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C32-AMINO DERIVATIVES OF THE IMMUNOSUPPRESSANT ASCOMYCIN

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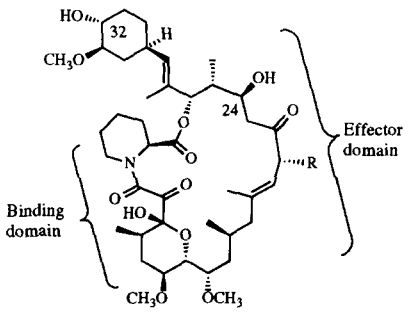
Abstract: Various C32-amino derivatives were investigated as replacements for the C32-hydroxyl group of ascomycin and its C24-deoxy analog. The syntheses of these amino derivatives and their biological evaluation are reported herein. © 1997 Elsevier Science Ltd.

Since the reported discovery of the immunosuppressive agent FK-506 in 1987,[2] FK-506 and its C21-ethyl analog ascomycin (FK-520, **1**) have been the target of extensive scientific research.[3] These naturally occurring tricyclic 21-membered macrocyclic lactones have been shown to possess exceptional immunosuppressive activity and therapeutic potential in the treatment of organ transplant rejection and autoimmunity.[4] However, their poor bioavailability and toxicity[5] prompted us to explore the structure–activity relationships of these structurally intriguing macrocycles.



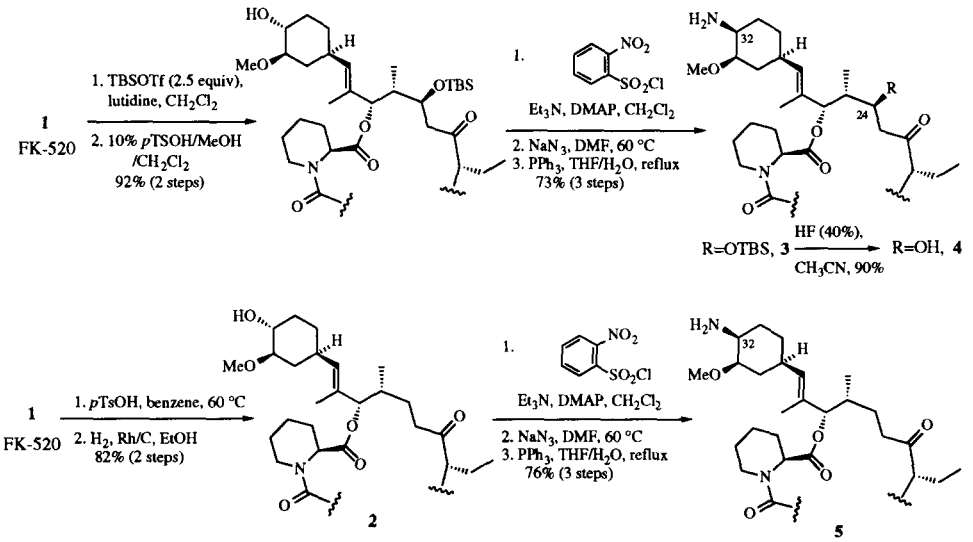
R = allyl FK-506
R = ethyl Ascomycin (FK-520), **1**

Since the isolation of the binding protein for FK-506 (FKBP-12) by affinity chromatography,[6,7] the structure of FKBP-12 has been determined by nuclear magnetic resonance[8–10] and the FK-506/FKBP-12 complex determined by X-ray crystallographic techniques.[11] These studies suggested that these agents are divided into two molecular domains: a binding domain with high affinity for FKBP-12 containing the tricarbonyl region and an effector domain (the right half) that is primarily responsible for its interaction with its biological target, calcineurin. The function of the cyclohexyl moiety is not clearly understood,[12–14] although a recent publication has suggested that appropriate substituents (e.g., an indolyl) at the C32-position of the cyclohexyl may in fact enhance the interaction of the macrolide/FKBP complex with calcineurin.[15,16]

As a part of our research on these structures, we have synthesized a series of amino and amido derivatives at the C32-position and evaluated their immunosuppressive activity in an in vitro T-cell proliferation assay.

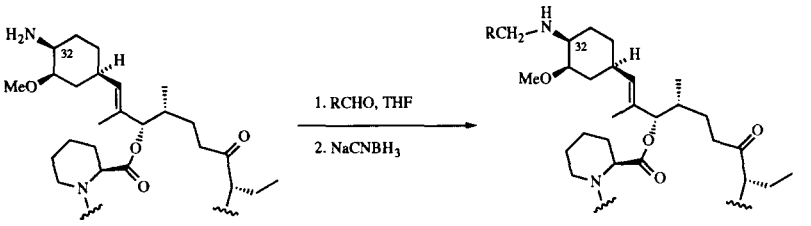
Chemistry: The syntheses of C32-*epi*-amino-ascomycin (**4**) and C32-*epi*-amino-C24-deoxy-ascomycin (**5**) are illustrated in Scheme I. In order to achieve better selectivity for the sulfonylester formation at C32-position, the competing C24-hydroxyl group was first protected as a TBDMS-ether in two steps as follows. Both the C32- and C24-hydroxyl groups were protected as silyl ethers and then the C32-TBDMS group was selectively removed by treatment with *p*-toluenesulfonic acid in methanol. With this C24-OTBDMS ascomycin as a starting material, the C32-hydroxy group was esterified with *o*-nitrobenzenesulfonyl chloride and the resulting sulfonylester was reacted with sodium azide to give the C32-*epi*-azido derivative as a single stereoisomer. Reduction of the azide with triphenylphosphine in aqueous THF followed by removal of the silyl group at C24 gave C32-*epi*-amino ascomycin (**4**).[17] Alternatively, for the preparation of compound **5**, C24-deoxy-ascomycin, **2**, was prepared by a known method[18] and the C32-hydroxyl group was converted to the corresponding amine by the same methodology as illustrated above.

Scheme 1



With C32-*epi*-amino-C24-deoxy-ascomycin (**5**) in hand, a series of alkyl- and arylmethylamino derivatives was prepared by reductive alkylation (Scheme II, compounds **6–12**).[19] In general, amine **5** was reacted with two equivalents of the requisite aldehyde in THF in the presence of powdered molecular sieves (3 Å) and after 1 h of stirring, sodium cyanoborohydride in THF was added to reduce the pre-formed imine. The final compounds were obtained after appropriate deprotection and purified by either preparative TLC or flash silica chromatography (Scheme II).

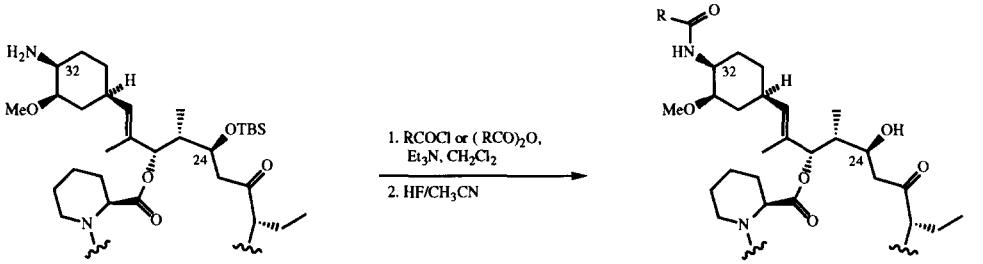
Scheme II



2 6-12

For the preparation of acylamino derivatives, amine **3** was reacted with either the requisite acid chloride (compounds **13–16**) or anhydride (compounds **17** and **18**) in the presence of triethylamine. The final amide products were obtained after hydrogen fluoride mediated deprotection of the silylether (Scheme III).

Scheme III

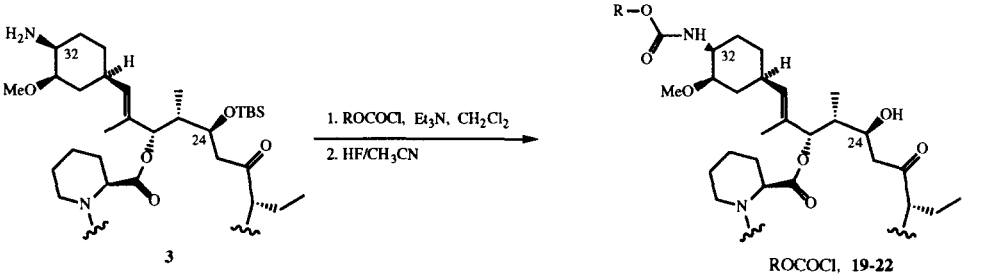


3

RCOCl, **13-16**
$(RCO)_2O$, **17** and **18**

A series of carbamate derivatives **19–22** was prepared by treatment of **3** with an appropriate chloroformate in the presence of triethylamine followed again by deprotection (Scheme IV).

Scheme IV

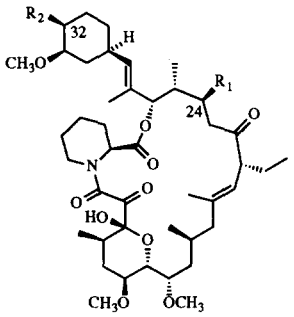


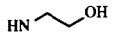
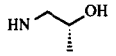
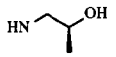
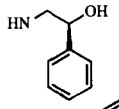
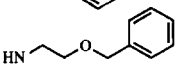
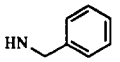
3

ROCOCl, **19-22**

Results and discussion: The compounds prepared in this study were tested in a T-cell proliferation assay[20] and compared to the natural product ascomycin, which bears a C-32 hydroxyl group. Table I illustrates the in vitro immunosuppressive activity of C32-N-alkyl and arylmethyl derivatives. Substitution of C32-hydroxyl group with the epimeric amino moiety led to an approximate ten-fold loss in potency,[21] however, this loss was partially recovered with the removal of the C24-hydroxyl group (compound **5**). The addition of dimethyl, benzyl or 2-hydroxyethyl groups (**6** , **7** and **12**, respectively) attenuated the potency, while the introduction of a *R*-2-hydroxypropyl group (**8**) afforded a slight increase in activity. The *S*-isomer (**9**) was approximately three-fold less active than the *R*-isomer. Replacement of the methyl group in the 2-hydroxypropyl derivative (**8**) with a phenyl moiety (**10**) or masking the hydroxyl group as a benzyloxy group (**11**) decreased the immunosuppressive activity. These results suggest that there is both a stereochemical and steric factor associated with substituents at the C32-position of the cyclohexyl ring.

Table 1: In Vitro immunosuppressant activity of C32-amine derivatives of ascomycin and its C24-deoxy analog (3)

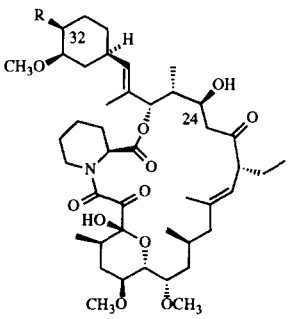


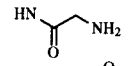
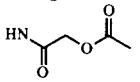
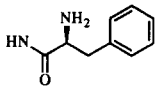
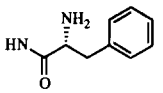
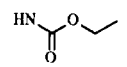
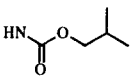
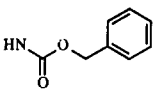
Compound	R_1	R_2	T Cell IC_{50} (nM)
Ascomycin	OH	····OH	0.69
3	H	····OH	0.65
4	OH	NH_2	7.3
5	H	NH_2	2.2
6	H	$N(CH_3)_2$	2.9
7	H		4.4
8	H		1.2
9	H		3.8
10	H		11.4
11	H		13
12	H		3.3

To reduce the basicity of the C32-amino group while retaining its hydrogen bond ability, a series of N-acyl (**13–18**) and carbamate derivatives (**19–22**) were prepared and evaluated for biological efficacy (Table 2). In general amides at this position did not improve activity relative to amine **4**, although formamide **13** did exhibit a 2.5-fold increase in potency. Replacement of the formyl group with an α-aminoacetyl (**14**) or cyclopropylcarbonyl (**16**)

group decreased the activity whereas the acetoxyacetyl group (**15**) retained similar potency in the T-cell assay. It is interesting to note that the addition of a (*R*)-benzyl group at the α-position of aminoacetyl group of **14** (*R*-phenylalanine derivative **17**) enhanced its biological activity five-fold relative to its (*S*)-isomer **18** thus suggesting that potentially the C32-substituent of the macrolide may interact with calcineurin in its tertiary complex with macrolide/FKBP.[13] In an effort to combine the enhanced activity found in **13** and **15**, the acyl moiety was further extended with a series of carbamate derivatives **19–22**. It was found that the carbamates in general exhibit higher potency than that of the parent amine **4** with the exception of *sec*-butylcarbamate derivative **21**.

Table 2: In Vitro immunosuppressant activity of C32-N-acyl and carbamate derivatives of ascomycin



Compound	R	T Cell IC_{50} (nM)
4	NH_2	7.3
13		2.9
14		16.9
15		3.8
16		13
17		3.3
18		16.3
19		2.3
20		3.8
21		10.9
22		5.5

In summary, a series of C32-*epi*-amino derivatives of ascomycin and its C24-deoxy analog was prepared and their immunosuppressive activity evaluated in an in vitro T-cell assay. Replacement of the C32-hydroxyl group

with an epimeric amino group resulted in an attenuated immunosuppressive response which could be partially overcome in the C24-deoxy series. Modification of the amino substituent permitted the identification of analogs (e.g., carbamate **19**) whose immunosuppressive activity approached that of the parent natural product. Introduction of a basic amine group into the C32-position of ascomycin obviously altered the physico-chemical properties of this macrolide. Any effects this change in physical properties has on in vivo efficacy and toxicity will be the subject of a future publication.

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