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Preparation of Functionalized Ethers of Cyclosporin A.

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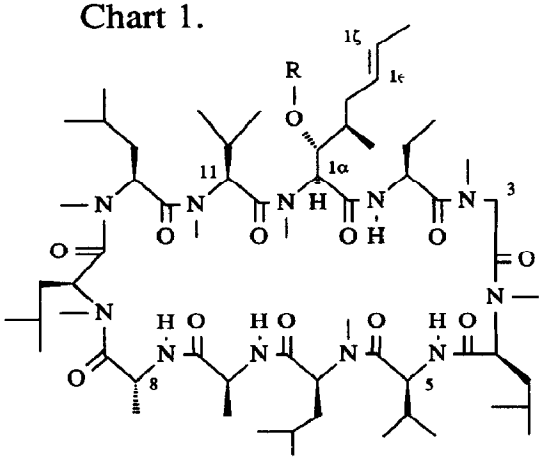
Abstracts: *Cyclosporin A (1) was O-alkylated selectively using phase transfer catalyzed reactions. With allyl chloride, metallyl chloride and propargyl bromide this lead to the ethers 2, 3 and 4, respectively. With t-butyl bromoacetate the product was the ester 5 which was reduced to the alcohol 6. With 2-(bromomethyl-propenoic acid methyl ester the ether 7 was obtained which was converted to 8. The allylic ether alcohol 10 was prepared from 9. This in turn was accessible from 1 and 1-chloro-2-chloromethyl-2-propene.*

Cyclosporin A[1] (1), the active ingredient of Sandimmune®, is a powerful immunosuppressant[2] preventing allograft rejections in animals[3] and humans[4]. It is a natural product[5] also accessible through total synthesis[6]. The mechanism of action[7] is based on the inhibition of the production of lymphokines such as interleukine-2 (IL-2). Mainly these lymphokines are secreted by the activated T helper cells thus stimulating the clonal expansion of activated T cells. These in turn are capable of distinguishing self from non-self in their response against antigens presented to the immune system in association with major histocompatibility complex (MHC) class I or class II gene products. Although it is recognized that cyclosporin A inhibits the transcription of lymphokines, the exact mechanism[8] is not clear. Cyclosporin A binds tightly to cyclophilin[9], the postulated receptor, which in all likelihood is identical with the enzyme peptidyl-prolyl *cis-trans* isomerase[10]. The cyclophilin-cyclosporin A complex in turn binds to and inhibits the Ca^{2+} and calmodulin dependent phosphatase calcineurin[11].

The limited number of functional groups[12], not including the amides present in cyclosporin A, led us to search for possibilities toward selective alkylations of its only hydroxy group. Initial attempts to form ethers with sodium hydride as base in various solvents did not produce tangible results. This was not totally surprising in light of the fact that cyclosporin A required the presence of 4-dimethylaminopyridine even for a simple acetylation of the hydroxy group with acetic anhydride in pyridine[13]. Possibly, the

Chart 1.



	R	mp.	α_D (MeOH)
1)	H		
2)	$CH_2CH=CH_2$	219-220	-201.1
3)	$CH_2C(=CH_2)CH_3$	204-206	-198.2
4)	$CH_2C\equiv CH$	224-225	-192.8
5)	$CH_2COOt\text{-}Bu$		-172.5
6)	CH_2CH_2OH	215-217	-208.9
7)	$CH_2C(=CH_2)COOMe$	136-137	-201.9
8)	$CH_2CH(CH_3)CH_2OH$	200-202	-212.8
9)	$CH_2C(=CH_2)CH_2Cl$	160-162	-196.9
10)	$CH_2C(=CH_2)CH_2OH$	200-202	-188.8

secondary hydroxy group of cyclosporin A is more sterically hindered than would a priory be expected just considering the amino acid 1 (MeBMT, numbering see Chart 1). This might be attributed to the folding of the cyclosporin moiety. In the ground state[14] (NMR and X-ray analysis) this side chain is folded between MeLeu-6 and MeLeu-4 into the cavity of the β-sheet. The presence of other, less populated conformations are detectable by NMR spectroscopy. These conformations can be observed when the spectra of cyclosporin A are measured in solvents or solvent mixtures of various polarities. In general the ground state conformation is predominant in non polar solvents like chloroform while other conformations are observed in more polar solvents like methanol[15]. Possibly, the reactivity of the hydroxy functionality is dependent upon access to one particular, yet unknown, conformation.

We found phase transfer catalyzed reactions[16] to be the only method to achieve O-alkylations of cyclosporin A (1). Our first attempt was the preparation of the allyl ether 2 from cyclosporin A and allyl chloride in a mixture of methylene chloride[17] and 40% potassium hydroxide in the presence of one equivalent of benzyltriethylammonium chloride during 24 hours. The pure amorphous allyl ether 2 was isolated in 55% yield following column chromatography; after crystallization compound 2 was obtained in 25% yield. In the NMR spectrum[18] of 2, the OCH_2 group gave rise to multiplet corresponding to the AB part of an ABX spin pattern. The predominant conformation of 2 in chloroform solution was judged by NMR to correspond to the conformation that was observed for cyclosporin A itself under similar conditions. An X-ray analysis[19] of 2 revealed this to be true for the solid state as well. Thus compound 2 represents the first cyclosporin with an alkylated hydroxy group.

The phase transfer catalyzed alkylation with methallyl chloride gave similar results. The resulting crystalline product 3 was isolated in 55% yield after column chromatography.

The propargyl ether 4 was prepared under similar conditions with propargyl bromide being the alkylating agent. The product was isolated in 13% yield following column chromatography and could be obtained in crystalline form. NMR spectroscopy of a chloroform solution of 4 indicated the presence of 15% of a second conformational isomer.

Treating cyclosporin A similarly with methyl iodide, however, led to a complex mixture of poly methylated products having similar polarities.

Next we studied the reaction of t-butyl bromoacetate with cyclosporin A under phase transfer conditions. Not surprisingly, this alkylating agent required longer reaction times. After 48 hours the desired product 5 was produced in only about 6% yield. Some fractions of the chromatogram of the crude contained secondary alkylation products which were not investigated further. The ester function of 5 was reduced, albeit slowly, to the alcohol 6 in the presence of lithium borohydride. The reduction product was chromatographed over silica gel and then crystallized from ether and pentane to give 6 as a solid.

Methyl bromoacetate with cyclosporin A (1) did not produce reasonable amounts of an alkylation product. On the other hand, higher reaction rates were observed for the alkylation of 1 with methyl 2-(bromomethyl)-acrylate[20]. This reaction was carried out in the presence of 18 mol% of benzyltriethylammonium chloride. After 12 hours, the methyl ester 6 was isolated in 40% yield as a crystalline product following column chromatography over silica gel (54% yield). When 7 was treated with lithium borohydride, concomitant reduction of both the double bond and the ester function led to the alcohol 8. This was obtained in crystalline form despite the presence of a 1:1 mixture of diastereoisomers as evidenced by the NMR spectrum of 8.

When chloro-2-chloromethyl-2-propene served as the alkylating agent the product 9 was isolated in 50% yield as a crystalline material. The chloride in 9 was replaced by acetate in a mixture of sodium acetate in DMF at 110°C in the presence of tetrabutylammonium bromide. The resulting acetate was hydrolyzed directly to the alcohol 10 which was obtained and characterized as a solid.

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