inhibition of proliferation of PMA and Ionomycin stimulated murine T cells.[11]
[c]Bismuthine formation and coupling carried out using benzyl-protected phenol.
[d]Bismuthine formation and coupling carried out using TBDMS-protected phenol.



3 **5** **6**

TABLE 2: *IN VITRO* ACTIVITY OF 31- AND 32-O-ARYL ETHERS OF L-683,742

Ar	Yield[a] (%)	5 IC_{50}[b] (nM)	6 IC_{50}[b] (nM)	Ar	Yield[a] (%)	5 IC_{50}[b] (nM)	6 IC_{50}[b] (nM)
3	--	1.63	--	4-CH_3O-phenyl	47	0.9	1.2
phenyl	37	3.2	5.2	4-HO-phenyl[c]	68	0.9	1.6
4-CH_3-phenyl	33	2.6	6.0	----	--	--	--

[a]Combined isolated yields of coupled products **5** and **6** from arylation reaction.
[b]IC_{50} for inhibition of proliferation of PMA and Ionomycin stimulated murine T cells.[11]
[c]Bismuthine formation and coupling carried out using TBDMS-protected phenol.

The observed structure-activity relationship offers some intriguing possibilities. As stated above, the correlation between the electronic character of the substituents on the aromatic ring and the *in vitro* activity is significant. This correlation may be partly, but not entirely, due to altered ability to bind to FKBP. For example, in a competitive binding assay[12] ascomycin (**2**) has 4-fold better binding to FKBP than the p-hydroxyphenyl derivative **4 l** (1.6 nM and 6.3 nM, respectively) yet they are nearly equipotent in the T cell proliferation assay. Alternatively, the observed effects may be due to altered cell penetration or distribution. Perhaps the most intriguing possibility is that the electronic character of the phenyl ring alters interaction with calcineurin, the known target of the drug/FKBP complex. Further investigation into this area is necessary before the underlying causes of the observed structure-activity relationship are elucidated.

In summary, this communication reports the preparation of 31- and 32-O-aryl ethers of the immunosuppressive macrolides ascomycin and L-683,742 and highlights the utility of pentavalent bismuth chemistry in medicinal chemistry. The compounds synthesized were active in an *in vitro* assay measuring inhibition of T-cell proliferation. Substitution at both the 31-and 32-positions was tolerated and the activities of the aryl ethers show a trend of electron donating para substitution leading to more active compounds. The synthesis and activities of other interesting analogs prepared using this chemistry will be reported.

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