Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 15, pp. 1725-1728, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00281-2

Modifications of the MeBmt Side Chain of Cyclosporin A

Marcel K. Eberle,* Anne-Marie Jutzi-Eme, and François Nuninger

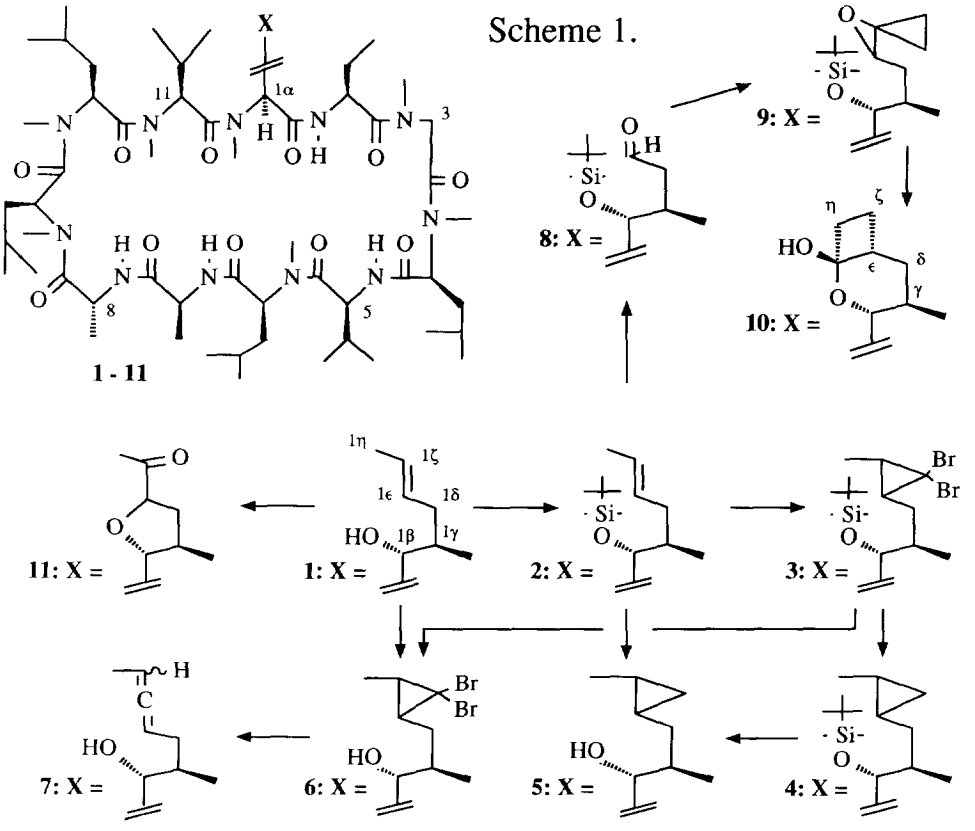
Preclinical Research, Sandoz Ltd, Basel, Switzerland

Abstract: *Dibromocarbene was added to the double bond of* **2** *with formation of dibromocyclopropyl cyclosporin* **3** *as a single isomer. Reduction of* **3** *gave* **4**. *Hydrolysis with fluoride led to the unprotected cyclosporin* **5**. *Removal of the silyl protecting group from* **3** *gave* **6**, *which could also be obtained directly from* **1**. *The dibromocyclopropyl compound* **6** *was transformed to the allene* **7** *(mixture of diastereoisomers). The aldehyde* **8** *was converted to* **9** *and rearranged to* **10** *(single isomer). Oxidation of* **1** *with Jones reagent gave the ketone* **11** *(single isomer).*

Cyclosporin A (**1**), the active component of Sandimmune, is a powerful immunosuppressant preventing allograft rejections in animals and humans. Cyclosporin A binds tightly to cyclophilin, the postulated receptor, which in all likelihood is identical with the enzyme peptidyl-prolyl *cis-trans* isomerase. The cyclophilin-cyclosporin A complex in turn binds to and inhibits the Ca^{2+} and calmodulin dependent phosphatase calcineurin (see e.g. literature citations in references 1b,c and 8).

The MeBmt {*N*,4-dimethyl-4(*R*)-[2(*E*)-butenyl]-L-threonine} side chain (amino acid 1) of cyclosporin A (**1**) (for the numbering see Scheme 1) has been the target of various chemical alterations.[1] Here we would like to report some of our own results carried out in an effort to modify the amino acid 1 of the cyclosporin skeleton. We were especially interested in the stereocontrol exerted by the backbone of cyclosporin A (**1**) during transformations involving the carbon-carbon double bond. In order to eliminate possible side reactions, the hydroxyl group was protected with a silyl group. For the preparation of the *tert*-butyl dimethylsilyl protected cyclosporin A we had to use the more reactive triflate. In the presence of triethylamine the desired compound **2** was obtained in 84% yield after crystallization[2] (mp 187-188 °C). Dibromocarbene, generated in a two phase system,[3] was added to **2** leading to the dibromocyclopropyl homolog **3**, isolated as a single diastereoisomer of unknown stereochemistry in about 20% yield after chromatography. The halogen atoms were removed reductively in the presence of tributyltin hydride.[4] It

Scheme 1.



was noticed that the replacement of the first bromide occurred very fast, while the removal of the second bromide took place much more slowly. Thus, the silylated cyclopropyl derivative **4** was obtained in 40% yield after heating the dibromo compound **3** in toluene in the presence of the reducing agent. Hydrolytic cleavage of the silyl protecting group in the presence of tetrabutylammonium fluoride in tetrahydrofuran gave in 57% yield the hitherto unknown derivative **5**, a cyclosporin with a cyclopropyl group in place of the original double bond.

The unprotected dibromocyclopropyl alcohol **6**, isolated in 80% yield after treatment of **3** in the presence of fluoride, was found to be a stable compound as well. This could also be prepared in 15% yield from the unprotected cyclosporin A (**1**) under similar conditions used for the preparation of **3**. Treatment of **6** with excess of methyl lithium at low temperature (-70 to -35 °C, then quenched with water) resulted in the formal elimination of bromine with concomitant formation of the allene **7** in 31% yield. The latter was obtained as a mixture of diastereoisomers although the starting material was isomerically pure according to

our analytical data. Attempts to carry out the analogous ring opening reaction with **3** were not successful.

The protected cyclosporin A (**2**) in methylene chloride was treated with ozone at -70 °C, then with dimethylsulfide. The aldehyde **8** was isolated in 65% yield after purification. The condensation of this aldehyde with commercial cyclopropyldiphenylsulfonium tetrafluoroborate in a mixture of methylene chloride and 40% sodium hydroxide was completed after 1.5 h. Our initial attempts to perform this transformation in homogenous DMSO/KOH solution, the usual choice of solvent for this type of reaction,[5] yielded the expected product in traces only. Treatment of the aldehyde **8** with the sulfonium tetrafluoroborate in a two phase system gave the spiro compound **9** in 59% yield as a mixture of diastereoisomers. This was mainly based on mass spectral data since the proton NMR spectrum of **9** was not very informative. Nevertheless the ^{13}C spectrum of **9** supported the assigned structure since the expected absorptions due to the cyclopropyl group were observed in sets of two. Direct rearrangement of the spiro cyclopropyl epoxide in the presence of stannic chloride,[5] followed by fluoride treatment, led to the isomerically pure cyclobutanone derivative **10** in 43% yield after chromatography on silica gel. The presence of a single isomer was indicated by the proton NMR spectrum of this compound. The signals of the seven N-methyl groups were all observed as sharp singlets. The ^{13}C spectrum of the compound **10** indicated the presence of the hemiketal form in chloroform solution based on the presence of a signal at 97.5 ppm and the absence of any signal above 175 ppm . The stereochemistry for the two new stereogenic centers was determined by double resonance NMR spectroscopy. After irradiation of the 1-β-H (δ 3.8 ppm) NOEs with the following protons or group of protons were observed: with the 1-γ-Me group at δ 0.7 ppm, with one of the 1-δ protons (axial) at δ 1.38 ppm and with the 1-ϵ proton at δ 2.35 ppm. The latter was assigned to the proton at the junction of the presumably cis fused four and six membered rings. Additional NOEs were observed between the 1-ϵ proton and the 1-η syn proton on the four membered ring and a weak NOE with the 1-γ methyl group. Molecular modeling calculations seem to indicate that a tetrahydropyran fused with a four membered ring as indicated by **10** should be thermodynamically more stable with the amino acid side chain and the methyl group both being in an equatorial and the hydroxy group of the hemiketal being in an axial position. The observed NOEs between the 1-γ-Me group at δ 0.7 ppm and the 1-ϵ proton at δ 2.35 ppm seem to indicate the presence of a boat form for the tetrahydropyran ring in **10**. Attempts to oxidize cyclosporin A (**1**) in acetone at room temperature with Jones reagent gave the ketoether **11** in 44% yield as a single diastereoisomer. This result may be explained by assuming that the first site of attack by the oxidizing agent is not the secondary hydroxy group but the double bond of the

MeBmt side chain instead, the iodoether formation[6] of cyclosporin A serving as a precedent. The newly formed ether alcohol would then be oxidized further to the ketoether **11**. The stereochemistry of the new stereogenic center has not been determined.

With the exceptions of the allene **7** and the spirocompound **9**, all compounds were obtained as single isomers. This may be attributed to the conformational rigidity of **1** with the double bond playing an integral part.

The new cyclosporins were tested *in vitro* [MLR (Mixed Lymphocyte Reaction, MD (Michel-Dutton, Humoral Immune Response) and Il-2 (Inhibition of Il-2 Release)] and compared[7] with **1**. The most active compounds were **5** and **7**. Both were found to be about 2 - 3 times less active than the parent compound **1**. Compounds **9**, **10** and **11** were found to be at least 100 times less active than cyclosporin A. For more information about the procedures used in these tests see our results recently published for cyclosporin-8 derivatives.[8]

References:

*) To whom correspondence concerning this paper should be addressed.

1) a) Eberle, M. K.; Nuninger, F. *J. Org. Chem.*, **1995**, *60*, 2610. b) Eberle, M. K.; Nuninger, F. *Tetrahedron Lett.*, **1994**, *35*, 6477. c) Eberle, M. K.; Nuninger, F. *J. Org. Chem.*, **1992**, *57*, 2689. d) Aebi, J. D.; Deyo, D. T.; Sun, C. Q.; Guillaume, D.; Dunlap, B. E.; Rich, D. H. *J. Med. Chem.*, **1990**, *33*, 999. e) Rich, D. H; Sun, C. Q.; Guillaume, D.; Dunlap, B. E.; Evans, D. A.; Weber, A. E. *J. Med. Chem.*, **1989**, *32*, 1982. f) Galpin, I. J.; Mohammed, A. K. A.; Patel, A. *Tetrahedron*, **1988**, *44*, 1783. g) Aebi, J. D.; Guillaume, D.; Dunlap, B. E.; Rich, D. H. *J. Med. Chem.*, **1988**, *31*, 1805. h) Galpin, I. J.; Karim, A; Mohammed, A. K. A.; Patel, A. *Tetrahedron Lett.*, **1987**, 6517. i) Rich, D. H; Dhaon, M. K.; Dunlap, B. E.; Miller, S. P. F. *J. Med. Chem.*, **1986**, *29*, 978.

2) All new compounds were characterized by TLC, HPLC (reversed phase), 360 MHz ^{1}H NMR and by high resolution mass spectroscopy. Rotations (measured in methanol) **4**: α_D= -171.8 (c=0.25); **5**: α_D= -146.7 (c=0.70); **7**: α_D= -174.4 (c=0.56); **9**: α_D= -164.9 (c=0.68); **10**: α_D= -168.9 (c=0.28); **11**: α_D= -177.7 (c=0.47).

3) Keller, W. E. *Phase Transfer Reactions* (Fluka Compendium); Georg Thieme: Stuttgart, New York, **1986**, Vol. 1, pp 112 - 122.

4) Seyferth, D., Yamazaki, H., Alleston,, D. L. *J. Org. Chem.*, **1963**, *28*, 703.

5) a) Trost, B. M., Bogdanowicz, M. J. *J. Amer. Chem. Soc.*, **1973**, *95*, 2038. b) Trost, B. M., Bogdanowicz, M. J. *Tetrahedron Lett.*, **1972**, 887. c) Trost, B. M., Bogdanowicz, M. J. *J. Amer. Chem. Soc.*, **1971**, *93*, 3773.

6) Petcher, T. J.; Weber, H.-P.; Rüegger, A. *Helv. Chim. Acta*, **1976**, *59*, 1480.

7) We are grateful to Peter Hiestand for performing these tests.

8) Eberle, M. K., Hiestand, P., Jutzi-Eme, A.-M., Nuninger, F., Zihlmann, H. R. *J. Med. Chem.* **1995**, *38*, 1853.

(*Received in Belgium* 14 *December* 1994; *accepted* 28 *June* 1995)