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# Stereoselective Total Synthesis of (−)-Depudecin

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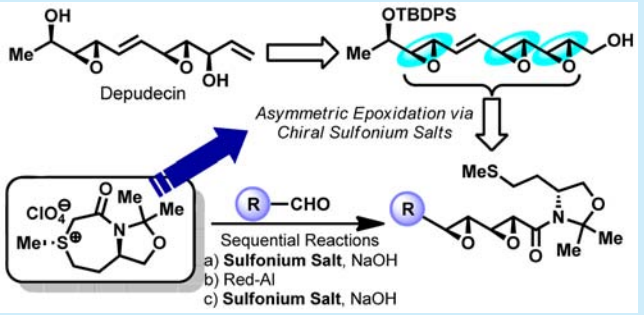
Supporting Information

**ABSTRACT:** The total synthesis of the natural product depudecin, an antiangiogenic microbial polyketide with inhibitory activity against histone deacetylases, is reported. Characterized by a highly oxidized 11-carbon chain containing two epoxides conjugated through a *trans*-disubstituted olefin, its total synthesis was efficiently accomplished by a novel asymmetric methodology of epoxide formation based on a new class of chiral sulfonium salts, allowing for the construction of the oxirane rings in an efficient and stereoselective fashion.




In 1992, Matsumoto and co-workers discovered depudecin (**1**) (Figure 1) from the culture broths of the fungus *Alternaria brassicicola* as part of a screening program directed toward the search for antitumor agents with detransforming activity.[1,2] Rapidly, this new metabolite became the focus of biologists and biochemists due to its fascinating cell differentiation−modulation activity and its unprecedented structure. Its structure features the presence of two oxirane rings separated by a *trans* double bond, which was determined and confirmed by the same authors using X-ray diffraction analysis of the corresponding bis(1*S*)-(−)-camphanate derivative.[2] Some years later, depudecin (**1**) was also isolated from the weed pathogen *Nimbya scirpicola* by Tanaka et al., who demonstrated its phytotoxicity toward the host plant of the fungus, *Eleocharis kuroguwai*, and toward other tested plants.[3]

The ability of depudecin to revert the transformed morphology of tumor cells to a normal cell rendered it a valuable and outstanding molecular probe for the investigation of signaling pathways involved in many biological processes, such as the organization of actin.[4] Notably, Schreiber et al. identified the molecular target(s) of depudecin and found evidence that this potential antitumor drug belongs to an expanding group of molecules capable of inhibiting histone deacetylases (HDAC).[5] In contrast to the previously identified inhibitors of HDAC[6] such as trichostatin A (**2**), trapoxin (**3**), and largazole (**4**) (Figure 1), depudecin (**1**) possesses a unique chemical structure that might increase its selectivity toward these biological targets.

Histone deacetylases (HDACs), the enzymes responsible for the removal of the acetyl group from lysine residues of histones and other proteins, play a key role in the regulation of gene expression and chromatin assembly.[7] There are 18 HDAC isoforms in four phylogenetic groups (classes I−IV). The enzymatic action of HDACs explains their involvement in a plethora of biological functions that include regulation of the cell cycle and mitosis, DNA damage response, cellular stress response, protein degradation, cytokine signaling, immunity and inflammation, angiogenesis, apoptosis, and cell invasion. Therefore, inhibition of HDACs represents a novel strategy in human cancer therapy. In fact, there are currently three HDAC inhibitors (HDACi) (vorinostat, belinostat, and romidepsin) FDA-approved as anticancer agents.[8] In addition, HDACs represent valuable tools for unravelling the mechanisms and functions of these enzymes, which to date remain unclear.[9]

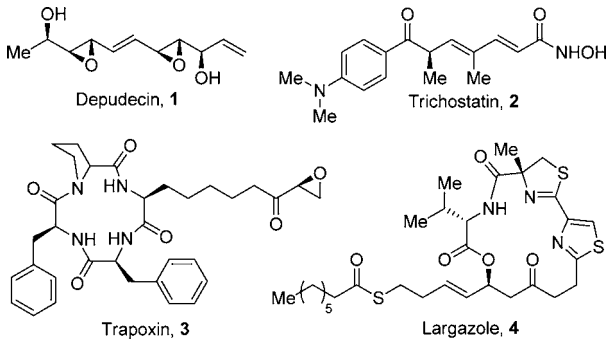



**Figure 1.** Molecular structures of depudecin and other representative HDAC inhibitors.

As proof of the potential utility of depudecin in cancer therapy, as a consequence of its inhibitory capacity against HDAC, Oikawa et al. described its antiangiogenic activity *in vivo*.[10] In addition to these biological studies, the biosynthetic origin of depudecin has similarly encouraged remarkable interest leading to the identification of the six-gene clusters that encode the enzymes responsible for the biosynthesis of depudecin.[11]

Despite the enticing structural and biological features of depudecin, only one total synthesis of (−)-depudecin has been

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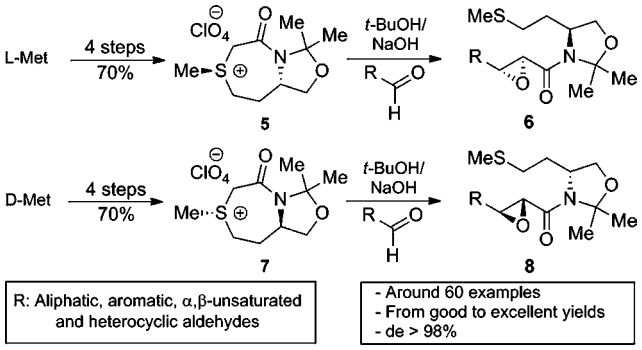
reported thus far by the Schreiber group according to a strategy that also provided access to depudecin-related compounds.[12] The total synthesis proceeded in 24 steps with an overall yield of 0.7% and possessed two key drawbacks, the use of an excess of mercuric chloride for final deprotection steps and the necessity of an oxidation−reduction sequence to obtain the *syn*-diol systems, resulting in a long, linear, and environmentally unfriendly synthesis. Nevertheless, this synthetic work provided access to sufficient amounts of (−)-depudecin, which was employed for biological studies that led to the elucidation of its mechanism of action. In addition, the synthesis provided depudecin-related intermediates, such as mono-methylthiomethyl- and bis-methylthiomethyl-protected ethers derivatives, which were biologically inactive, demonstrating that both the epoxide and hydroxyl groups were essential for the detransforming activity of depudecin.

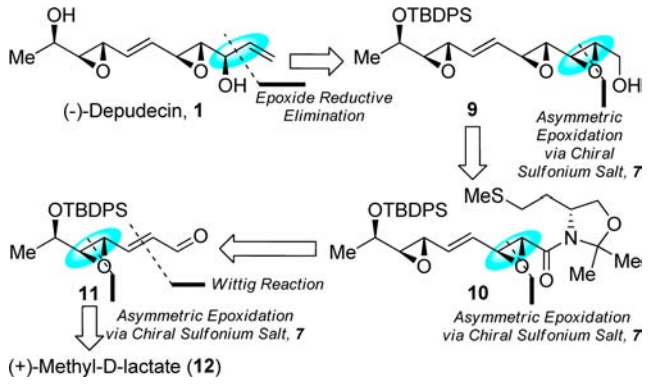
Given the potential that depudecin represents in the field of antitumorals and as a molecular probe to better understand the enzymatic mechanisms of action of HDAC, we decided to embark on the design of an efficient and readily accessible route to its synthesis. The route would be based on the use of a new class of chiral sulfonium salts[13] **5** and **7** for the construction of the oxirane rings found in the natural product. This methodology utilizing asymmetric sulfur ylide mediated epoxidation has proven to be a highly efficient means for the construction of epoxy amides of types **6** and **8**, displaying generality, scope, and stereoselectivity.[14,15] Furthermore, it has been successfully applied to the synthesis of various natural products of biological interest, such as the bengamides,[16] the cyclodepsipeptides globomycin and SF-1902 $A_5$,[17] and the sphingoid-type bases such as clavaminol H, phytosphingosine, sphinganine, or sphingosine[18] (Scheme 1).

**Scheme 1. Cyclic Sulfonium Salts Derived from L- and D-Methionines: Synthesis and Reactivity**



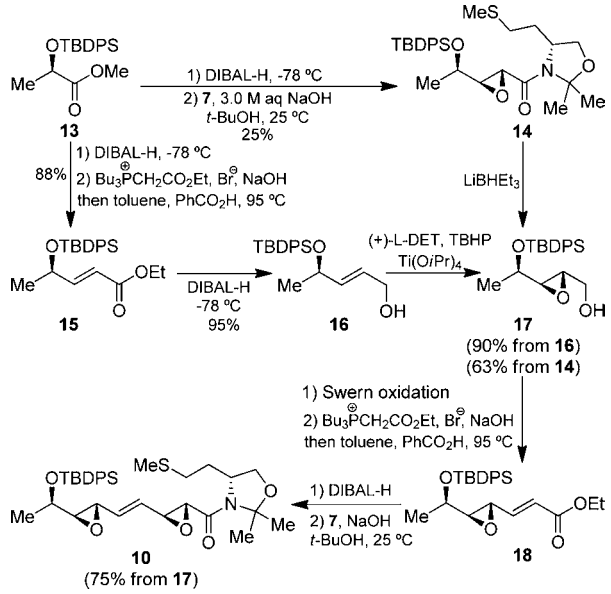

For the preparation of (−)-depudecin (**1**), we conceived of a linear approach, based on the aforementioned methodology of asymmetric epoxidation for the sequential construction of the oxirane rings. Thus, according to the retrosynthetic analysis depicted in Scheme 2, (−)-depudecin (**1**) could be obtained from triepoxy alcohol **9** through a reductive opening process in the synthetic direction. This key advanced precursor **9** could be obtained from diepoxy amide **10** via asymmetric epoxidation mediated by the sulfonium salt **7**. Diepoxy amide **10**, in turn, was traced back to the *α,β*-unsaturated *γ,δ*-epoxy aldehyde **11**, which was envisioned to be readily accessed from the commercially available (+)-methyl-D-lactate (**12**) via reaction of its corresponding protected aldehyde with sulfonium salt **7**.

**Scheme 2. Retrosynthetic Analysis of (−)-Depudecin**




The synthesis of the required key precursor **10** proceeded as shown in Scheme 3. Thus, beginning from (+)-methyl-D-lactate

**Scheme 3. Synthesis of Key Diepoxy Amide 10**




**12**, the alcohol was protected as the silyl ether **13** and the ester reduced to the aldehyde. The crude aldehyde was then reacted with the sulfonium salt **7** to obtain the epoxy amide **14**, albeit in a poor yield (25%). Several attempts were made to improve the yield of epoxy amide **14**, with no success. In light of these discouraging results, we decided to utilize the Sharpless methodology[19] to prepare the required starting epoxide. Thus, the crude aldehyde obtained from **13** was subjected to a Wittig reaction, according to the modified Martin conditions,[20] to generate the corresponding *trans-α,β*-unsaturated ester **15** in 88% overall yield. The synthesis of the key epoxy alcohol proceeded with reduction of the ester **15** into the allylic alcohol **16** by treatment with DIBAL-H followed by a Sharpless asymmetric epoxidation (SAE) with (+)-L-DET to afford the desired epoxy alcohol **17** in 90% yield. Conveniently, the synthesis of the epoxy alcohol allowed confirmation of the stereoselectivity of the asymmetric epoxidation reaction with sulfonium salt **7** in the preparation of epoxy amide **14**. To this aim, **14** was transformed into epoxy alcohol **17** by treatment with lithium triethylborohydride (Super-H) in 63% yield. The comparison of the spectroscopic and physical properties of **17** obtained by both routes confirmed the high stereoselectivity of



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Table 1. $^{1}H$ (400 MHz) and $^{13}C$ NMR (100 MHz) Data of Natural and Synthetic (−)-Depudecin in $CDCl_3$[a]

| | natural (−)-depudecin[b] | | synthetic (−)-depudecin[c] | | synthetic (−)-depudecin[d] | |
|---|---|---|---|---|---|---|
| position | $\delta_H$ (multi, $J$, Hz) | $\delta_C$ | $\delta_H$ (multi, $J$, Hz) | $\delta_C$[e] | $\delta_H$ (multi, $J$, Hz) | $\delta_C$ |
| 1 | 1.29 (d, 6.5) | 20.05 | 1.32 (d, 6.5) | 20.00 | 1.30 (d, 6.5) | 20.1 |
| OH | | | 1.77 (d, 6.0) | | 1.80 (d, 6.0) | |
| OH | | | 1.90 (d, 6.4) | | 1.94 (d, 6.3) | |
| 2 | 3.72 ($dq_{br}$, 6.5, 4.7) | 67.34 | 3.74−3.79 (m) | 64.05 | 3.74 ($dq_{br}$, 6.5, 4.5) | 64.2 |
| 3 | 2.90 (dd, 4.7, 2.2) | 64.50 | 2.92 (dd, 4.5, 2.2) | 62.39 | 2.90 (dd, 4.5, 2.2) | 62.5 |
| 4 | 3.37 (m) | 55.67 | 3.39−3.41 (m) | 55.28 | 3.38 (ddd, 5.6, 3.5, 2.2) | 55.4 |
| 5 | 5.69 (m) | 132.55 | 5.71−5.73 (m) | 132.01 | 5.70 (m) | 132.1 |
| 6 | 5.70 (m) | 132.06 | | 131.49 | | 131.6 |
| 7 | 3.42 (m) | 55.27 | 3.44−3.46 (m) | 54.87 | 3.42 (ddd, 5.6, 3.5, 3.74) | 55.0 |
| 8 | 3.00 (dd, 4.5, 2.2) | 67.85 | 3.03 (dd, 4.2, 2.2) | 66.88 | 3.01 (dd, 4.3, 2.2) | 67.0 |
| 9 | 4.10 (dddd, 5.5, 4.5) | 71.96 | 4.15 (d, 5.4) | 71.46 | 4.13 (m) | 71.6 |
| 10 | 5.92 (ddd, 17.1, 10.5, 5.5) | 136.55 | 5.95 (ddd, 17.2, 10.6, 5.5) | 136.12 | 5.93 (ddd, 17.3, 10.6, 5.5) | 136.2 |
| 11 | 5.29 (ddd, 10.5, 1.4) | 117.50 | 5.29 (dt, 10.6, 1.2) | 117.07 | 5.27 (dt, 10.6, 1.4) | 117.2 |
| | 5.38 (ddd, 10.5, 1.4) | | 5.41 (dt, 17.3, 1.3) | | 5.39 (dt, 17.3, 1.4) | |

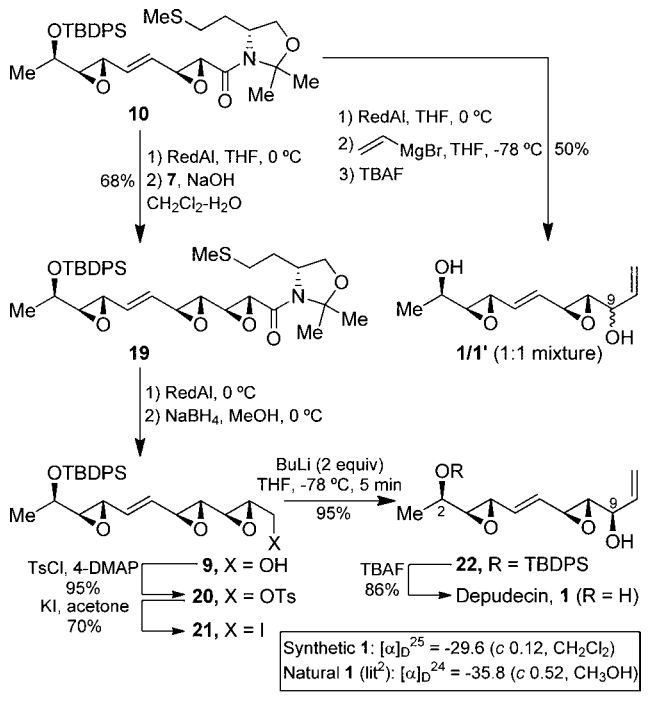
[a]Chemical shifts (ppm) referenced to $CDCl_3$ ($\delta_H$ 7.26; $\delta_C$ 77.00). [b]Data reported by Matsutani in ref 2. [c]Data reported in the present article for synthetic (−)-depudecin. [d]Data reported by Schreiber in ref 12. [e]$^{13}C$ NMR spectra was recorded on a 600 MHz instrument (150 MHz).

our epoxidation methodology, estimated to be in a diastereomeric excess (de) greater than 98% (Scheme 3). It is noteworthy to highlight the exquisite stereocontrol displayed by this class of sulfonium salts, even for mismatched pairs such as the case of the aldehyde derived from **13** and sulfonium salt **7**. In this case, as with others previously reported by us,[13,18] the chirality of the starting aldehyde did not override the asymmetric induction exerted by the sulfonium salt, resulting in the complete formation of epoxy amide **14**, which corresponds to the unfavored Felkin−Ahn product,[21] and no detection of its diastereoisomer. As continuation of our synthesis, we then proceeded with the synthesis of the diepoxy amide **10** from epoxy alcohol **17** by a synthetic sequence that involved the following: (1) oxidation of epoxy alcohol **17** into the aldehyde by treatment with $SO_3$·Pyr complex,[22] (2) a Wittig reaction, (3) reduction of the resulting ester **18** to the corresponding $\alpha,\beta$-unsaturated aldehyde **11** by treatment with DIBAL-H, and finally, (4) reaction of **11** with the sulfur ylide derived from sulfonium salt **7**. To our delight, this four-step sequence efficiently provided the essential fragment **10** in 75% overall yield and as a single diastereoisomer (Scheme 3).

In an effort to reduce the number of steps, a straightforward addition of the appropriate vinyl Grignard reagent was explored. To this aim, epoxy amide **10** was treated with RedAl and the resulting epoxy aldehyde reacted with vinylmagnesium bromide, followed by desilylation with TBAF of the crude mixture. Although several conditions were tested, all of them resulted in an inseparable mixture of isomers corresponding to depudecin (**1**) and its C-9 epimer **1′** in a 1:1 ratio and in low yields, likely due to the decomposition of the starting epoxy aldehyde. This disappointing and unexpected result led us to resume the initial strategy by synthesizing the triepoxy amide **19**. Thus, **19** was obtained as a single diasteroisomer in 68% yield over two steps by reaction of the corresponding epoxy aldehyde, resulting from the treatment of **10** with RedAl with sulfonium salt **7** according to the two-phase method ($CH_2Cl_2$/aq NaOH).[23] This highly valuable triepoxy amide was then converted into the targeted triepoxy alcohol **9** by reaction with RedAl, followed by treatment with $NaBH_4$, in 90% overall yield (Scheme 4).

With the triepoxy alcohol **9** in hand, we proceeded toward the completion of the synthesis of (−)-depudecin. To this end, we initially converted compound **9** into the tosylate derivative **20**, which was then subjected to the action of KI in dry acetone to provide iodide **21** without difficulty. With the aim of obtaining the allylic alcohol **22** in a stereoselective fashion, we explored various methodologies for reductive opening (i.e., Zn/EtOH; $Ph_3P/I_2$, LDBB),[24] identifying that treatment with BuLi at −78 °C[25] for 5 min provided the best method for the preparation of **22**, which was obtained in 95% yield. Once the precursor **22** was prepared, final desilylation was achieved by treatment with TBAF to obtain (−)-depudecin (**1**) in 85% yield as a single diastereoisomer (Scheme 4), whose spectroscopic and physical data matched with those reported for the natural product (Table 1).

Scheme 4. Completion of the Synthesis of (−)-Depudecin



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In conclusion, we have established and completed the synthesis of (−)-depudecin (**1**) in 17 steps and 19% overall yield, providing a highly efficient and streamlined synthetic route for depudecin. The route is a significant improvement over the previously reported synthesis and makes this interesting and unexplored natural product readily available for further biological investigations. In addition, this synthetic scheme is amenable to modifications, paving the way for the design of depudecin analogues. The synthesis and biological evaluation of depudecin-based analogues as novel HDAC inhibitors with novel chemical properties and potential isoform specificity represent our priorities in current and future investigations.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b02697.

Experimental procedures, spectral data, and NMR spectra for all new products (PDF)

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### Notes

The authors declare no competing financial interest.

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