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ALKYL ETHER ANALOGS OF THE FK-506 RELATED, IMMUNOSUPPRESSIVE MACROLIDE L-683,590 (ASCOMYCIN)

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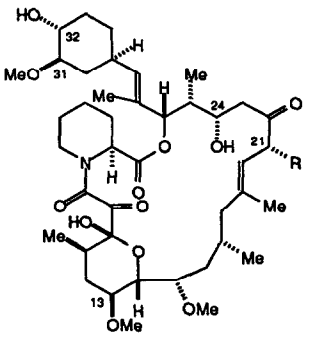
Abstract: C32-O-Ether derivatives of L-683,590 have been prepared by alkylation with various alkyl, alkenyl, and alkynyl 2,2,2-trichloroacetimidates. These analogs exhibit good immunosuppressive activity *in vitro*, as measured in a T cell proliferation assay. In the case of cinnamyl ethers, this activity correlates with the lipophilicity (π value) of the aromatic substituents.

The clinical importance of a potent and safe immunosuppressant is becoming increasingly evident as the present therapeutic agents, notably cyclosporin A (CsA)[1] and FK-506[2], find utility in the treatment of diseases beyond organ transplant rejection (e.g., psoriasis, rheumatoid arthritis, etc).[3] It is thought that most autoimmune diseases can be treated effectively with immunosuppressants, provided the safety profile of such agents would permit long term administration. Along with organ transplantation, FK-506 **1** is currently under clinical evaluation for several autoimmune indications; however, it is not clear whether the neuro- and nephrotoxicity exhibited by this drug will allow for use in the treatment of chronic autoimmune diseases.[4]



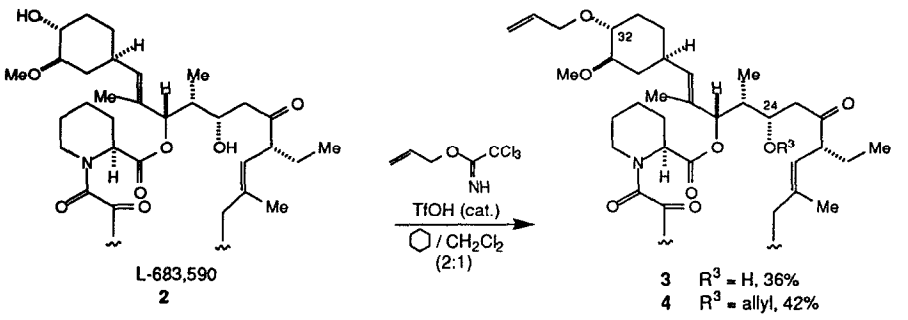
		R	TCell (IC_{50}, nM)
1	FK-506	allyl	0.29
2	L-683,590	ethyl	0.69

CsA and FK-506 act to suppress the immune system by inhibiting the proliferation of antigen stimulated T cells. While they block the same calcium-dependent signal transduction pathway in T cells[5], FK-506 is 100-fold more potent *in vitro*.[6] The goal of our research is to discover semi-synthetic analogs of FK-506 or the structurally related L-683,590 (ascomycin)[7] **2** that maintain the superior activity of this class of immunosuppressants while displaying fewer side-effects. As part of an ongoing chemical derivatization effort, a series of C32-O-alkyl ether analogs of L-683,590 were prepared with the object of modulating the lipophilicity of this compound.

Chemistry

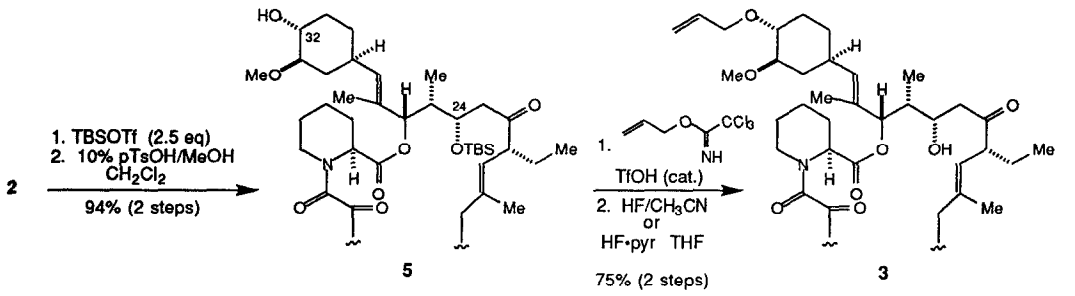
Etherification of the hydroxyl groups in compounds such as L-683,590 requires a method that is non-basic and thermally mild to avoid complicating side-reactions.[8] The acid catalyzed reaction of alkyl 2,2,2-trichloroacetimidates with alcohols provides the corresponding ethers at room temperature and under essentially neutral conditions.[9] This procedure has been used to couple a variety of alkyl groups with alcohols, and has been employed extensively as a mild and reliable means of delivering alcohol protecting groups. We have found this method to be a convenient one for the preparation of ether derivatives of L-683,590 and related compounds.[10] As shown in Scheme 1, treatment of **2** with allyl 2,2,2-trichloroacetimidate[9b] (2 equiv.) and trifluoromethanesulfonic acid (TfOH, 0.2 equiv.) in cyclohexane/methylene chloride (2:1) provided a mixture of C32-O-allyl- and bis(C24, C32-O-allyl)-L-683,590, **3** and **4**, in 78% yield.[11]

Scheme 1



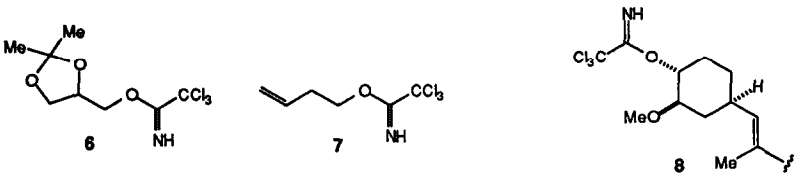
A modest degree of regioselectivity favoring alkylation of the C32 hydroxyl group is observed in these transformations; however, in many cases it was found desirable to protect the competing C24-OH as the t-butyldimethylsilyl ether prior to alkylation. This was accomplished by the two-step, bis-silylation/mono-desilylation protocol depicted in Scheme 2. Alkylation followed by deprotection of the C24-OTBS group by one of the hydrogen fluoride-mediated methods produced the C32-O-ether analogs.

Scheme 2



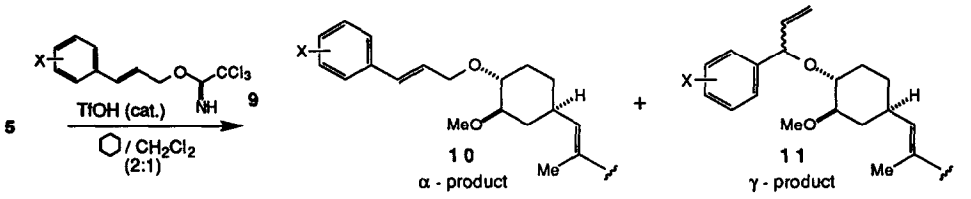
The reactivity of an alkyl 2,2,2-trichloroacetimidate is dependent on the ability of the alkyl group to support an incipient partial positive charge along the reaction coordinate. For instance, *p*-methoxybenzyl 2,2,2-trichloroacetimidate is a very reactive alkylating agent[9d], whereas we have found that reagents such as **6** and **7** do not react under the normal conditions. In the latter cases, prolonged exposure with L-683,590 and TfOH gave the

trichloroacetimidate-transfer product **8**.[12] Thus, our alkylations are limited to the use of benzylic, allylic, or other non-primary, alkyl 2,2,2-trichloroacetimidate reagents.



In the case of certain allylic 2,2,2-trichloroacetimidates, there exists an issue of alkylation regiochemistry. When alcohol **5** was treated with various, substituted cinnamyl 2,2,2-trichloroacetimidate reagents **9** under the standard conditions, ether adducts arising from both α- and γ-alkylation, **10** and **11**, were obtained (Scheme 3). The α-alkylation pathway was generally favored in the cinnamyl series (1-3:1), however, aromatic substituents were found to influence this ratio, with electron-withdrawing groups (X in **9** = 4-F, -Cl, -Br) resulting in a greater proportion of α-product. Cinnamyl derivatives such as **10** could be further modified by hydrogenation (H_2, Rh/C cat., EtOH) to provide the corresponding phenpropyl ethers.

Scheme 3

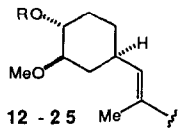


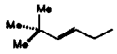
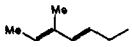
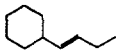
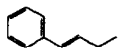
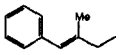
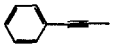
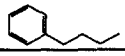
Results and Discussion

A series of C32-O-ether derivatives of L-683,590 were prepared to examine the effect of this modification on immunosuppressant activity (Table 1). Simple alkyl, alkenyl, and alkynyl ether derivatives (**12-14**) exhibit an approximate ten-fold loss in potency in a T cell proliferation assay[13] compared to the parent alcohol L-683,590. The benzyl ether derivative **15** was found to be a marginally better immunosuppressant *in vitro*, with this activity being sensitive to α-branching (**16**). Two heteroaromatic analogs (**17, 18**) were found to be less active than their benzyl counterpart. Several 3'-substituted allyl ethers were also examined (**19-22**). While general alkyl and alkenyl substitution at this site did not improve activity relative to allyl ether **13**, the cinnamyl ether analog **22** exhibited a demonstrable (4-fold) gain in potency. Several cinnamyl-like ethers containing modified aromatic tethers were prepared to investigate the role of the *trans* propenyl linking chain (**23-25**). These were found to possess slightly inferior immunosuppressive activity compared to **22**, suggesting that among this series the propenyl tether is optimum.

An investigation of the C32-O-cinnamyl ether derivatives of L-683,590 was conducted in an attempt to increase the potency of this class. It was found that varying the substituents (X) on the aromatic ring resulted in an 18-fold range of *in vitro* activities, Table 2. Halogen substitution (**26-30**) was found to have an adverse

Table 1: *In vitro* immunosuppressive activity of C32-O-ether analogs of L-683,590

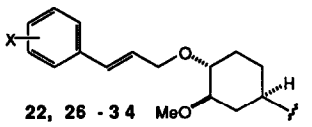


Compound	Ether group (R)	T Cell[13] IC_{50} (nM)
2	H	0.7
12		12.5
13		7.4
14		6.1
15		3.0
16		11.3
17		13.9
18		7.1
19		14.2
20		6.9
21		5.5
22		1.9
23		5.5
24		2.7
25		3.5

effect on potency relative to the parent cinnamyl ether 22, whereas, incorporation of a hydroxyl substituent at the 3- or 4- position (33, 34) served to increase immunosuppressive activity to a level approximating that of the parent natural product L-683,590, 2.

Analysis of the data from this study reveals a striking correlation between the *in vitro* potency of these analogs and the π-value[14] associated with the aromatic substituent. As shown in Figure 1, analogs containing substiuents with low π-values are much more active as immunosuppressants than those with the highest π.

Table 2: *In vitro* immunosuppressive activity of C32-O-cinnamyl ether analogs of L-683,590



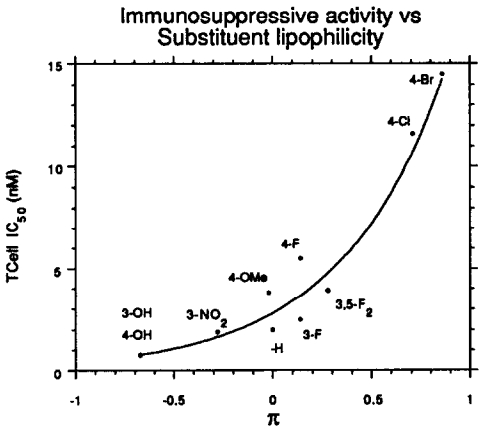
22, 26 - 34

Compound	X-	π–value[14]	T Cell[13] IC_{50} (nM)	FKBP12[16] EC_{50} (nM)
26	4-Br	0.86	14.5	23.0
27	4-Cl	0.71	11.6	15.8
28	3,5-F_2	0.28	3.9	--
29	4-F	0.14	5.5	--
30	3-F	0.14	2.5	--
22	H	0.0	1.9	29.7
31	4-OMe	-0.02	3.8	--
32	3-NO_2	-0.28	1.9	34.3
33	3-OH	-0.67	0.8	--
34	4-OH	-0.67	0.8	20.5
L-683,590 **2**	--	--	0.7	1.6

Further, a correlation exists amongst the entire group, where a non-linear relationship is observed between substituent π-value and potency. A connection between bioactivity, both *in vitro* and *in vivo*, and compound lipophilicity has been observed within many drug classes.[15] While other factors (e.g., electronic, steric) may also be operating in this system, the observed correlation of immunosuppressive activity with substituent π-value suggests that the lipophilicity of this region exerts a significant influence on *in vitro* potency.

It is of interest that the *in vitro* immunosuppressive activity of these compounds does not directly correlate with their ability to bind the major cytosolic receptor, FKBP12, as measured in a competitive binding assay, Table 2.[16] For example, analogs **26** and **34** bind to FKBP12 with equal affinities (ca. 20 nM), while their immunosuppressive activities differ 18-fold. Further, a 13-fold difference exists in the binding EC_{50} values of **34** and **2**, yet they possess equivalent immunosuppressive activity. The enhanced

Figure 1:



immunosuppressive activity of L-683,590 analogs such as **34** is perhaps due to an alteration in cellular penetration, intracellular localization, or even the establishment of a new and favorable interaction with the target protein for this system, calcineurin.[17,10]

In summary, a series of structurally diverse C32-O-alkyl ether analogs of L-683,590 were prepared and evaluated as immunosuppressants in an *in vitro* T cell proliferation assay. This study resulted in the identification of a class of C32-O-cinnamyl ether analogs with *in vitro* potency essentially equivalent to that of the parent natural product. The *in vivo* evaluation of some of these analogs for immunosuppressive efficacy and toxicity will be the subject of a future report.

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11. Satisfactory ^{1}H NMR and mass spectral data were obtained on all reaction products.
12. This phenomenon has been reported previously and termed "reimidation": Danklmaier, J.; Honig, H. *Liebigs Ann. Chem.* **1989**, 665-669. It is noteworthy that reagents such as **6** and **7** could serve a useful purpose for the *acid-catalyzed* formation of 2,2,2-trichloroacetimidates from alcohols, complementing the standard base promoted synthesis. An attempt to use **8** itself as an alkylating agent (allyl alcohol, TfOH (cat.), C_6H_{12}/CH_2Cl_2) gave no reaction.
13. Assay was conducted using murine splenic T cells activated with PMA and ionomycin. In all cases the inhibition observed was reversed by the addition of exogenous IL-2, see: Dumont, F.J.; Staruch, M.J.; Koprak, S.L.; Melino, M.R.; Sigal, N.H. *J. Immunol.* **1990**, *144*, 251.
14. Fujita, T.; Iwasa, J.; Hansch, C. *J. Am. Chem. Soc.* **1964**, *86*, 5175. π is defined: $\pi = \log P_X - \log P_H$; where: P_H is the octanol-water partition coefficient of the parent compound, and P_X is the partition coefficient of the derivative.
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