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# Efficient Synthesis of [$^3$H]-Sanglifehrin A via Selective Oxidation/Reduction of Alcohols at $C_{31}$ and $C_{35}$

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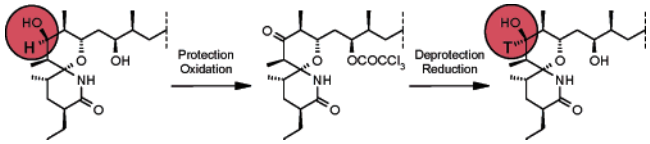


Sanglifehrin A is a novel complex natural product showing strong immunosuppressive activity and remarkably high affinity for cyclophilin A. To assess its pharmacokinetic properties in vivo, an efficient synthetic route was developed to introduce a tritium label in position $C_{35}$ of sangliferin A via an oxidation/reduction strategy. The synthetic approach is particularly attractive, because the $C_{35}$-oxo intermediate **7** is available in good yield on large scale and the reducing agent, lithium tri-*sec*-butylborotritide, is readily available. An attempt to apply a similar strategy to the alcohol in position $C_{31}$ led primarily to $C_{31}$-*epi*-hydroxy sanglifehrin A under a variety of conditions.

Sanglifehrin A (SFA) was isolated from *Streptomyces flaveolus* in 1995.[1] The substance is a potent immunosuppressant and has a remarkably high affinity for an intracellular binding protein called cyclophilin A ($IC_{50}$ = 2−4 nM).[2] In addition to its interesting biological activity, SFA has a unique and complex molecular structure, which consists of a 22-membered macrocycle, bearing in position 23 a nine-carbon tether terminated by a highly substituted spirobicyclic moiety. The macrolide contains an (*E*,*E*)-diene, a short polypropionate fragment, and a tripeptide unit composed of valine and two unusual amino acids, piperazic acid and *m*-tyrosine. One of the most remarkable features of the molecule is certainly the complex spirobicyclic oxaazaspiro[5.5]undecanone system extending from $C_{33}$ to $N_{42}$. This substructure, which exhibits seven stereocenters, six of which are contiguous, is unique for the sanglifehrin class of compounds. The structural attractiveness of sanglifehrin, combined with its biological activity, generated broad interest in industrial and academic laboratories. Their efforts resulted in the preparation of several fragments[3] and finally culminated in the total syntheses by Nicolaou et al.[4] and Paquette et al.[5]

The promising immunosuppressive properties of sanglifehrin A prompted us to undertake a complete study of its pharmacokinetic properties. Toward this purpose, a tritium or carbon-14 radiolabeled isotopomer of sanglifehrin A was needed. Typically, for such complex natural products, the radiolabel could be introduced either by a synthetic approach[6] or via a fermentation process.[7] We decided to explore tritium labeling via a synthetic route first. Indeed, we reasoned that a straightforward two-step oxidation/reduction strategy could be applied to either position $C_{31}$ or $C_{35}$. The reduction of one or both positions was expected to proceed stereoselectively due to the steric effect of the adjacent methyl groups.

Herein, a detailed account of the regioselective oxidation of the hydroxyl groups at either $C_{31}$ or $C_{35}$ as well as the stereoselective outcome of the reduction of the corresponding ketones is described. This study led us to a straightforward and high-yielding synthesis of sanglifehrin A tritium labeled in position $C_{35}$ on large scale.

The overall synthetic route, which allows the preparation of [$^3$H]-labeled sanglifehrin A, is shown in Scheme 1.

Intramolecular cyclization of the $C_{53}$ ketone of sanglifehrin A with both hydroxy groups at $C_{15}$ and $C_{17}$ under mild acidic conditions provided in quantitative yield ketal **1**. The stable ketal moiety was used as a protecting group for this sensitive portion of the molecule. Attempts to oxidize directly intermediate **1** bearing a free phenol moiety were unsuccessful. Therefore, *m*-tyrosine was selectively acetylated in the presence of acetic anhydride and catalytic amounts of DMAP to afford **2** quantitatively. The $CH_2Cl_2$/pyridine ratio was critical for the

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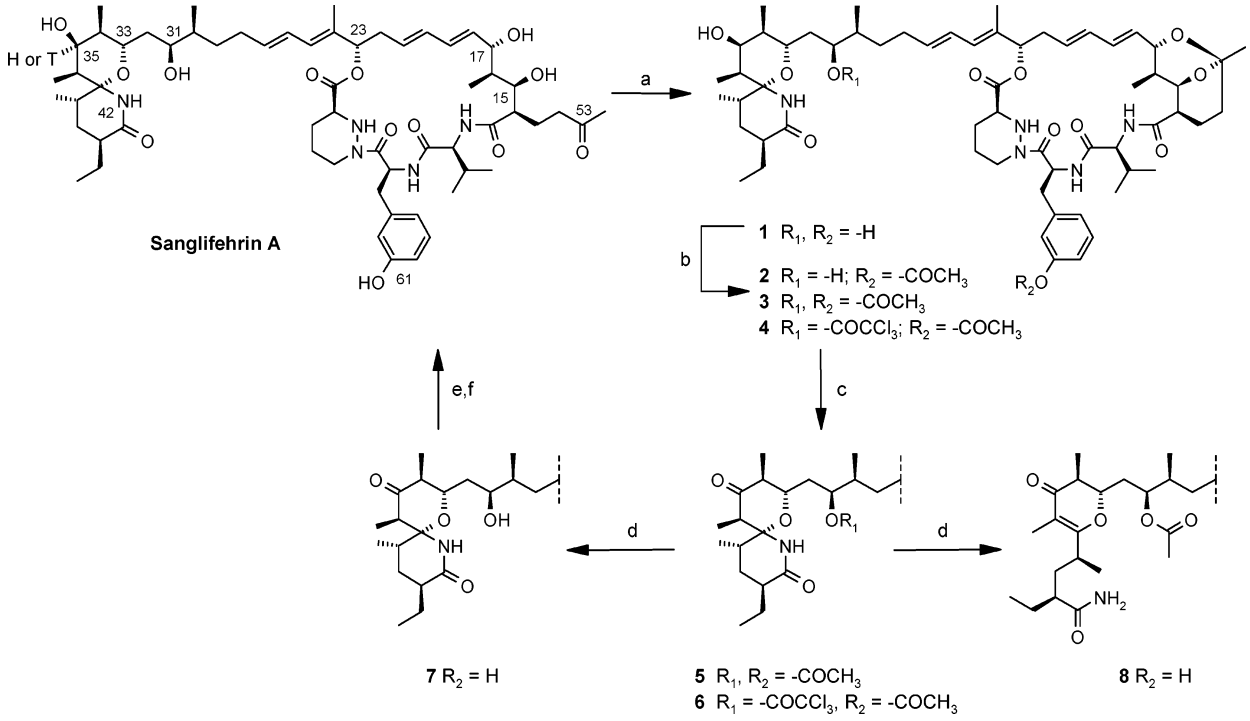
**SCHEME 1. Synthetic Approach toward Sanglifehrin A Tritium Labeled in Position $C_{35}$[a]**




[a] Reagents and conditions: (a) TFA (catalytic), $CH_3CN$, quantitative; (b) for (**2**): $Ac_2O$, DMAP (catalytic), $CH_2Cl_2$/pyridine 27:1, quantitative; for (**3**): $Ac_2O$, DMAP (catalytic), $CH_2Cl_2$/pyridine 9:1, 65%; for (**4**): (i) same as for (**2**), (ii) $(Cl_3CO)_2O$, DMAP (catalytic), $CH_2Cl_2$/pyridine 31:1, 67% (two steps); (c) Dess−Martin's periodinane, $CH_2Cl_2$, (**5**, 91% and **6**, quantitative); (d) 1.0 M aqueous $NaHCO_3$/MeOH 10:1, (**7**, 55% and **8**, 30%); (e) L-Selectride, THF, −20 °C, 50%; (f) see ref 3g.

chemoselectivity. By increasing the concentration of pyridine, the $C_{31}$,$C_{61}$-diacetylated product **3** is formed in 65% yield. Oxidation of the alcohol at $C_{35}$ with Dess−Martin's periodinane[8] afforded ketone **5** in excellent yield (91%). Deprotection of **5** under mildly basic conditions led to rapid removal of the phenolic acetate. Unfortunately, under prolonged alkaline treatment, amide **8** was isolated in 30% yield instead of the desired fully deacetylated ketone **7**. The ring-opening of the spirobicyclic subunit through α,β-keto elimination appeared to be faster than deprotection of the sterically hindered $C_{31}$ acetate. Removal of this protecting group under reductive conditions, e.g. $NaBH_4$ in EtOH or L-Selectride in THF, was not successful either, because the macrolide ester was reduced more rapidly than the $C_{31}$ acetate. Therefore, we turned our attention to the more labile trichloroacetate protecting group. Starting from *mono*-acetate **2**, selective trichloroacetylation followed by oxidation afforded intermediate **6** in excellent yield. Deprotection of **6** under mildly alkaline conditions led to the desired ketone **7**, the precursor for the stereoselective reduction, in 55% yield. As predicted by the model, all reducing agents reacted exclusively from the less hindered face of the molecule producing the desired equatorial alcohol. The highest yield (51%) was obtained with L-Selectride. The $^1H$ NMR spectra of the compound obtained by reduction of **7** were identical with the spectra of intermediate **1** obtained previously by acidic treatment of sanglifehrin A. Final deprotection of the intramolecular ketal **1** was performed as reported previously.[3g]

The synthetic route described in Scheme 1 was used to produce successfully sanglifehrin A labeled with tritium in position $C_{35}$. The approach is particularly attractive for two reasons. The precursor **7** is readily available from sanglifehrin A in five steps and 37% overall yield and the radiolabeled material needs to be carried through two synthetic steps only. The reducing agent of choice for tritium labeling was lithium tri-*sec*-butylborotritide, the tritiated analogue of L-Selectride, because it is readily available from lithium tritide and tri-*sec*-butylborane.[9,10]

In parallel, we tested a similar two-step strategy on the alcohol in position $C_{31}$. To this end, we submitted *mono*-acetate **2** to a series of oxidative conditions (Scheme 2).

Under Swern conditions or with pyridinium dichromate as an oxidant, the reaction was sluggish and led mostly to decomposition products. On the other hand, the $C_{31}$,$C_{35}$-diketone **10** could be obtained by using either tetrapropylammonium perruthenate[11] (TPAP) in the presence of 4-methylmorpholine *N*-oxide as co-oxidant (42% yield) or Dess−Martin's periodinane[8] (72% yield). Finally, selective oxidation of the $C_{31}$ alcohol was ob-

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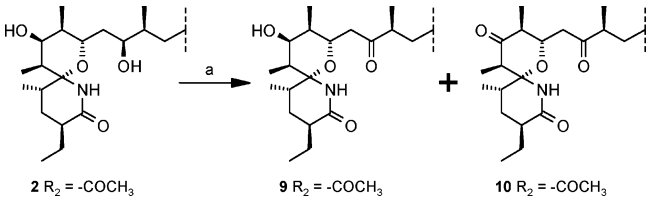
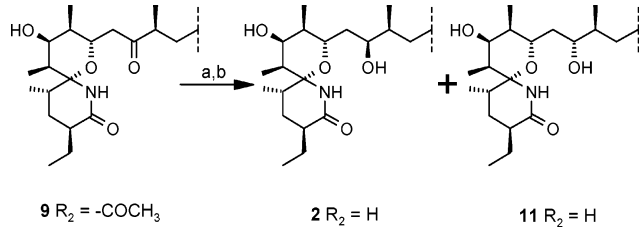
**SCHEME 2. Selective Oxidation of Alcohols at $C_{31}$ and $C_{35}$[a]**




[a] Reagents and Conditions: (a) DMF, DMSO (5%), IBX, (**9**, 60%), or Dess−Martin's periodinane, $CH_2Cl_2$, (**10**, 72%).

**SCHEME 3. Stereoselectivity of the Reduction of the Ketone at $C_{31}$[a]**




[a] Reagents and Conditions: (a) 1.0 M aqueous $NaHCO_3$/MeOH 10:1, quantitative; (b) $NaBH_4$, EtOH, 0 °C, (**1**, 16% and **11**, 39%).

served predominantly when 2-iodoxybenzoic acid (IBX),[12] the precursor of Dess−Martin's periodinane, was used as an oxidant in DMF containing 5% DMSO. Under these mild conditions, ketone **9** was isolated in 60% yield. After alkaline deprotection of the phenol, several reductive conditions were tested on the $C_{31}$ ketone **9** (Scheme 3).

Reduction with $NaBH_4$ in EtOH afforded a 1:2 mixture in favor of $C_{31}$-*epi*-hydroxy sanglifehrin A **11** as assessed by comparing the $^1$H NMR spectra with the spectra of **1**. Running the $NaBH_4$ reduction in the presence of cerium trichloride (Luche's conditions) did not modify the isomeric ratio, even though an effect of organolanthanide reagents on the stereochemical outcome of ketone reduction has been reported.[13] The more sterically hindered reducing agent, L-Selectride, afforded exclusively **11** based on HPLC analysis. No reaction was observed by using DIBAL-H at low temperature. As no conditions were found to reduce the $C_{31}$ ketone to the alcohol with the desired stereoselectivity, sanglifehrin A tritium labeled in position $C_{31}$ or doubly labeled in positions $C_{31}$ and $C_{35}$ is not accessible via the oxidation/reduction strategy.

In conclusion, the availability of radiolabeled material is essential to do a full study of the pharmacokinetic properties of promising new development compounds. Herein, we report on an efficient two-step oxidation/reduction strategy to introduce a tritium label in position $C_{35}$ of complex natural product sanglifehrin A. In this case, the synthetic approach is particularly attractive because (1) the precursor for the reduction step, intermediate **7**, is available in five steps and 37% overall yield from sanglifehrin A on large scale, (2) the reduction is fully stereoselective, (3) the reducing agent, lithium tri-*sec*-butylborotritide, used to introduce the tritium is cheap and readily available at almost theoretical specific activity, and (4) the radioactive material is involved in two synthetic steps only. In addition to this approach, possibilities to apply a similar strategy to position $C_{31}$ of sanglifehrin A were explored. The alcohol at position $C_{31}$ could be regioselectively oxidized with IBX in DMF containing 5% DMSO. Unfortunalely, reduction of the corresponding ketone led either to an epimeric mixture or, in the case of L-Selectride, selectively to the $C_{31}$-*epi*-hydroxy sanglifehrin A.

## Experimental Section

**Tritiated Sanglifehrin A.** Lithium tritide was obtained from 252 $\mu$L of *n*-butyllithium (1.6 M in hexane, 0.4 mmol) and 90 $\mu$L of TMEDA (0.6 mmol) at an initial pressure of 812 hPa of tritium gas. After stirring at 23 °C for 1 h the LiT had formed and the pressure dropped to 650 hPa indicating the uptake of 0.4 mmol of tritium. The mixture was frozen to −196 °C and the excess tritium was taken back. Hydrogen was admitted to a pressure of 400 hPa and the mixture was warmed. Then 400 $\mu$L of 1.0 M tri-*sec*-butylborane in hexane (0.4 mmol) was added, whereupon the LiT went into solution. After 30 min, 170 mg of 35-Oxo Sanglifehrin A Ketal **7** in 1.5 mL of dry THF was added dropwise at −15 °C. After stirring for 1 h the reaction was quenched with 3 mL of acetonitrile/water 1:1. After 5 min the mixture was frozen to −196 °C and the tritium−hydrogen mixture formed by hydrolysis of the reagent was taken back into a uranium trap. After evaporation of the solvents the residue was lyophilized with methanol to remove volatile and exchangeable tritium.

The total activity of the crude reaction mixture was 82.58 GBq (19.4% based on butyllithium, 47.9% based on **7**). Preparative HPLC separation on RP18 column with use of a mixture of acetonitrile and water (37 to 90% of acetonitrile within 10 min) gave 43.29 GBq of radiochemically pure tritiated Sanglifehrin A Ketal **2**.

Ketal (3.7-GBq) was then deprotected with a mixture of 100 $\mu$L of THF, 100 $\mu$L of water, and 20 $\mu$L of 2 N HCl. After 30% conversion the reaction mixture was submitted to preparative HPLC and the unchanged starting material was resubmitted to the deprotection. After 5 cycles a total yield of 1.095 GBq of [$^3$H]-Sanglifehrin A (29.6%) at a specific activity of 0.943 TBq/mmol and a radiochemical purity of 97.34% was obtained.

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**Supporting Information Available:** Full experimental details and spectral characterization of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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