Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 1609–1612

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis of Derivatives of the Novel Cyclophilin-Binding Immunosuppressant Sanglifehrin A with Reduced Numbers of Polar Functions

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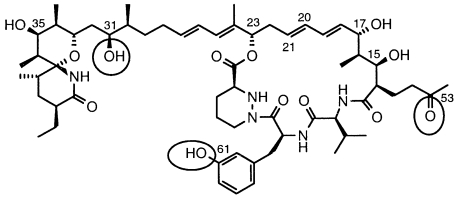
Received 4 December 2000; accepted 27 April 2001

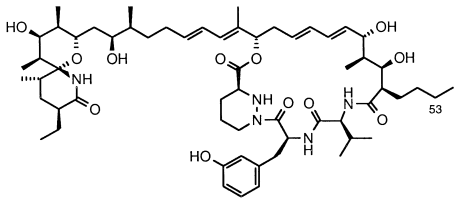
Abstract—The syntheses and the biological activities of 53-deoxo sanglifehrin A (**2**) and 61-deoxy octahydrosanglifehrin A (**3**) are described. Compound **2** shows intracellular cyclophilin (CyP)-binding and immunosuppressive activity in the mixed-lymphocyte reaction (MLR) similar to that of sanglifehrin A (**1**). Compound **3** is much less active in the MLR despite unchanged intracellular CyP-binding. This indicates that the 53-keto group is not necessary for immunosuppressive activity, while the 61 hydroxy group is required. © 2001 Published by Elsevier Science Ltd.

Sanglifehrin A (**1**) was isolated at Novartis from a fermentation broth of the actinomycete strain *Streptomyces flaveolus* and is the most abundant representative of a series of related immunosuppressive natural products identified by screening for novel cyclophilin-binding metabolites.[1] Compound **1** exhibits a 20-fold higher affinity for cyclophilin (CyP) than cyclosporine A in a cell-free binding assay. However, its immunosuppressive activity is 10-fold lower as determined by the mixed lymphocyte reaction (MLR).[1a] Compound **1** does not inhibit the phosphate activity of calcineurin, the target of CyP/cyclosporine A complex, and does not affect IL-2 gene expression indicating a different mode of action than cyclosporine A.[1a]

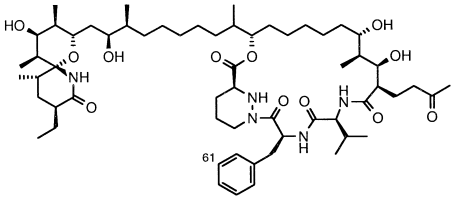
Sanglifehrin A consists of a 22-membered macrocycle, bearing in position 23 a nine-carbon chain terminated by a unique spirobicyclic moiety. The macrocycle



sanglifehrin A, **1**



53-deoxo sanglifehrin A, **2**



61-deoxy octahydro sanglifehrin A, **3**

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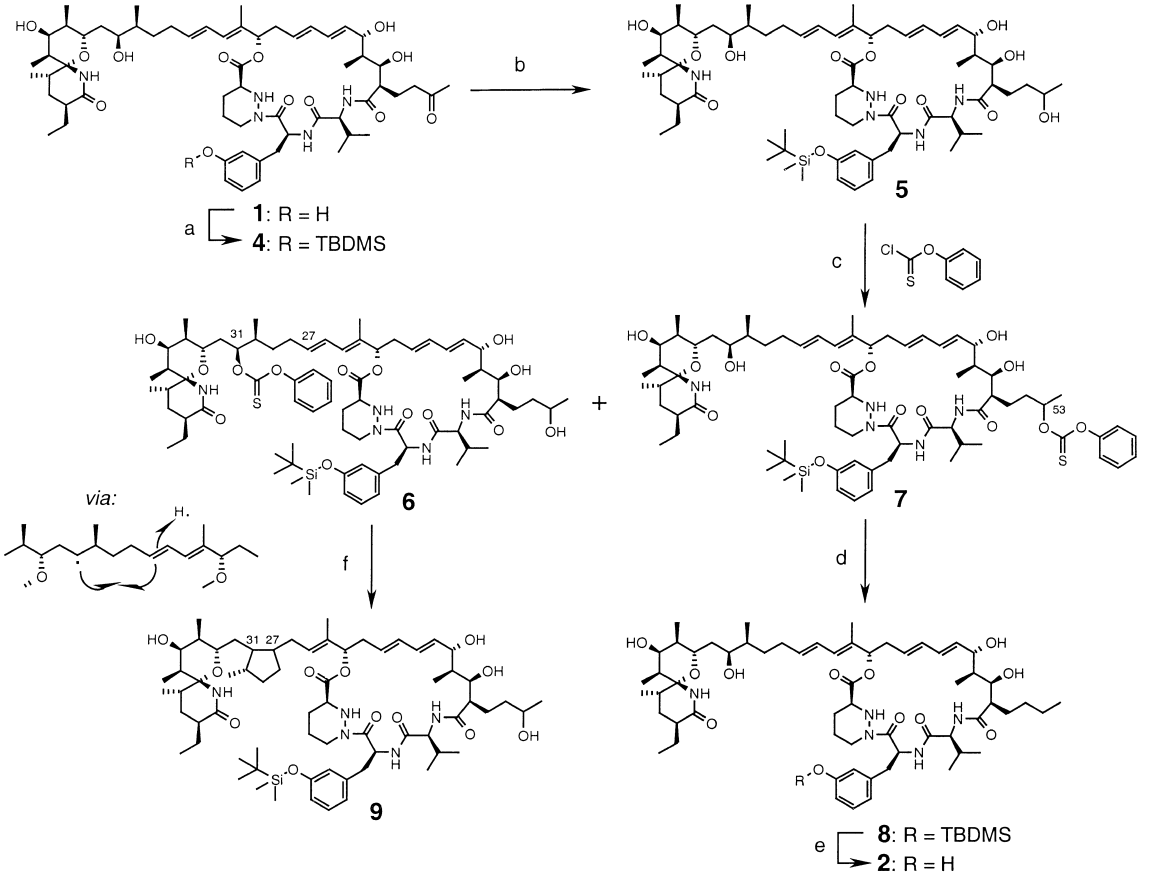
PII: S0960-894X(01)00293-1

contains a tripeptide subunit consisting of valine and the unusual amino acids *meta*-tyrosine and piperazic acid.

Our aim was to determine which functional groups of sanglifehrin A (**1**) are essential for immunosuppressive activity as well as to improve the biological activity. The relatively low immunosuppressive activity of **1** in comparison to its high affinity for CyP could be the result of poor cell permeation due to the presence of polar hydroxy and keto functions. We anticipated that reducing the number of polar groups could lead to increased cell permeation and would reveal their importance for the immunosuppressive activity. To this end, we followed synthetic approaches[2] as well as natural product derivation. In this paper we report the syntheses of 53-deoxo sanglifehrin A (**2**) and 61-deoxy octahydro sanglifehrin A (**3**) as well as attempts towards preparing 31-deoxy sanglifehrin A.

For the synthesis of 53-deoxo sanglifehrin A (**2**) the phenolic hydroxy group of **1** was silylated by treatment with *N*-*tert*-butyldimethylsilyl-*N*-methyltrifluoroacetamide to give **4** in 94% yield (Scheme 1). $NaBH_4$ reduction of the ketone **4** gave the alcohol **5** as a 1:1 epimeric mixture. Treatment of **5** with phenylchlorothionoformate led to the 31-thionocarbonate **6** and the 53-thionocarbonate **7** in 11 and 18% yield, respectively. Barton-deoxygenation of **7** was effected by treatment with Bu_3SnH in the presence of AIBN to give **8** in 36% yield. Final desilylation led to 53-deoxo sanglifehrin A (**2**).[3] Attempted deoxygenation of **6** under the same conditions as above was unsuccessful. Instead, a diastereomeric mixture of the five-membered ring products **9** was obtained, which had formed through reaction of the intermediate radical with the 26–27 double bond in a 5-*exo*-trig reaction. No further attempts were made towards 31-deoxy sanglifehrin A.

In order to remove the phenolic hydroxy group of **1**, a Pd catalyzed reduction of the corresponding triflate using formic acid as hydrogen donor was envisioned. Thus, **1** was first converted to its triflate **10** using *N*-phenylbis(trifluoromethanesulfonimide)[4] in 77% yield (Scheme 2), which was treated with catalytic amounts of $Pd(OAc)_2$ and 1,1′-bis(diphenylphosphino)ferrocene (dppf) and excess formic acid.[5] No reaction occurred at room temperature and at 50 °C **10** decomposed. The poor reactivity was attributed to the complexation of the palladium catalyst to the 1,3-diene systems. Therefore we decided to remove the double bonds. Thus, **1** was hydrogenated using palladium on charcoal to give octahydro sanglifehrin A (**11**) in 34% yield as an epimeric mixture at position 24. By the same method as for **1**, **11** was converted to its triflate **12**. Treatment of **12** with a combination of catalytic amounts of $Pd(OAc)_2$



Scheme 1. Reagents and conditions: (a) *N*-*tert*-butyldimethylsilyl-*N*-methyltrifluoroacetamide (2 equiv), acetonitrile, 50 °C, 3 h, 94%; (b) $NaBH_4$ (1.5 equiv), MeOH, 0 °C, 30 min, quant; (c) $ClCSOC_6H_5$ (2 equiv), DMAP (cat), $ClCH_2CH_2Cl$, 3 days, rt, **6**: 11%, **7**: 18%; (d) Bu_3SnH (2 equiv), AIBN (0.25 equiv), benzene, reflux, 45 min, 36%; (e) TBAF (1.5 equiv), THF, 0 °C, 30 min, quant; (f) Bu_3SnH (2 equiv), AIBN (0.25 equiv), benzene, reflux, 45 min.

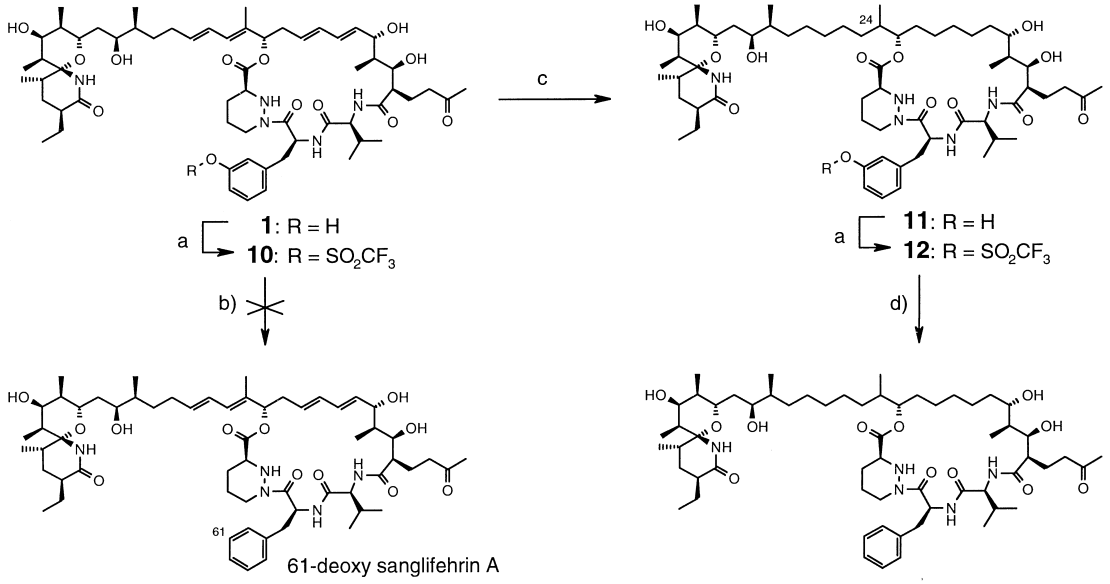
and (dppf)$PdCl_2$ dichloromethane 1:1 complex and excess formic acid now led to the desired 61-deoxy octahydro sanglifehrin A (**3**)[6] in 81% yield.

The biological activities of the compounds with respect to CyP-binding and immunosuppression are summarized in Table 1. CyP-binding was analyzed in both a cell-free assay and a cellular assay. Cell-free CyP-binding was assessed by competition of the compounds with the interaction of CyP-A and **1**.[7] Intracellullar CyP-binding was determined in a competitive format with whole cells and ^{3}H-cyclosporine A.[8] The ratio cell-free/intracellular CyP-binding is a way to estimate the cell permeation of sanglifehrin derivatives. For example, if cell-free binding decreases, that is if the cell-free IC_{50} increases, and if intracellular binding is unchanged, the ratio cell-free/intracellular binding will be >1 and is interpreted as an increased cell-permeation. Likewise, a ratio <1 indicates a decrease in cell-permeation. The immunosuppressive activity of the compounds was assessed by MLR as described.[1a] To compensate for variation of absolute IC_{50} values between independent experiments of **1** and its derivatives, **1** was used as a reference in each experiment. Thus, the IC_{50} values given are relative values compared to sanglifehrin A (**1**) ($rIC_{50} = IC_{50}$ of compound/IC_{50} of **1**).

53-Deoxo sanglifehrin A (**2**) shows similar values in cell-free and intracellular CyP-binding and hence no improved permeation (cell-free/intracellular Cyp-binding ratio 0.8). Thus, the removal of the 53-keto group of **1** does not improve cell permeation. Octahydro sanglifehrin A (**11**) has a 26-fold weaker affinity for CyP than sanglifehrin A (**1**). The decreased binding affinity of **11** compared to **1** could be due to the increased flexibility of the compound.

61-Deoxy octahydro sanglifehrin A (**3**) has a relative IC_{50} of 300 in the cell-free CyP assay, which indicates that the removal of the phenolic hydroxy group results in an additional 11-fold loss in binding activity. However, the intracellular CyP-binding of **11** and **3** is roughly unchanged, which indicates a better permeation of both compounds in comparison to **1** (cell-free/intracellular CyP-binding ratio of 65 and 270, respectively).

The MLR activity of **2** is slightly increased indicating that the 53-keto group is not essential for immunosuppressive activity. Compound **11** is almost equipotent to **1** in the MLR despite a 26-fold loss in cell-free CyP binding. This indicates that the increased permeation compensates to some degree for the reduced CyP binding. Alternatively, the immunosuppressive activity



Scheme 2. Reagents and conditions: (a) Tf_2NPh (3.6–5.4 equiv), EtN(*i*-Pr)$_2$ (20 equiv), $ClCH_2CH_2Cl$, rt, 3–6 days, **10**: 85%, **12**: 77%; (b) $Pd(OAc)_2$ (0.1 equiv), dppf (0.1 equiv), HCOOH (12.6 equiv), EtN(*i*-Pr)$_2$ (25.2 equiv), DMF, rt, 5.5 h, no reaction; (c) Pd/C, H_2, EtOH, 35 min, rt, 34%; (d) $Pd(OAc)_2$ (0.1 equiv), (dppf)$PdCl_2CH_2Cl_2$ 1:1 (0.1 equiv), HCOOH (12.6 equiv), EtN(*i*-Pr)$_2$ (25.2 equiv), DMF, rt, 5.5 h, 81%.

Table 1. Biological activities of compounds

Substance	CyP binding		'Permeation' ratio	MLR[1a]
	Cell-free[7] rIC_{50}	Intracellular[8] rIC_{50}	cell free/intracellular	rIC_{50}
1	1	1	1	1
2	1.5±0.1	2.0±0.6	0.8	0.5±0.1
11	26±2	0.4±0.2	65	1.7±0.5
3	300±50	1.1±0.1	270	22±4

Mean relative IC_{50} values (rIC_{50})±standard error of 3–4 experiments are shown. Absolute IC_{50} values of sanglifehrin A (**1**) are as follows: cell-free CyP-binding, 0.001–0.011 μM; intracellular CyP-binding, 4–16 μM; and MLR, 0.07–0.42 μM.

might be independent of CyP binding and the region of **1**, which interacts with a yet unknown target, is not affected by hydrogenation. The overall immunosuppressive activity of **3** is decreased in comparison to **11** indicating that the 61 hydroxy group is crucial for immunosuppressive activity. This information is consistent with our view that the tripeptide unit is the structural motive determining the CyP binding and immunosuppressive activity.[2d]

In summary, our results show that very selective chemical derivations can be performed on this complex natural product. The 53-deoxo compound **2** shows similar activities compared to **1**, which indicates that the 53-keto group is not necessary for immunosuppressive activity and its removal does not alter cell permeation. On the contrary, the 61-deoxy compound **3** shows decreased MLR activity compared to **11** despite increased cell permeation, which indicates that the 61 hydroxy group is crucial for immunosuppressive activity. Further work was undertaken to increase the cell permeation while maintaining or even increasing the immunosuppressive activity of sanglifehrin A (**1**). Results will be reported elsewhere.

Acknowledgements

The authors thank L. Walliser, D. Wagner, U. Strittmatter-Keller and S. Fuchs for their excellent technical assistance.

References and Notes

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3. Spectral data for compound **2**. The ^{1}H NMR interpretation was corroborated by an HSQC spectrum. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.25 (s, 1H), 8.14 (d, J=6.5 Hz, 1H), 7.89 (s, 1H), 7.44 (d, J=9.0 Hz, 1H), 7.06 (t, J=7.7 Hz, 1H), 6.59 (dd, J=1.0 Hz, J=7.0 Hz, 1H), 6.57 (d, J=7.2 Hz, 1H), 6.51 (s, 1H), 6.21 (dd, J=11.4 Hz, J=15.0 Hz, 1H), 6.13 (dd, J=11.4 Hz, J=15.0 Hz, 1H), 6.01 (dd, J=10.8 Hz, J=13.8 Hz, 1H), 5.97 (t, J=11.4 Hz, 1H), 5.68 (td, J=7.2 Hz, J=14.4 Hz, 1H), 5.58 (m, 3H), 5.40 (m, 2H), 5.23 (d, J=9.4 Hz, 1H), 4.79 (d, J=3.5 Hz, 1H), 4.49 (d, J=11.4 Hz, 1H), 4.18 (m, 1H), 4.05 (m, 2H), 3.90 (m, 1H), 3.82 (m, 1H), 3.69 (t, J=9.6 Hz, 1H), 3.56 (m, 1H), 3.51 (m, 1H), 2.78–2.23 (m, 7H), 2.13–1.08 (m, 29H), 1.71 (s, 3H), 0.93–0.75 (m, 24H, 8×aliphatic CH_3 including 53-CH_2–CH_3), 0.62 (d, J=6.7 Hz, 3H); $C_{60}H_{93}N_5O_{12}$, MS_{calcd} for M−H requires 1074.68, found 1074.6.

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6. Spectral data for compound **3**. The ^{1}H NMR interpretation was corroborated by an HSQC spectrum. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.48 (s, br, 1H), 7.90 (s, 1H), 7.38–7.12 (m, 4H), 5.80 (m, 1H), 5.59 (d, J=4.8 Hz, 1H), 5.15–4.65 (m, 3H), 4.18 (s, 1H), 4.12 (m, 1H), 3.98 (d, J=6.0 Hz, 1H), 3.70 (m, 2H), 3.58 (s, br, 1H), 3.50 (m, 1H), 3.22 (m, 1H), 2.90–2.60 (m, ca. 6H), 2.50–2.30 (m, 2H), 2.18 (m, 1H), 2.07 (s, 3H), 2.10–1.00 (m, ca. 44H), 0.95–0.70 (m, 21H), 0.62 (d, J=6.7 Hz, 3H); although **3** is a mixture of diastereomers this is not apparent from the NMR spectrum; $C_{60}H_{99}N_5O_{12}$, MS_{calcd} for M−H requires 1080.73, found 1080.7.

7. The cell-free CyP-binding was determined in an enzyme-linked immunosorbent assay analogous to the assay described in Schneider, H.; Charara, N.; Schmitz, R.; Wehrli, S.; Mikol, V.; Zurini, M. G.; Quesniaux, V. F.; Movva, N. R. *Biochemistry* **1994**, *33*, 8218 with the exception that cyclosporine conjugated to bovine serum albumin was replaced by sanglifehrin coupled to bovine serum albumin.

8. Intracellular CyP-binding was assessed in a competitive format with whole cells and ^{3}H-cyclosporine A as described in Baumann, G.; Andersen, E.; Quesniaux, V.; Eberle, M. K. *Transplant. Proc*. **1992**, *24*, 43.