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A Synthesis of the C1-N12 Tripeptide Fragment of Sanglifehrin A

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Abstract: The synthesis of the C_1-N_{12} tripeptide of the novel immunosuppressant sanglifehrin A is described. Evans oxazolidinone methodology was used to install the C8 stereocentre of the *meta*-tyrosine sub-unit.
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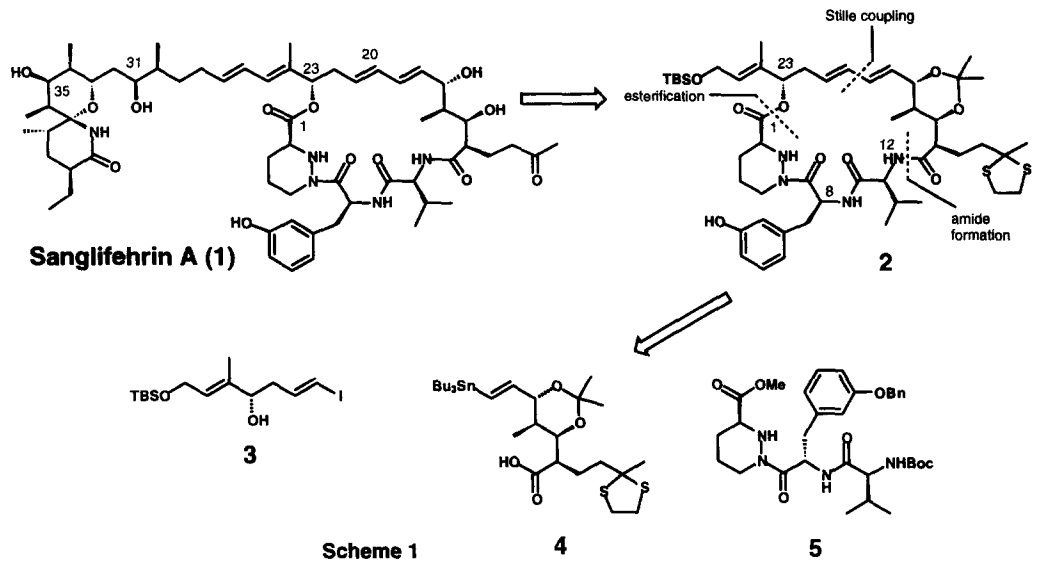
Sanglifehrin A **1**, isolated at Novartis from a fermentation broth of the Actinomycete strain *Streptomyces flaveolus*, is a representative of a series of immunosuppressive natural products identified by screening for novel cyclophilin binding metabolites.[1] Sanglifehrin A exhibits a 20-fold higher affinity for cyclophilin A than cyclosporin A and its immunosuppressant activity is around 8-fold lower as determined by the mixed lymphocyte reaction (MLR). However, in contrast to cyclosporin A, sanglifehrin A inhibits both T-lymphocytes and B-lymphocytes.

The novel molecular architecture of sanglifehrin A comprises a unique spiro array and a mixed polypropionate-peptide derived lactone linked *via* an *E,E*-diene system. Notably, the 24 membered macrocyclic lactone contains the unusual piperazic acid and *meta*-tyrosine residues. It is believed that the conformational rigidity supplied by the piperazic acid sub-unit may play a key role in controlling the binding of sanglifehrin A with cyclophilin.[2] In an effort to probe the mechanism of action of sanglifehrin A further and to produce compounds with higher immunosuppressive activity, we decided to embark upon a total synthesis of sanglifehrin A.

We disclose herein our synthesis of tripeptide **5**, the C_1-N_{12} fragment of sanglifehrin A.



As can be seen in Scheme 1, the retrosynthetic analysis indicated that the C_1-C_{26} fragment **2** would be constructed by Stille coupling[3] of vinyl iodide **3** and stannane **4**, after the separate union of both these sub-fragments to tripeptide **5**.

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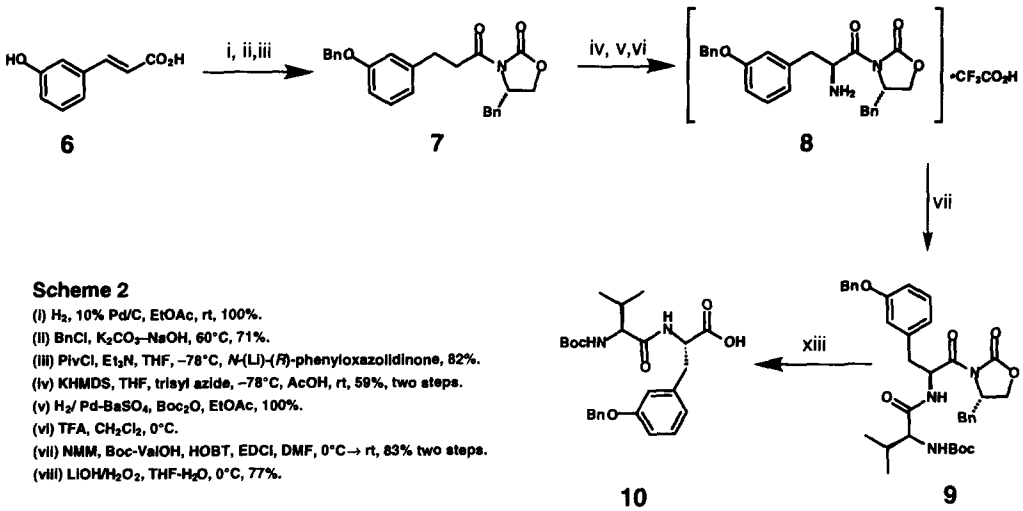
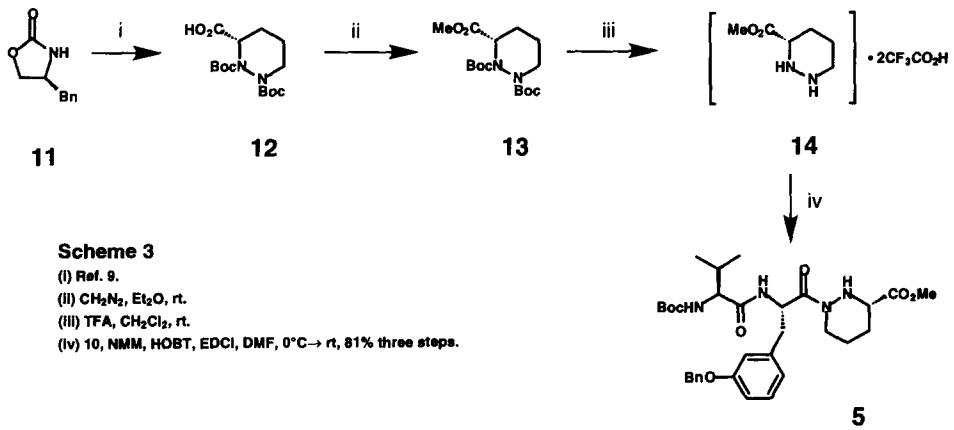
The synthesis commenced with the selective hydrogenation of commercially available 3-hydroxy cinnamic acid **6**. The phenolic function was protected as its benzyl ether in good yield (85% overall for the two steps). Installation of the benzyl oxazolidinone chiral auxiliary *via* the pivaloyl mixed anhydride method proved satisfactory (82%).[4] α-Amination of imide **7** was accomplished *via* the two step procedure of (a) generating the α-azide functionality and (b) catalytic reduction to the corresponding amino group. Thus, treatment of imide **7** with potassium hexamethyldisilazide (THF, –78°C) and quenching with trisyl azide proceeded in moderate yield with good stereocontrol (59%, d.r. = 95:5).[5] The remainder of the mass balance was unreacted imide **7**. Catalytic reduction of the azide group *in the presence of Boc anhydride*[6] was followed by trifluoroacetic acid-mediated Boc deprotection. Crude trifluoroacetate salt, **8**, was coupled to *N*-Boc protected valine using EDCI/HOBT[7] in the presence of N-methylmorpholine[8] to give the C_7-N_{12} sector of sanglifehrin A, **9**, in a very satisfying 83% yield over the two steps. Lithium peroxide hydrolysis[9] excised the oxazolidinone cleanly to generate the corresponding carboxylic acid **10** in good yield (77%) and with no observable epimerisation of the α-stereocentre, as determined by ^{1}H NMR analysis.[10]



Scheme 2

(i) H_2, 10% Pd/C, EtOAc, rt, 100%.
(ii) BnCl, K_2CO_3–NaOH, 60°C, 71%.
(iii) PivCl, Et_3N, THF, –78°C, *N*-(Li)-(*R*)-phenyloxazolidinone, 82%.
(iv) KHMDS, THF, trisyl azide, –78°C, AcOH, rt, 59%, two steps.
(v) H_2/ Pd-$BaSO_4$, Boc_2O, EtOAc, 100%.
(vi) TFA, CH_2Cl_2, 0°C.
(vii) NMM, Boc-ValOH, HOBT, EDCI, DMF, 0°C→ rt, 83% two steps.
(viii) LiOH/H_2O_2, THF-H_2O, 0°C, 77%.

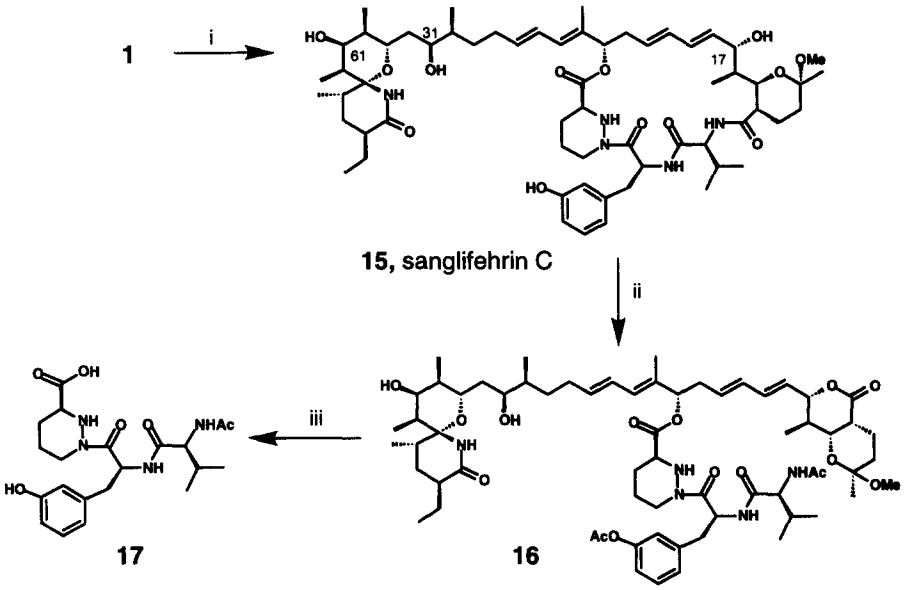
Bis-Boc protected piperazic acid **12** was smoothly generated from oxazolidinone **11** according to Hale's procedure[11] and converted to the corresponding methyl ester **13**. Deprotection with trifluoroacetic acid afforded salt **14**, which was coupled with carboxylic acid **10** to give tripeptide **5** in high yield (81% from **12**).[12] Notably, coupling of the crude piperazic acid salt occurred exclusively at the β-amine position.



Scheme 3

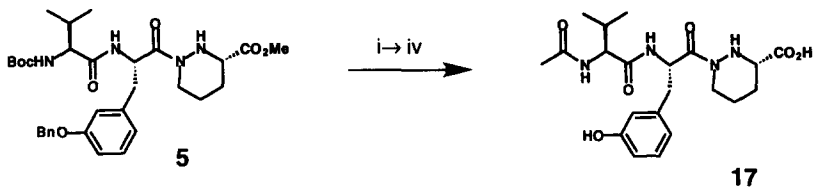
(i) Ref. 9.
(ii) CH_2N_2, Et_2O, rt.
(iii) TFA, CH_2Cl_2, rt.
(iv) 10, NMM, HOBT, EDCI, DMF, 0°C→ rt, 81% three steps.

Treatment of sanglifehrin A with methanol under mildly acidic conditions provided sanglifehrin C, **15**. Acetylation provided a mixture of the C17, 31, 61-(O)-triacetate of **15** (53%) and lactone **16** (34%). This latter product arising from hemi-acetal rearrangement and ring opening was ideally suited for excising the tripeptide portion. Extended treatment with mild base led to saponification of the C_1-ester linkage and liberated tripeptide fragment **17**.



Scheme 4
(i) MeOH, PPTS, rt, 100%.
(ii) Ac_2O (4 eq.), Pyr. (11 eq.), DMAP, CH_2Cl_2, 14d, rt, 34%.
(iii) MeOH, K_2CO_3, rt, 14d, 52%.

Synthetic tripeptide **5** was converted through a short series of reactions to provide synthetic **17**, which was shown to be identical with "natural" derivative **17** by ^1H and ^{13}C NMR and HPLC co-injection.[13]



Scheme 5
(i) TFA, CH_2Cl_2, 0°C.
(ii) DMAP, Ac_2O, Pyr., 0°C→rt, 100% two steps.
(iii) LiOH, THF-H_2O, 0°C→rt, 100%.
(iv) H_2, 10%-Pd/C, EtOAc, 85%.

In summary, we have illustrated a short synthesis of the C_1-N_{12} tripeptide fragment of sanglifehrin A. The route is capable of producing multi-gram quantities of intermediates. The flexibility of the above approach allows possible variation for the introduction of alternative protecting groups and functionality within the fragment for biological testing of analogues. Work directed towards the synthesis of vinyl iodide **3**, stannane **4** and, ultimately, lactone **2** will be reported in due course.

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References

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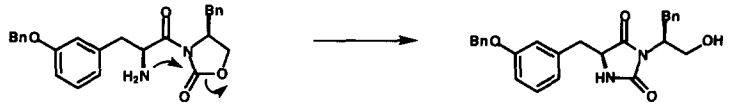
6. Catalytic reduction in the absence of Boc-anhydride leads to opening of the oxazolidinone by the resultant α-amino functionality as shown.



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8. The coupling of the corresponding free base generated *in situ* is sufficiently rapid such that oxazolidinone ring opening (*cf.* Ref. 6) does not occur.
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10. All new compounds were fully characterised by ^{1}H and ^{13}C NMR, IR and Microanalysis.
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12. Data for **5**:- ^{1}H NMR (D_6-DMSO, 400MHz), δ =7.66 1H [d, *J* = 11.2 Hz, *NH*], 7.32-7.50 5H [m, *Ar*], 7.16 1H [m, *Ar*), 6.68-6.92 [m, *Ar*], 5.55 1H [dd, *J* = 5.6, 11.2, *-CONHCH(Bn)CO-*], 5.25 1H [d, *J* = 11.2 Hz], 5.15 1H d, [*J* = 8.2, 11.3Hz, *ArOCH$_2$Ar*], 3.90 1H [s(br)], 3.76 1H [m, *Val-αH*], 3.65 3H, [s, *CO$_2$CH$_3$*], 3.15 2H [s(br)], 2.80 1H [dd, *J* = 5.6, 12.2 Hz, *-CHCH$_2$Ar*], 2.70, 1H [dd, *J* = 8.3, 12.2 Hz, *-CHCH$_2$Ar*], 1.92 1H [m, *Val-βH*], 1.54-1.8 4H [m], 1.48 9H [s, *CO$_2$(CH$_3$)$_3$*], 0.75 6H [d, *J* = 5.6 Hz, *Val-γH*], 0.74 6H [d, *J* = 5.6 Hz, *Val-γH*].
$[\alpha]_D^{22}$ = –26.12 (c = 6.05, MeOH). Microanalysis expected for $C_{32}H_{44}N_4O_7$:- C64.41, H 7.43, N 9.39, O 18.77. Obtained:- C 64.23, H 7.49, N 9.30, O 19.06.
13. Data for **17**:- ^{1}H NMR (D_6-DMSO, 400MHz), δ =8.08 1H [s(br), *NH*], 7.68 1H [d, *J* = 10.2 Hz, *NH*]], 6.97 1H [m, *Ar*], 6.50-6.33 3H [m, *Ar*], 5.40 1H [dd(br), *J* = 5.6, 13.0 Hz, *-NHCH(Bn)CO*], 4.45 1H [d, *J* = 11.2 Hz,*-NHCH(*CO_2H)-], 4.20 1H [d, *J* = 11.2 Hz, *NH*], 4.12 1H [dd, *J* = 7.44, 7.44 Hz, *Val-αH*], 2.9-2.6 4H [m] 1.96 1H [m, *Val-βH*], 1.75 2H [m, *piperazic CH$_2$*], 1.68 2H [m, *piperazic CH$_2$*], 0.75 6H [d, *J* = 5.6 Hz, *Val-γH*].
$[\alpha]_D^{22}$ = –37.13 (c = 5.36, MeOH). Microanalysis expected for $C_{21}H_{30}N_4O_6$:- C 58.05, H 6.96, N 12.89, O 22.09. Obtained:- C 58.09, H 6.99, N 12.93, O 22.13.