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ISOLATION OF AN ISOMER OF FK-506 FROM FERMENTATION OF *Streptomyces tsukubaensis* AND ITS CHEMICAL SYNTHESIS FROM FK-506

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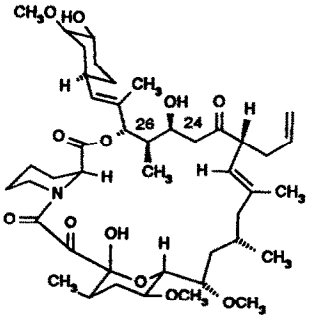
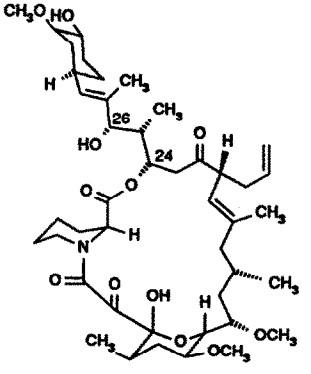
Summary: From the fermentation broth of *Streptomyces tsukubaensis* 9993 an isomer **2a** of the immunosuppressant FK-506 **1a** was isolated as a minor metabolite. From NMR- and FAB-MS data follows that in **2a** the size of the macrocycle is reduced by two carbon atoms due to a shift of the lactone-acyl from O-26 to O-24. The compound was synthesized from FK-506 and proved to be identical with the natural product **2a**.

The macrolide FK-506 (**1a**) is a new powerful immunosuppressant,[1] which has been isolated from the fermentation broth of the soil actinomycete



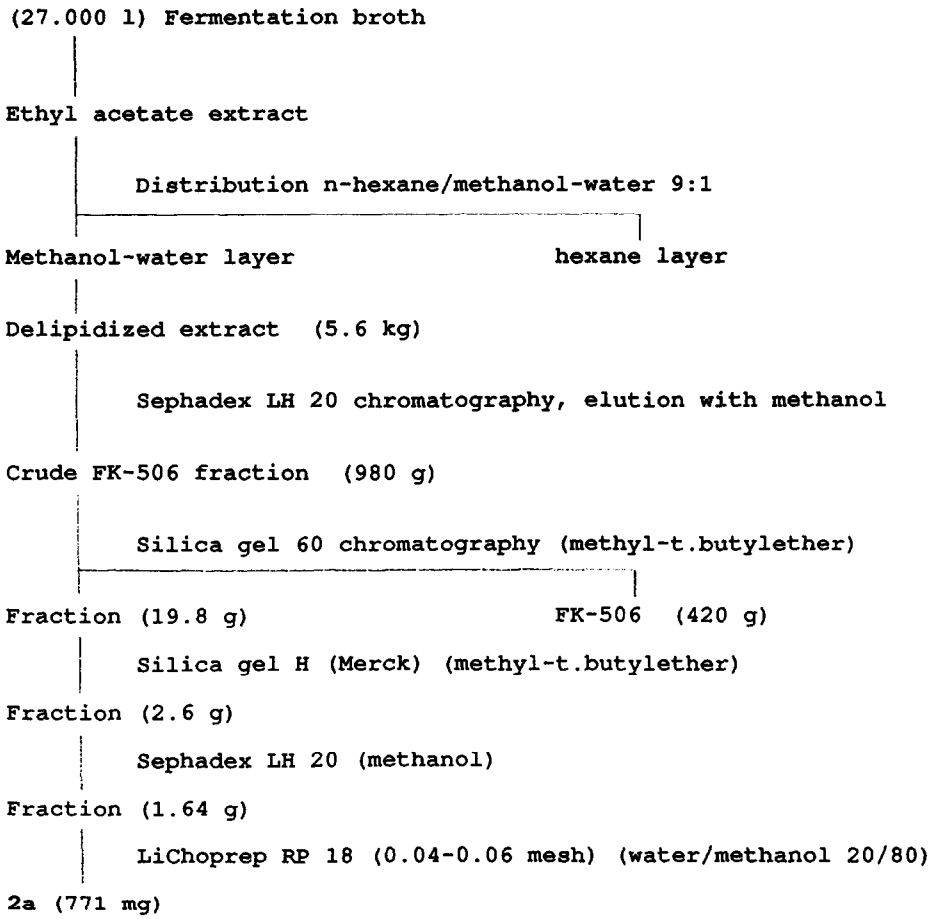
1a *(FK-506)*



2a *(Iso-FK-506)*

Streptomyces tsukubaensis 9993 together with several closely-related minor metabolites.[2] It has a similar mode of action as cyclosporin A, although totally distinct in structure and binding to a different

cytosolic receptor.[3] Currently FK-506 is in broad clinical evaluation for the prevention and treatment of organ transplant rejection.[4] We now report the isolation of a new metabolite **2a** from *S. tsukubaensis*, an isomer of FK-506 wherein the lactone-acyl is connected to the oxyfunction in position 24 instead of 26, and its chemical synthesis, starting from FK-506.[5]



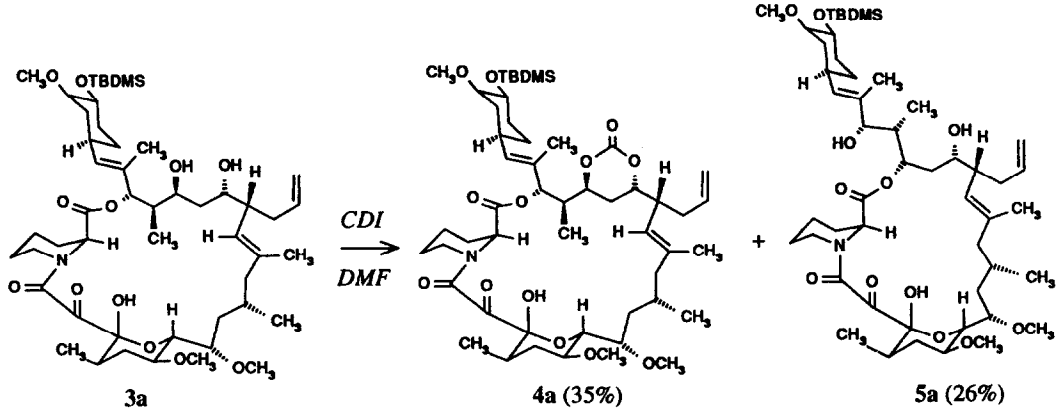
Scheme 1: Isolation of the FK 506 isomer **2a**

During the isolation of FK-506 from the ethyl acetate extract of the fermentation broth of *Streptomyces tsukubaensis* according to Scheme 1, we

obtained side fractions which by HPLC analysis on LiChrosorb RP18 showed the presence of a new unidentified component, having a longer retention time than FK-506 (11.70 min vs 10.66 min for FK-506). The compound was isolated and purified by sequential chromatography on silica gel H, Sephadex LH20 and LiChroprep RP18 (Scheme 1) to give an amorphous colourless material with a melting point of 103-105°C.

As with FK-506 the FAB-MS spectrum of the new metabolite is characterized by peaks at 786 (FK-506: MH^+-H_2O) and 768 (FK-506: MH^+-2H_2O) $^m/_z$ indicating that the compound is an isomer of FK-506. The ^{13}C-NMR spectrum closely resembles the spectrum of FK-506 (comp.table 1) with both, the same total number of C-atom signals and the same characteristic pattern of C(=O)-, C(-O) and C(=C)-signals, being observed. The ^{1}H-NMR spectrum ($CDCl_3/CD_3OD$ 5:1[6]) features a doublet at δ = 3.91 (J = 1.5 Hz). By decoupling experiments it has been assigned to H-26 (FK-506 in $CDCl_3$: H-26 at δ = 5.33). The H-24 signal is found at δ = 5.29 (FK-506: H-24 at δ = 3.94). Thus the H-24 and H-26 signals exchanged positions indicating an acyl-shift from O-26 to O-24.

These data suggest that the new metabolite has the structure **2a**, being an isomer of FK-506 where the size of the macrocycle is reduced by two carbon atoms.



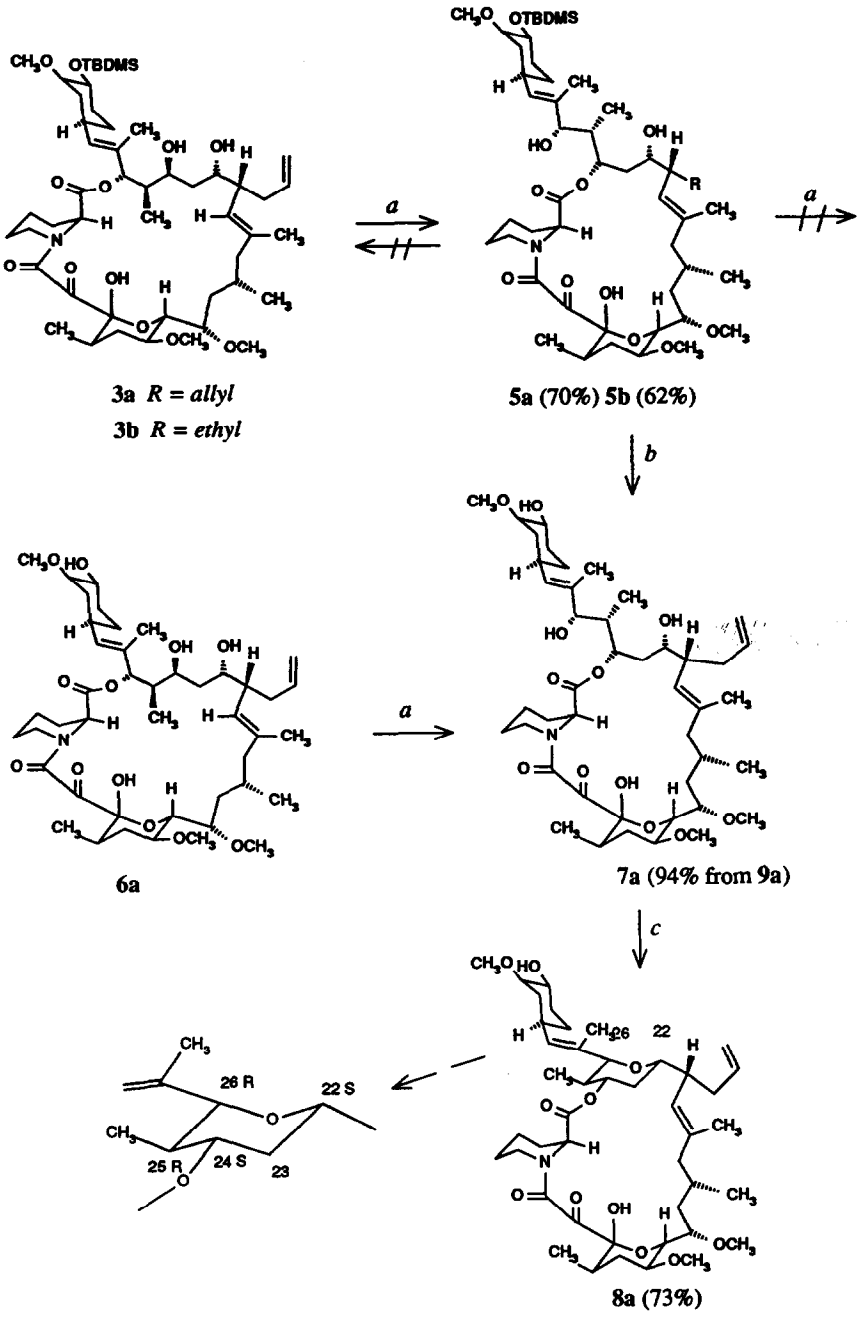
Scheme 2.

To confirm this structure we embarked on the synthesis of **2a** starting

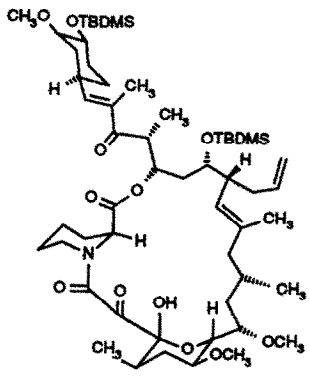
Table 1: ^{13}C- and ^{1}H-NMR data of FK-506 (1a), iso-FK-506 (2a), 33-t.-butyldimethylsilyl-22(S)-dihydro-FK-506 (3a) and 33-t.-butyldimethylsilyl-22(S)-dihydro-iso-FK-506 (5a)

	1a[a]		2a[b]		3a[a]		5a[c]	
Position	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	168.9		168.9		169.1		170.8	
2	56.5		56.4		56.9	4.67	56.3	5.22
3	27.5		26.3		27.3		28.0	1.94
								2.20
4	21.0		20.7				21.8	1.35
								1.80
5	24.4		24.9		24.6		24.5	1.45
								1.75
6	39.1		38.2				39.4	2.90
						4.43		4.48
8	164.6		162.3		165.0		165.3	
9	196.1		196.5		196.1		196.5	
10	97.0		99.8		96.9		96.2	
11	34.9		33.9				34.6	
11Me	16.1		16.5		16.2		16.3	
12	32.5		33.5		32.4		32.7	1.46
13	73.5		73.5		73.8		73.5	3.35
13OMe	56.2							
14	72.7		71.3		72.6	3.63	72.3	3.67
15	75.1		74.0		75.3	3.58	74.9	3.57
15OMe	56.7							
16	32.9		31.6		32.8		31.5	0.95
								1.40
17	26.1		26.6		26.5		26.1	1.60
17Me	20.3		20.3		20.6		20.7	0.90
18	48.5		50.5		49.3		49.6	1.70
								2.16
19	138.8		139.7		136.1		135.9	
19Me	15.8		16.5		15.3		15.3	1.60
20	122.4		123.7		126.3		127.5	4.80
21	52.7		53.3		43.7		43.8	2.71
22	212.4		211.6		71.8		69.7	3.06
23	43.4		41.2	2.65			34.7	
				3.17				
24	69.8	3.94	71.5	5.29	70.4	3.96	73.2	5.22
25	39.7		37.2	2.02	40.1		40.2	1.80
25Me	9.4		8.2		8.9		8.9	0.96
26	77.3	5.33	77.3	3.91 D	77.0	5.31	78.3	3.86
				1.5 Hz				
27	13.9		14.1		14.5		12.3	1.60
28	132.2		136.3		132.2		134.5	
29	129.8		127.8		128.6		132.3	5.15
30	34.7		35.0				35.1	2.26
31	34.8		35.1				36.5	0.95
								1.95
32	84.1		84.4		84.1		84.3	2.95
32OMe	56.5							
33	73.4		73.4		75.2		75.2	3.40
34	31.2		31.4		33.7		33.9	1.36
								1.88
35	30.5		31.0		30.9		30.8	1.06
								1.60
36	35.1		36.6				32.3	1.84
								2.46
37	135.5		135.1		137.5		137.8	5.72
38	116.5		116.8		115.6		115.0	4.92
								4.99

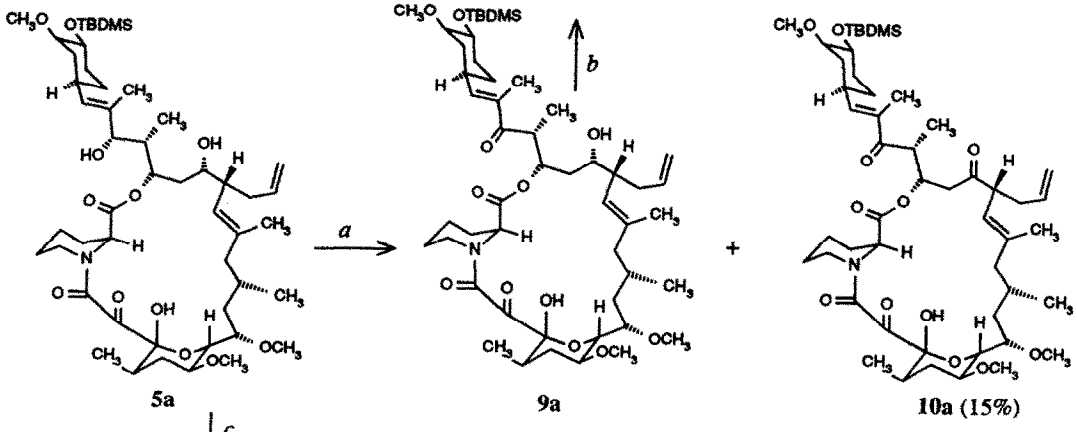
a) In $CDCl_3$, major conformer; b) In $CDCl_3/CDOD_3$ 5:1; c) In $CDCl_3$.



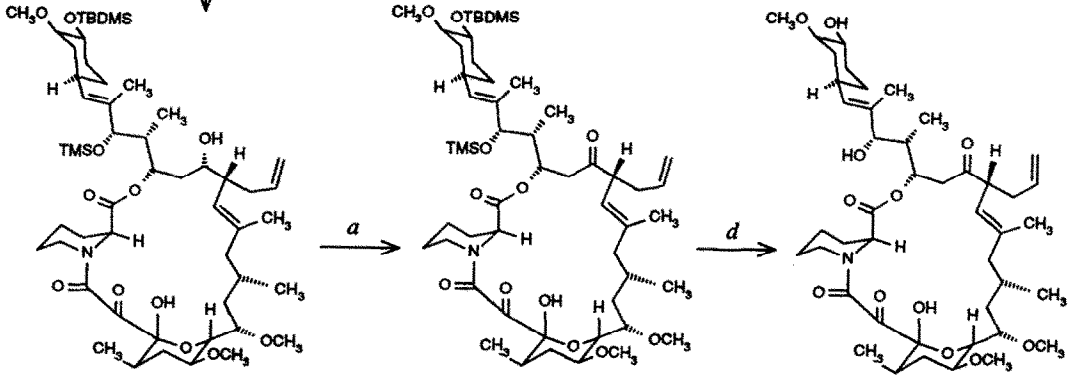
Scheme 3. Reaction conditions: *a Im (or DMAP, Py), DMF; b 2%HF, CH_3CN; c pTsOH, toluene.*



11a (47%, from 5a)



5a **9a** **10a (15%)**



12a (57%) **13a (69%)** **2a (88%)**

Scheme 4. Reaction conditions: *a NMO, TPAP, 4Å mol sieve, CH_2Cl_2; b $^tBuMe_2SiOTf$, lutidine; c Me_3SiOTf, lutidine; d 1N HCl, CH_3CN.*

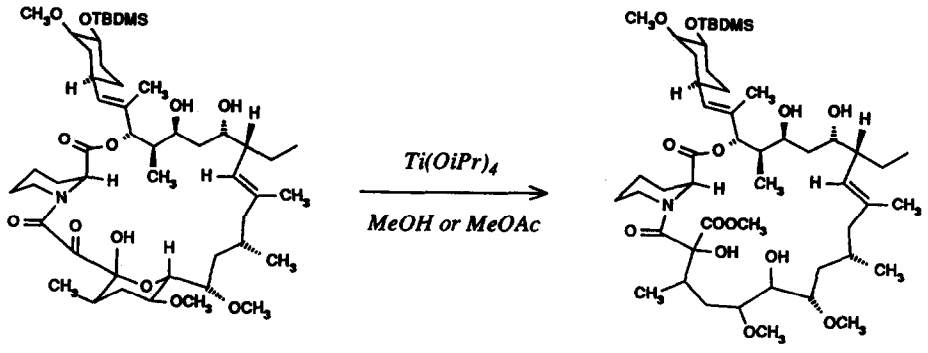
from FK-506 (1a). We had already earlier detected independently, that a compound of the same structural type as 2a is formed in a reaction of a 22-dihydro-FK-506 derivative: When 33-O-silylated 22-dihydro-FK-506 (3a) was treated with carbonyldiimidazole to give the cyclic carbonate 4a a byproduct was obtained which was identified as the 22-dihydro-iso-FK-506 derivative 5a (Scheme 2). As in the case of FK-506 and 2a the ^{13}C-NMR spectra of 3a and 5a strongly resemble each other (Table 1). In $CDCl_3$ only signals of one single conformation (cis-amide: δ C-2 56.3 ppm, C-6 39.4 ppm) are seen. In the ^{1}H-NMR spectrum of 5a as for 2a a doublet appears at δ = 3.86 (J = 6Hz), which is identified as the H-26 signal by decoupling experiments (coupling with H-25, δ = 1.80). The signal of H-24 is located at δ = 5.22 and superimposed on the H-29 signal. A 2D-CH-correlation study confirmed these assignments (data not shown). Since 3a is the major isomer obtained from 33-O-silylated FK-506 (14a) by reduction with triacetoxyborohydride (Scheme 6), the configuration at C-22 in 3a and 5a was assumed to be S.[5,7]

Anticipating that the 1,3-acyl shift had been induced by imidazole generated during the carbonylation reaction, we treated 33-O-silylated 22-dihydro-FK-506 3a with imidazole in DMF at 50°C. 5a was formed in a slow reaction but without major byproducts. Work up after seven days gave a 62% yield of 5a together with unchanged 3a (Scheme 3). The reaction could in principle also be carried out with unprotected dihydro-FK 506 (6a), but the chromatographic isolation of the product proved to be more difficult due to smaller differences in the r_F-values. 4-Dimethylpyridine (DMAP) could be used instead of imidazole. With stronger nonnucleophilic bases like diazabicycloundecane (DBU) additional reactions occurred, on which we will report separately.[8] Interestingly with the dihydro derivative 3b, the 21-ethyl analogue of 3a, the base catalyzed acyl shift is proceeding even slower: only 47% yield of 5b and 47% recovered starting material were isolated after seven days with DMAP in DMF at 50°C as compared to 70% yield of 5a from 3a under identical conditions.

Selective oxidation of the 22-oxyfunction in 5a to carbonyl failed: Treatment of 5a with N-methyl-morpholine-N-oxide (NMO) and tetrapropyl-ammonium-perruthenate (TPAP) or with periodinane[9] (Dess-Martin reagent) resulted in preferential oxidation of the 26-oxyfunction. A mixture of the 26-carbonyl- 9a and the 22,26-dicarbonyl-derivative 10a was obtained which could be separated only after silylation of the remaining oxyfunction in 9a to give 11a (Scheme 4). The problem was solved by selective protection of the 26-OH group of 5a, which could be achieved

with trimethylsilyltriflate/lutidine in dichloromethane at -70°C, whereas with bis-trimethylsilylacetamide in toluene the 22- and 26-O-silylated products were obtained in about equal amounts. Oxidation of the protected intermediate **12a** with NMO/TPAP and subsequent deprotection yielded **2a** which proved to be identical with the fermentation product upon TLC and ^{1}H-NMR comparison.[10]

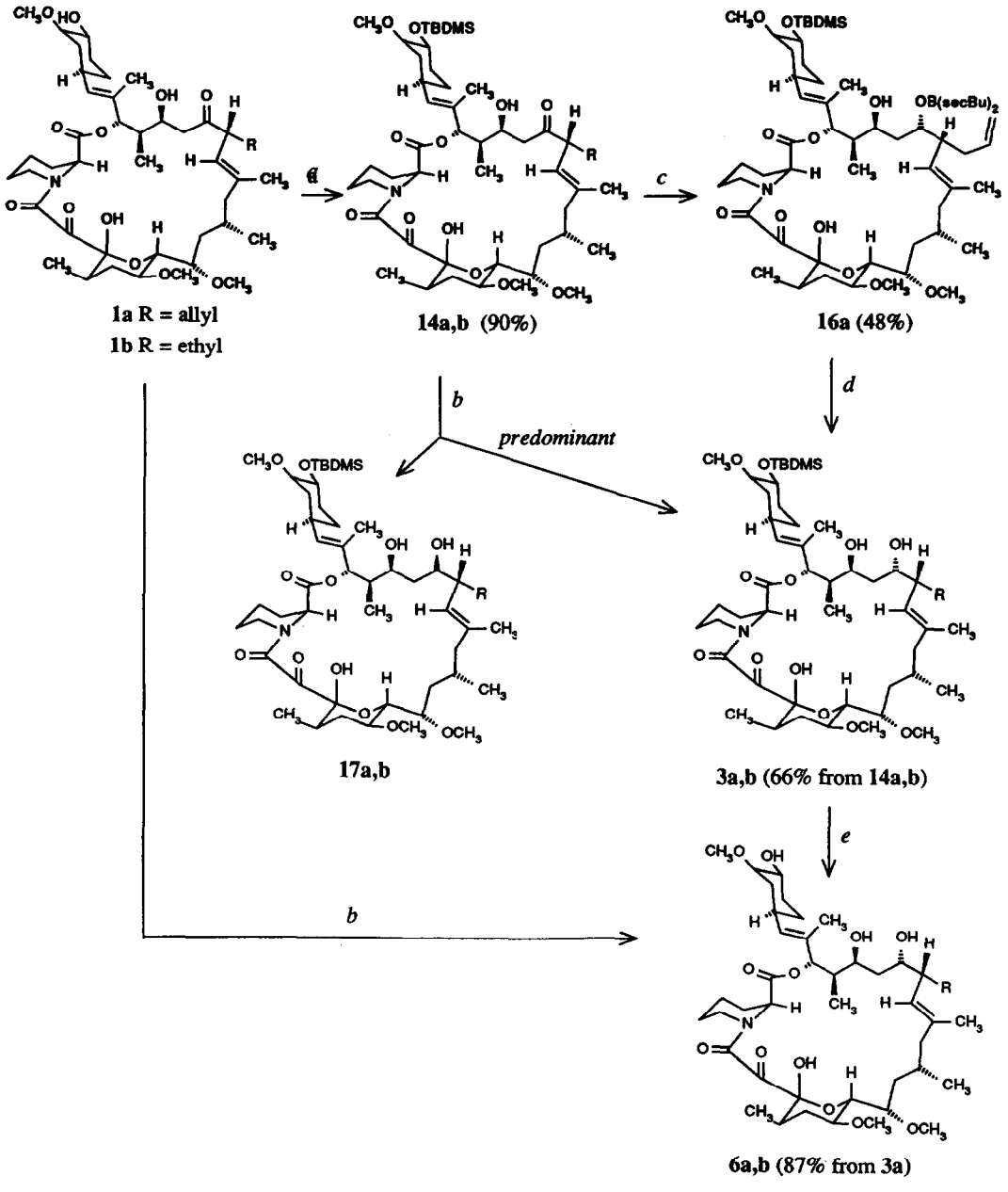
All attempts to isomerize FK-506 (**1a**) or its 21-ethyl analogue (**1b**) directly to **2a,b** were unsuccessful. FK-506 or its 33-O-tert.butyldimethylsilyl derivative **14a** did not rearrange when heated with DMAP in DMF.[11] No reaction was observed either with **1b** in the presence of tetraisopropoxy-titanium in THF or dichloromethane. When intermolecular transesterification of **14b** to the methylester was attempted (tetraisopropoxytitanium/methanol or -/methyl acetate) benzilic acid rearrangement to **15b** took place instead,[12] presumably as result of an attack of methyltitanate at the 9-carbonyl of **1b** (Scheme 5).



14b **15b**

Scheme 5.

With the 22-dihydro-iso-FK-506 derivatives of **5a,b** no further acyl shift to O-22 or backshift to O-26 was observed when they were heated with imidazole or DMAP in DMF at 50°C. Under acidic conditions, i.e. p-toluenesulfonic acid in toluene, cyclisation of **5a** or **7a** to the 22,26-epoxide **8a** occurred (Scheme 3). From an ^{1}H-NMR analysis it follows, that all substituents on the newly formed tetrahydropyran ring of **8a** are in equatorial position. It is assumed that the configuration at C-25 remaines unchanged throughout the series of reactions starting from FK-506. Therefore the configurations at the other positions on the



Scheme 6. Reaction Conditions: *a* $^tBuMe_2SiCl$,*Im*,*DMF*; *b* $Me_4NBH(OAc)_3$ *HOAc*/CH_3CN; *c* $LiBH(secBu)_3$, *THF*; *d* $NaBO_3$, *THF*; *e* 2%*HF*, CH_3CN.

tetrahydropyran moiety follow to be as shown in Scheme 3. This confirms, that the major product from the reduction of FK-506 with the Evans reagent (Scheme 6) is the anti-(S)-diastereomer **3a**.[5,7b]

Furthermore **3a** was compared with the product obtained by reduction of FK-506 with Lithiumselectride, for which the 22(S) configuration has been assigned.[7] In our hands the reaction of FK-506 with Lithiumselectride in THF at -70°C gave mainly a boronic acidester **16a**[13] together with small amounts of **3a**. The unsubstituted dihydro derivative was obtained from **16a** by treatment with sodiumperborate and was shown to be **3a** by TLC- and ^{1}H-NMR comparison.

EXPERIMENTAL

^{1}H- and ^{13}C-NMR-spectra were recorded at 250 MHz (Bruker WM 250). The concentration was about 20mg in 0.5ml $CDCl_3$. The solvent was used as internal standard (δ $CDCl_3$ = 77.0). SF = 62.9 MHz, SW = 15000 Hz, SI = 32k, AQ = 1.081 sec, PW = 6 sec (corresponding to a 60° pulse), NS = 40000, EM with LB = 1. Proton BB decoupled with DP = 10H.
C-H correlation spectra were recorded with the Bruker standard program XHCORRDC.AU. SW2 = 6000Hz, SI2 = 4K, SW1 = 700 Hz, NE = 64 a 512 scans, before Fourrier transformation filled with zero's to SI1 = 256 and in both dimensions multiplied with a pi/4 shifted line bell window function.

33-O-tert.Butyldimethylsilyl-22(S)-dihydro-FK-506 (3a)

a) By reduction with tetramethylammonium-triacetoxyborohydride. To 33-O-tert.butyldimethylsilyl-FK-506 (**14a**)[1a,14] (23.4g, 25.5 mmol) in 220ml acetonitrile and 80ml acetic acid a solution of tetramethylammonium-triacetoxyborohydride (7.37g, 28 mmol) in 25ml acetonitrile was added dropwise with stirring at -5°C. After 5 h stirring at -5 to 0°C the reaction was quenched by addition of water. The mixture was concentrated in vacuum and the residue partitioned between dichloromethane and water. The organic layer was dried ($MgSO_4$) and the solvents evaporated. Chromatography of the residue (toluene/ethyl acetate 1:1) gave 15.6g (66%) **3a** along with 2.1g starting material and 1g of a fraction containing starting material and the 22(R)-isomer **17a**.

3a: NMR data see table 1. FAB-MS (m/z): 902 (MH^+-H_2O), 884 ($-H_2O$), 578, 568, 266.
17a: ^{13}C-NMR ($CDCl_3$), two conformers, signals of the major conformer, δ 169.1 (C-1), 165.8 (C-8), 136.2 (C-19), 125.5 (C-20), 98.7 (C-10), 43.3 (C-21), 36.8 (C-23).

b) By reduction with Lithiumselectride. A solution of Lithiumselectride (0.57g ,3 mmol) in 3ml THF was added dropwise to 33-O-tert.butyldimethylsilyl-FK-506 (**14a**) (1.83g ,2 mmol) in 3ml THF stirred at -70°C. After 2 h at -70°C the reaction was quenched by addition of saturated aqueous NH_4Cl and the mixture extracted with dichloromethane. The extract yielded after drying ($MgSO_4$) and evaporation of the solvent upon chromatography of the residue (toluene/ethyl acetate 4:1) 1.67g (48%) **16a** and 0.08g **3a**.
16a ^{1}H-NMR ($CDCl_3$): δ 5.07 (s,br H-26) 4.70 (broad d, J=4Hz, H-2e) 4.45 (d,br J=13Hz, 6e) 4.06 (m, H-22) 3.85 (m, H-24) 3.67 (d, J=8Hz, H-14) 3.61 (dd, J=2.5Hz, J=11Hz, H-15) 2.96 (m, H-32). ^{13}C-NMR ($CDCl_3$): δ 196.04 (C-9), 169.16 (C-1), 165.24 (C-8), 137.23 (C-37), 136.15 (C-19), 131.07 (C-28), 129.29 (C-29), 125.68 (C-20), 115.74 (C-38), 96.43 (C-10), 84.13 (C-32), 77.13 (C-26), 75.20 (C-33), 74.86 (C-15), 73.65 (C-13), 72.64 (C-14), 70.37 (C-24), 69.50 + 69.48 (C-22), 58.12 (OMe), 56.98 (OMe), 56.70 (C-2), 56.36 (OMe), 49.62 (C-18), 43.07 (C-21), 40.67 (C-25), 39.41 (C-6), 36.69 (C-23), 36.23 (C-31), 34.90 (C16,C-30), 34.69 (C-11), 33.77 (C-36), 33.31 (C-12,C-34), 30.94 (C-35), 27.69 (C-3), 26.36 (C-17), 26.23 + 26.16 (B-C-CH_2?), 25.86 (Si-^{t}Bu), 24.53 (C-5), 21.57 (C-4), 20.24 (C-17Me), 18.14 (Si-C), 16.34 (C-11Me), 15.71 (C-19Me), 15.30 + 15.24 (B-C-CH_3?), 14.60 (C-28Me), 13.53 + 13.43 (B-C-C-CH_3?), 10.02 (C-25Me), -4.52 (SiMe), -4.73 (SiMe). FAB-MS (m/z): 1112, 1094, 1076; calc. for $C_{58}H_{102}BNO_{12}Si$: 1043.
Treatment of **16a** (110mg, 0.1 mmol) with sodium perborate (45mg, 0.3 mmol) in 2ml of a 1:1 mixture of THF and water for 2h gave after chromatographic purification (toluene/ethyl acetate 2:1) 50mg (54%) **3a**.

33-O-tert.Butyldimethylsilyl-22(S)-dihydro-FR-900520 (**3b**) was similarly prepared from 33-O-tert.butyldimethylsilyl-FR-900520 (**14b**) by reduction with tetramethylammonium-triacetoxyborohydride in 66% yield. ^{1}H-NMR ($CDCl_3$), two conformers (3:2), signals of the major conformer (cis-amide): δ 5.30 (s, H-26), 4.65 (d, J=5Hz, H-2), 4.53 (s, 10-OH), 4.42 (d,br, J=14Hz, H-6e), 3.62 (dd, J=1Hz, J=4.5Hz, H-14), 3.58 (m, H-15). ^{13}C-NMR ($CDCl_3$), signals of the major conformer: δ 196.1 (C-9), 169.2 (C-1), 165.1 (C-8), 136.4 (C-19), 132.2 (C-28), 128.6 (C-29), 127.3

(C-20), 97.0 (C-10), 84.1 (C-32), 75.4 (C-15), 75.2 (C-33), 73.8 (C-13), 72.7 (C-14), 71.9 (C-22), 71.0 (C-24) 58.1 (OMe), 57.0 (OMe), 56.9 (C-2), 56.3 (OMe), 49.5 (C-18), 46.2 (C-21), 40.1 (C-25), 39.4 (C-6), 36.7 (C-23), 34.8 (C-30), 33.8 (C-34), 32.8 (C-16), 32.6 (C-12), 31.0 (C-35), 27.3 (C-3), 26.7 (C-17), 25.9 (Si-^{t}Bu), 24.6 (C-5), 23.7 (C-36), 21.3 (C-4), 20.7 (17Me), 16.3 (11Me), 15.4 (19Me), 14.6 (C-27, 12.5 (C-37, 9.2 (25Me).

22(S)-Dihydro-FK-506 (6a)

a) From FK-506 with tetramethylammonium-triacetoxyborohydride. The same procedure as above can also be applied to reduce unsubstituted FK-506. 80mg crude product, containing small amounts of starting material and a more polar compound as side components (TLC, ethylacetate), was obtained from 80mg FK 506. However the purification of the product is much more tedious as compared to the silylated derivative. In one chromatographic purification step (ethylacetate) only 20mg (25%) pure **6a** could be isolated.
^{1}H-NMR ($CDCl_3$), two conformers (3:2), signals of the major conformer: δ 1.62 (H-23a), 1.25 (H-23b), 3.92 (H-24), 1.90 (H-25), 5.30 (H-26).
^{13}C-NMR ($CDCl_3$): δ 195.9 (C-9), 169.3 (C-1), 164.9 and 165.4 (C-8), 137.4 and 136.9 (C-37), 136.3 and 135.7 (C-19), 126.2 and 126.8 (C-20), 97.0 and 98.4 (C-10), 71.7 and 70.9 (C-22), 43.9 and 44.1 (C-21), 36.8 and 36.9 (C-23).

b) From 33-O-tert.butyldimethylsilyl-22(S)-dihydro-FK-506 bydeprotection. 33-O-Tert.butyldimethylsilyl-22(S)-dihydro-FK-506 (**3a**) (276mg, 0.3 mmol) in 2ml acetonitrile was treated with 40% aqueous HF (20µl) for 1h at 10°C. The volatile components were evaporated in vacuum and the residue purified by chromatography (dichloromethane/ethanol 95:5) to give 241mg (87%) **6a**.

33-O-tert.Butyldimethylsilyl-22(S)-dihydro-FK-506-22,24-cyclic carbonate (4a) and 33-O-tert.butyldimethlysilyl-22(S)-dihydro-iso-FK-506 (5a)

33-O-tert.Butyldimethylsilyl-22(S)-dihydro-FK-506 (**3a**) (459mg, 0.5 mmol) was treated with carbonyldiimidazole (85mg, 0.5 mmol) in benzene (5ml) 12h at 50°C. The solvent was evaporated and the residue fractionated by chromatography (toluene/ethyl acetate 3:1) to give 165mg (35%) **4a** and

120mg (26%) **5a**.

4a: ^{1}H-NMR ($CDCl_3$): δ 5.14 (H-26), 5.07 (H-20), 4.58 (H-22), 4.40 (H-24), 2.57 (H-21), 2.0 (H-25), 1.98 (H-23), 1.82 (H-23'). ^{13}C-NMR ($CDCl_3$): δ 196.2 (C-9), 169.1 (C-1), 164.9 (C-8), 138.2 (C-19), 135.7 (C-37), 131.0 (C-29), 129.9 (C-28), 123.4 (C-20) 117.1 (C-38), 97.0 (C-10), 84.1 (C-32), 77.8 (C-26), 75.4 (C-15), 75.0 (C-33), 73.6 (C-13), 72.7 (C-14), 56.3 (C-2), 49.5 (C-18), 42.9 (C-21), 39.8 (C-25), 39.7 (C-6), 36.4 (C-23), 36.1 (C-36), 34.9 + 34.8 (C-30, C-31), 33.9 (C-11), 33.7 (C-16), 32.8 (C-12), 30.8 (C-25), 26.1 (C-17), 21.2 (C-4), 20.0 (17Me), 18.1 (Si^{t}Bu), 14.5 (C-27), 10.5 (25Me). FAB-MS (m/z): 946 (MH^+), 928 ($-H_2O$), 702, 266.

5a: NMR data ($CDCl_3$) see table 1. FAB-MS (m/z): 902 (MH^+-H_2O), 884 ($-H_2O$), 866 ($-H_2O$), 572, 266.

33-O-tert.Butyldimethylsilyl-22(S)-dihydro-iso-FK-506 (5a)

A solution of 33-O-tert.butyldimethylsilyl-22(S)-dihydro-FK-506 (3a) (1g,1.09 mmol) and 4-dimethylaminopyridine (133mg,1.09 mmol) in 10ml DMF was stirred 7 days at 50°C. The solvent was evaporated in vacuum and the crude product purified by chromatography (toluene/ethyl acetate 1:1) to give 0.7g (70%) **5a**. When imidazole was used instead of 4-dimethylamino-pyridine **5a** was obtained in 62% yield after 7 days at 50°C.

33-O-tert.butyldimethylsilyl-22(S)-dihydro-iso-FR-900520 (5b)

The same procedure was used as for the preparation of **5a** from **3a**, but 33-O-tert.butyldimethylsilyl-22(S)-dihydro-FR-900520 reacted slower as compared to the FK 506 analogue: 47% product together with 47% starting material were isolated after 7 days and 62.5% product together with 22% starting material after 10 days stirring with 4-dimethylaminopyridine in DMF at 50°C. 1H-NMR ($CDCl_3$): δ 5.15-5.22 (m, H-24, H-29), 5.0 (d,br J=3Hz, H-2), 4.75 (d, J=10Hz, H-20), 4.44 (d,br J=13Hz, H-6e), 4.10 (s,br OH), 3.84 (d, J=6Hz, H-26), 3.66 (d, J=9Hz, H-14), 3.59 (dd, J=11Hz, J= 2.5Hz, H-15), 3.42 (s) + 3.39 (s) + 3.29 (s) (OCH_3), 0.90 (s, Si^{t}butyl), 0.08 (s) + 0.07 (s) ($Si-CH_3$). FAB-MS (m/z): 890 (MH^+-H_2O), 872 ($-H_2O$), 560, 266.

22(S)-Dihydro-iso-FK-506 (7a)

a) From 33-O-tert.butyldimethylsilyl-22(S)-dihydro-iso-FK-506 (5a). 33-O-tert.Butyldimethylsilyl-22(S)-dihydro-iso-FK-506 (5a) (400mg, 0.43 mmol) in 10ml THF was treated with 8ml 1N HCl 30min at room temp. The mixture was neutralized with aqueous $NaHCO_3$ and extracted with diethyl ether. The extract was dried ($MgSO_4$) and the solvent was evaporated to give 325mg 7a (94%). NMR data: comp. to 5a (tab.1). FAB-MS (m/z): 788 (MH^+-H_2O), 770 (H_2O), 572, 266.

b) From 22(S)-dihydro-FK-506 (6a) (qualitative). Reacting 22(S)-dihydro-FK-506 (20mg, 0.03 mmol) with 3mg 4-dimethylamino-pyridine in 0.5ml DMF at 50°C for 16 h. leads to a product (educt/product ~3:1) which was identified as 5a by TLC comparison.

22,26-Epoxy-22(S)-dihydro-iso-FK-506 (8a)

22(S)-Dihydro-iso-FK-506 (120mg, 0.13mmol) and a few mg of p-toluene-sulfonic acid in 2ml toluene were stirred for 24h at 60°C. The reaction mixture was filtered and the solvent was evaporated. Chromatography (ethylacetate) of the residue yielded 86mg (73%) 8a. ^{1}H-NMR ($CDCl_3$): δ 4.58 (dd, J=10.2, J=10.4Hz, H-25, 3.33 (m, H-22), 3.19 (d, J=10.2Hz, H-26), 4.58 (dd, J=10.4, J=5.4Hz, H-24), 1.68 (ddd, J=5.4, J=12.0, J=2Hz, H-23e), 1.35 (ddd, J=10.4, J=12.0, J= 10.0Hz, H-23a). FAB-MS (m/z): 788 (MH^+), 770 (-H_2O), 572, 266.

22,33-O,O'-Bis-tert.butyldimethylsilyl-22(S)-dihydro-26-oxo-iso-FK-506 (11a) and 33-O-tert.butyldimethylsilyl-26-oxo-iso-FK-506 (10a)

33-O-tert.Butyldimethylsilyl-22(S)-dihydro-iso-FK-506 (5a) (254mg, 0.28 mmol) was treated with N-methyl-morpholine-N-oxide (71mg, 0.6 mmol) and a few mg tetrapropylammonium-perruthenate in 4ml dichloromethane in the presence of a 4A molsieve. After 1h stirring at room temp. the mixture was filtered and the solvent evaporated. The residue was purified by flash chromatography (toluene/ethyl acetate 4:1) and dissolved in 6ml dichloromethane. tert.Butyldimethylsilyltriflate (180mg, 0.75 mmol) and lutidine (90µl, 0.8 mmol) were added at 0°C and the mixture stirred overnight at RT. Routine workup gave 234mg crude product from which 135mg (47%) 11a and 38mg (15%) 33-O-tert.butyldimethylsilyl-26-oxo-iso-FK-506 (10a) were isolated by chromatographic separation (toluene/ethyl acetate 5:1).

11a ^{1}H-NMR ($CDCl_3$): δ 4.61 (H-2), 4.42 (H-6e), 3.11 (H-6a), 3.64 (d, J=9Hz, H-14), 3.59 (dd, J=11Hz, J=2.5Hz, H-15), 4.80 (d, J=9Hz, H-20), 5.07 (dd, J= 10Hz, J=2.5Hz, H-24), 6.24 (d, J=9Hz, H-29), 5.67 (ddt, J=17Hz, J=10Hz, J=7Hz, H-27), 4.97 (H-38tr), 4.91 (H-38cis), 3.40 + 3.38 + 3.28 (OCH_3).
10a ^{1}H-NMR ($CDCl_3$): δ 3.58 (d, J=9Hz, H-14), 2.93 (H-23a), 2.57 (H-23b) ($J_{23a/23b}$=17.5Hz, $J_{23a/24}$=9Hz, $J_{23b/24}$=2.5Hz), 5.55 ($J_{24/25}$, H-24), 3.70 (H-25), 1.07 (d, J=7Hz, 25-CH_3), 6.37 (d, J=9Hz, H-29).

33-O-tert.Butyldimethylsilyl-26-oxo-iso-FK-506 (10a) by oxidation of 5a with Dess-Martin reagent

Pyridine (32mg, 0.4mmol) and freshly prepared periodinane (84.8mg, 0.2mmol) were added to a solution of 33-O-tert.butyldimethylsilyl-22(S)-dihydro-iso-FK-506 (**5a**) (138mg, 0.15mmol) in 10ml dichloromethane and the mixture was stirred for 15min at room temp. After cooling to 0°C 2ml of saturated aqueous Na_2SO_3 were added. The organic layer was separated, dried ($MgSO_4$) and the solvent was evaporated. Upon chromatography on silica gel (toluene/ethylacetate 4:1) 57mg (41%) **10a** were obtained together with 60mg of a fraction which was a mixture of **10a** and the 26-oxo-22-dihydro-derivative **9a**.

33-O-tert.Butyldimethylsilyl-22(S)-dihydro-26-O-trimethylsilyl-iso-FK-506 (12a)

a) With trimethylsilyltriflate. Trimethylsilyltriflate (0.56g, 2.5 mmol) was slowly added at -70°C to a solution of 33-O-tert.butyldimethylsilyl-22(S)-dihydro iso-FK-506 (**5a**) (1.81g, 2mmol) and 2,6-lutidine (0.85g, 8 mmol) in 160ml dichloromethane and the mixture was stirred for further 30min. Aqueous $NaHCO_3$ was added and the organic layer separated, washed with water and brine, dried ($MgSO_4$) and evaporated. Chromatography of the residue (toluene/ethylacetate 10:1 to 4:1) gave 349mg (17%) 22,26-O,O'-bis(trimethyl-silyl)-33-O-tert.butyldimethylsilyl-22(S)-dihydro-iso-FK-506 and 1.11g (57%) (**12a**). ^{1}H-NMR ($CDCl_3$): δ 5.71 (ddt, J=17Hz, J=10Hz, J=7Hz, H-37), 4.9-5.15 (5H: H-2, H-24, H-29, H-38tr, H-38cis), 4.80 (d, J=9Hz, H-20), 4.52 (H-6e), 3.96 (d, J=1Hz, 10-OH), 3.55-3.70 (3H: H-14, H-15, H-26), 3.44 + 3.39 + 3.29 (3 OCH_3).

b) With bis-(trimethylsilyl)-acetamide. 33-O-tert.Butyldimethylsilyl-

22(S)-dihydro-iso-FK-506 (5a) (322mg, 0.35 mmol) was treated with bis-(trimethylsilyl)-acetamide (142mg, 0.7 mmol) in 0.5ml toluene for 1 h at room temp. After quenching with water and extraction with toluene the organic solution was dried and the solvent evaporated. Upon chromatography of the residue (toluene/ethylacetate 4:1) 80mg (23%) **12a** was obtained along with 75mg (22%) 33-O-tert.butyl-22(S)-dihydro-22-O-trimethylsilyl-iso-FK-506 and 160mg (43%) 22,26-O,O'-bis(trimethylsilyl)-33-O-tert.butyldimethylsilyl-22(S)-dihydro-iso-FK-506.
33-O-tert.butyl-22(S)-dihydro-22-O-trimethylsilyl-iso-FK-506:
^{1}H-NMR ($CDCl_3$): δ 4.81 (H-2), 4.48 and 3.03 (H-6), 3.69 (H-14), 3.62 (H-15), 4.78 (H-20), 2.51 (H-21), 3.30, $J_{22/23b}$=11Hz, H-22), 1.69 (H-23a)and 1.38 (H-23b) ($J_{23a/23b}$=13Hz, $J_{23a/24}$=10Hz), 5.13 ($J_{24/25}$= 2.5Hz, H-24), 1.79 ($J_{25/26}$=6Hz), 3.87 (H-26), 5.20 (H-29), 2.97 (H-32), 0.90 (t-butyl), 0.17 (Me_3Si), 0.08 (Me_2Si).

33-O-tert.Butyldimethylsilyl-26-O-trimethylsilyl-iso-FK-506 (13a)

To a solution of 33-O-tert.butyldimethylsilyl-22(S)-dihydro-26-O-trimethylsilyl-iso-FK-506 (12a) (65mg, 0.06 mmol) and N-methyl-morpholine-N-oxide (about 40mg, 34 mmol) in 2ml dichloromethane were added a few mg of tetrapropylammonium-perruthenate as well as pulverized 4A molsieve and the mixture was stirred overnight at RT. Filtration, evaporation and chromatography (toluene/ethylacetate 6:1) yielded 45mg (69%) 13a. ^{1}H-NMR ($CDCl_3$): δ 4.67 (broad d, J=4Hz, H-2), 4.46 (broad d, J=13Hz, H-6e), 3.62 (d, J=9Hz, H-14), 3.55 (dd, J=11Hz, J=2.5Hz, H-15, 2.75 (H-23a) and 2.63 (H-23b) ($J_{23a/23b}$=17.5Hz, $J_{23a/24}$=7.5Hz, $J_{23b/24}$=5Hz), 5.20 (H-24), 3.93 (d, J=6Hz, H-26), 5.67 (ddt (J=17Hz, J=10Hz, J=7Hz, H-37), 3.43 + 3.39 + 3.29 (3 OCH_3). FAB-MS: 900 (MH^+-Me_3SiOH), 882 (-H_2O), 718, 266, 226.

Iso-FK-506 (2a) by deprotection of 13a

33-O-tert.Butyldimethylsilyl-26-O-trimethylsilyl-iso-FK-506 (13a) (28mg, 0.03mmol) in 1ml acetonitrile was treated with 0.5ml 1NHCl 30min at room temp. Saturated aqueous $NaHCO_3$ solution was added and the mixture extracted with ethyl ether. The ether solution was dried ($MgSO_4$), the solvents were evaporated and the residue was purified by chromatography (ethyl acetate) to give 20mg (88%) **2a**.

Attempted rearrangement of 33-O-tert.butyldimethylsilyl-FK-506 or FK-506

a) With imidazole. After stirring a solution of 33-O-tert.butyldimethylsilyl-FK-506 (**14a**) (184mg) and imidazole (14mg) in 2ml DMF for 10 days at 50°C only unchanged starting material was recovered.

b) With tetraisopropoxytitanium. FK-506 (160mg, 0.2 mmol) was treated with tetraisopropoxytitanium (16ul, 0.05mmol) and 4A molsieve for 24h in dichloromethane (1ml) at reflux . According to TLC analysis the main component of the resulting reaction mixture is unchanged starting material. **2a** is not detected.

Attempted intermolecular transesterification

33-O-tert.Butyldimethylsilyl-FR-900520 (**14b**) (200mg, 0.2mmol) was treated with tetraisopropoxytitanium (114µl, 0.4mmol) and methanol (0.1ml, 25 mmol) in 2ml THF at reflux for 3h. The reaction was quenched by addition of 1N HCl, the reaction mixture filtered through Celite and the solvent evaporated. The residue was purified by chromatography (toluene/ethyl acetate 2:1) to give 77mg (38%) benzilic acid rearrangement product **15b**. ^{1}H-NMR ($CDCl_3$), two conformers (4:3): δ 3.83 + 3.63 (COOMe). ^{13}C-NMR ($CDCl_3$), signals of the major conformer: δ 209.4 (C-22), 172.1 (C-8), 169.5 + 169.0 (168.4?)(C-1, C-9), 139.3 (C-19), 136.5 (C-29), 131.6 (C-28), 123.8 (C-20), 84.2 (C-32), 82.9 (C-10?), 79.2 (C-14), 77.7 (C-13), 75.2 (C-33), 68.3 (C-24).

REFERENCES

1. a) Okuhara, M.; Tanaka, H.; Goto, T.; Kino, T.; Hatanaka, H. Eur.Pat.Appl. EP 184.162; Chem.Abstr. **1986**, 105, P 189454b; b) Kino, T.; Hatanaka, H.; Hashimoto, M.; Nishiyama, M.; Goto, T.; Okuhara, M.; Kohsaka, M.; Aoki, H.; Imanaka H. J.Antibiot. **1987**, 40, 1249.
2. a) Hatanaka, H.; Kino, T.; Asano, M.; Goto, T.; Tanaka, H.; Okuhara, M. J.Antibiot. **1989**, 42, 620; b) Fehr, T., Eur.Pat.Appl. EP 356399A; Chem.Abstr. **1990**, 113, P 113850x; c) Okuhara, M.;

Goto, T.; Hatanka, H.; Yuasa, S.; Nakatani, I. Eur.Pat.Appl. EP 353678; Chem.Abstr. **1990**, 113, P 113847b; d) Borris, R.P.; Wicker, L.S.; Zink, D.L.; Lieu, T.H. Eur.Pat.Appl. EP 358508; Chem.Abstr. **1990**, 113, P 38913q.

3. a) Thomson, A.W. Immunol Today **1989**, 10, 6; b) Carfield, C.E.; Musser, J.H. Ann.Reports Med.Chem. **1990**, 25, 195; c) Kocienski, P.; Stocks, M.; Donald, D.K In Chirality in Drug Design and Synthesis, Brown, C., Ed.; Academic Press, London **1989**, pp 131-165; d) Johansson, A.; Moller, E. Transplantation **1990**, 50, 1001; e) Chang, J.Y.; Sehgal, S.N.; Bansbach, C.C. Trends Pharmacol.Sci. **1991**, 12, 218.
4. McLeod, A.M.; Thomson, A.W. Lancet **1991**, 337, 25.
5. Grassberger, M.A.; Schulz, G.; Fehr, T. Ger.Offen. DE 3938-754; **1991**.
6. Whereas in $CDCl_3$ two sets of signals appear, only one set is observed after addition of CD_3OD ($CDCl_3$:CD_3OD = 5:1).
7. a) Coleman, R.S.; Danishefsky, S.J. Heterocycles **1989** 28, 157; b) Fisher, M.J.; Chow, K.; Villalobos, A.; Danishefsky, S.J. J.Org.Chem. **1991** 56, 2900.
8. Compare also ref. 3c
9. Dess, D.B.; Martin, J.C. J.Org.Chem. **1983**, 48, 4155.
10. The new isomer **2a** has a weaker immunosuppressive activity as compared to FK-506 (mixed lymphocyte reaction, mouse, IC-50 = 24ng/ml; FK-506: 0.02ng/ml).
11. Treatment of FK-506 with sodium hydride in refluxing THF induces cleavage of the macrocyclic ring due to retroaldol reaction: Tanaka, H.; Kuroda, A.; Marusawa, H.; Hatanaka, H.; Kino, T.; Goto, T.; Hashimoto, M. J.Am.Chem.Soc. **1987**, 109, 531.
12. Askin, D.; Reamer, R.A.; Jones, T.K.; Volante, R.P.; Shinkei, I. Tetrahedron Lett. **1989**, 30, 671.
13. From spectral data the third ligand on the boron atom can not be determined with certainty. Fisher M.J. et al. (ref. 6b) assume that the selectride reaction leads to a cyclic borinic ester, i.e. that the third ligand is the 24-oxy-function of the FK 506 skeleton, but no analytical data are given. We find in the ^{13}C-NMR spectrum of **16a** two C-22 signals (diastereomers due to R,S-B-sec.butyl), but only one single C-24 signal. The FAB-MS results (see Experimental) support neither of the two structures.
14. Okuhara, M.; Tanaka, H.; Goto, T.; Kino, T.; Hatanka, H. U.S. Pat. US 4,929.611; Chem.Abstr. **1991**, 114, 61843w.