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# Synthesis of New Biotin Derivatives

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**Abstract**—It is now established that the formation of C–S bonds during the conversion of dethiobiotin into biotin involves intermediate carbon radicals. To examine the behavior of dethiobiotin analogs that could lead to allylic and α-epoxyradicals, we have synthesized 4,5-dehydrodethiobiotins and the corresponding epoxides and 5,6-dehydrodethiobiotins. All these compounds have been labelled with $^{14}C$ on the carboxyl group. 

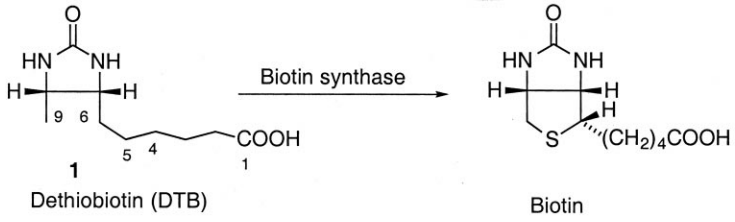
## Introduction

The last step of biotin biosynthesis, namely the introduction of sulfur into dethiobiotin (DTB) catalyzed by biotin synthase has long raised a very puzzling problem (Scheme 1).

However, in the last five years, significant progress has been made in the understanding of this complex mechanism. It is now well established that biotin synthase, an [Fe–S] protein is an S-adenosylmethionine (AdoMet) dependent enzyme.[1] The reductive cleavage of AdoMet generates a deoxyadenosyl radical which is responsible for homolytic C–H bond cleavage at positions 6 and 9 of DTB.[2] We have also recently shown that the sulfur source is very likely the [Fe–S] center of the protein.[3]

Much more work is, however, necessary to understand in detail this unprecedented mechanism. One of the approaches that we are considering is to look at the behavior of analogs of DTB containing radical clocks, namely epoxy and cyclopropyl groups α to the radical produced at C-6.[4]

We have already described the synthesis of their ethylenic precursors *Z* and *E*-4,5-dehydrodethiobiotin **2a** and **2b**.[5] Both compounds are recognized by the enzyme and were found to covalently bind to the protein when all the cofactors necessary for the reaction are present. They are the first mechanism-based inhibitors of biotin synthase.[5]




**Scheme 1.**

This interesting result encouraged us to undertake the synthesis of the epoxy and cyclopropyl derivatives **3a**, **3b** and **4a**, **4b**.

We also targeted the 5,6-dehydrodethiobiotins **5a**, **5b,** which could be of interest for the enzymatic studies (Scheme 2).

## Results and Discussion

### Synthesis of dehydrodethiobiotins

We report first a new synthesis of the intermediate alcohols **6a**, **6b** presenting several advantages over the previously described one, which was based on the construction of the imidazolidinone ring with pent-3-ynol as starting material.

*Keywords*: epoxides; labelling; phosphine ylids; biotin synthase.

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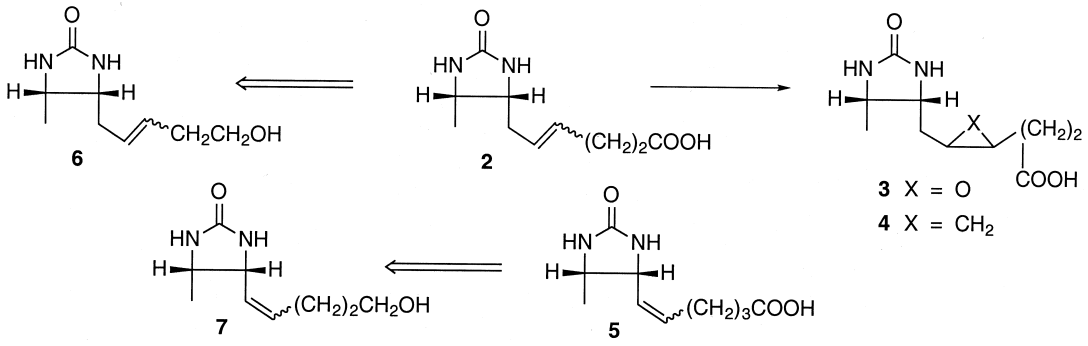


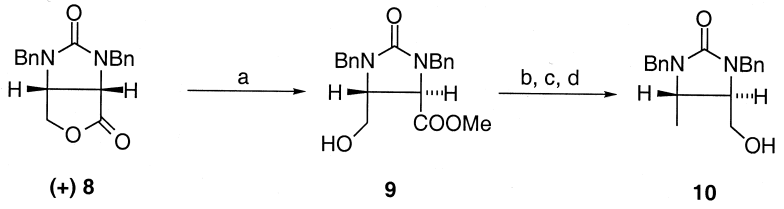

**Scheme 2. 2a** and **7a**, *Z* isomer; **2b** and **7b**, *E* isomer.

It was rather long with one non-stereospecific step leading to a 90:10 mixture of stereoisomers, which were extremely difficult to separate.[5]

A more attractive starting material was the enantiomerically pure lactone (+)**8**, an intermediate in the Hoffmann–La Roche total synthesis of biotin,[6] which we have successfully used in the past for the synthesis of 9-mercaptodethiobiotin.[7] However, our first attempts to introduce an unsaturated side chain failed because of the participation of the hydroxyl group generated at C-9. It was therefore reasonable to deoxygenate this position prior to any side-chain construction.

The first step consisted of the lactone ring opening (Scheme 3).

Under acidic conditions, (+)**8** was recovered unchanged, very likely because of an unfavorable equilibrium position. Basic conditions led to an opened product **9**. Due to the possibility of epimerization at C7, we checked the stereochemistry of **9**. The $^{3}J_{H_7-H_8}$ value, which could be an argument, is ambiguous: 4.5 Hz for *trans* DTB, 8 Hz for *cis* DTB, 6.1 Hz for **9**. The ambiguity was removed by X-ray analysis of **10**, obtained from **9** through a Barton-type deoxygenation followed by $LiAlH_4$ reduction. The 3D structure clearly establishes the *trans* relationship of the two substituents (Fig. 1).




**Scheme 3.** (a) MeONa, MeOH, reflux; HCl, 69%; (b) 4-FPTCl, pyridine, $CH_2Cl_2$, RT, 94%; (c) $Bu_3SnH$, AIBN, $PhCH_3$, reflux; (d) $LiAlH_4$, THF, 0°C, $H^+$, steps c, d 47%.

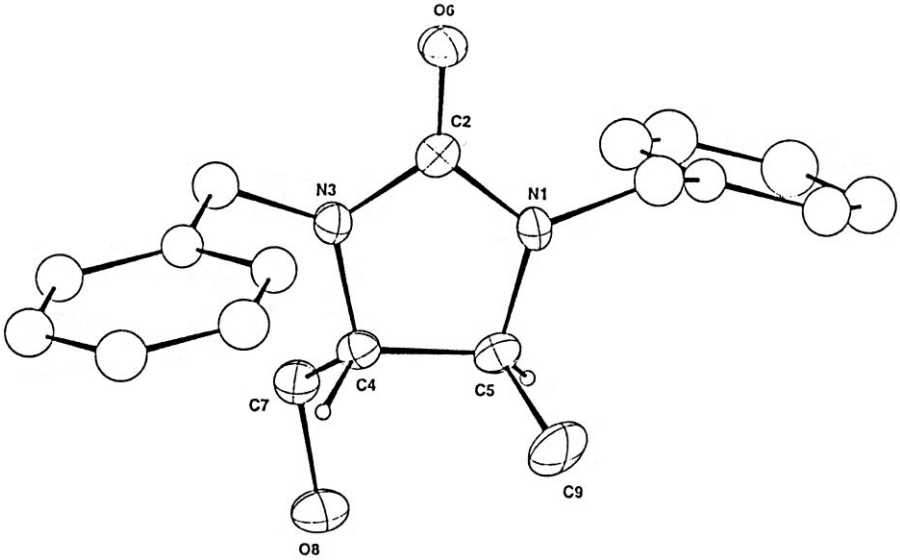
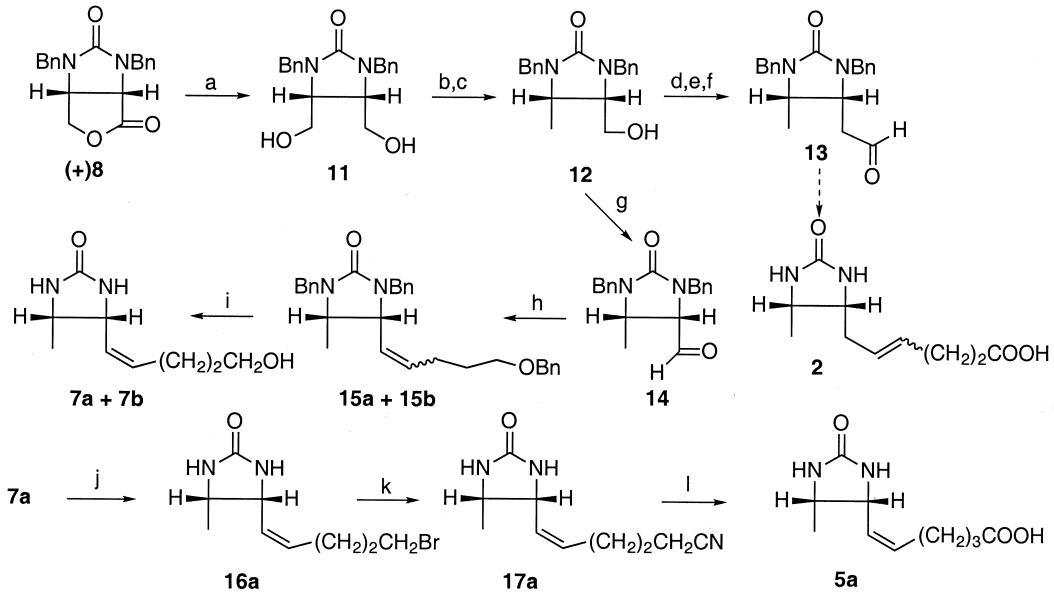



**Figure 1.** X-Ray crystal structure determination of compound **10**.




**Scheme 4.** (a) $LiAlH_4$, THF, 0°C, 98%; (b) 4-FPTCl, pyridine, $CH_2Cl_2$, −30°C, 49%; (c) $Bu_3SnH$, AIBN, $PhCH_3$, reflux, 57%; (d) MsCl, $NEt_3$, $CH_2Cl_2$, RT, 80%; (e) KCN, 70°C, 89%; (f) DIBAH, $PhCH_3$, −70°C, 70%; (g) $(COCl)_2$, DMSO, $NEt_3$, $CH_2Cl_2$, −78°C; (h) $Ph_3P^+(CH_2)_4OBn$, $Br^-$, nBuLi, THF, −60°C, steps g, h: 54%, *Z*/*E*=90:10; (i) Na, $NH_3$, EtOH, 40%, HPLC; (j) $Ph_3PBr_2$, pyridine, $CH_3CN$, RT; (k) KCN, DMSO, RT; (l) NaOH 1N, reflux, steps j, k, l: 41%.

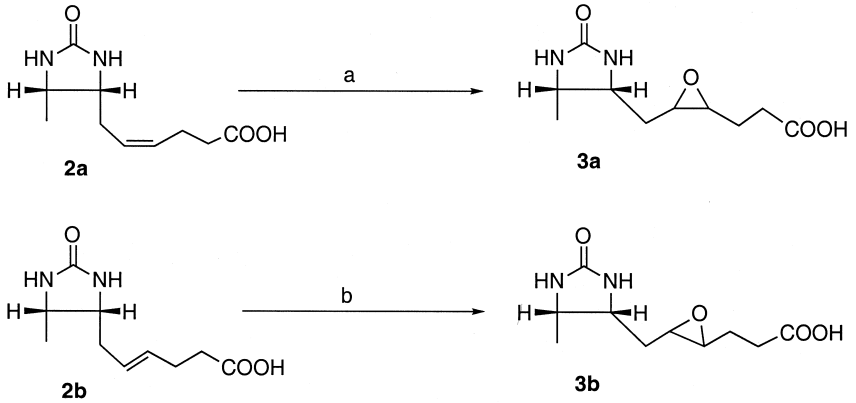
The *cis* stereochemistry on the imidazolidinone ring being a priori crucial for enzyme recognition, we decided to sacrifice the chirality and to fully reduce the lactone into the *cis* diol **11** (Scheme 4).

A Barton-type deoxygenation[8] gave the key intermediate **12**. **12** was transformed in three steps, mesylation, cyanation and DIBAH reduction into the aldehyde **13** which was converted into the two isomeric 4,5-dehydrodethiobiotins **2** as already described.[5]

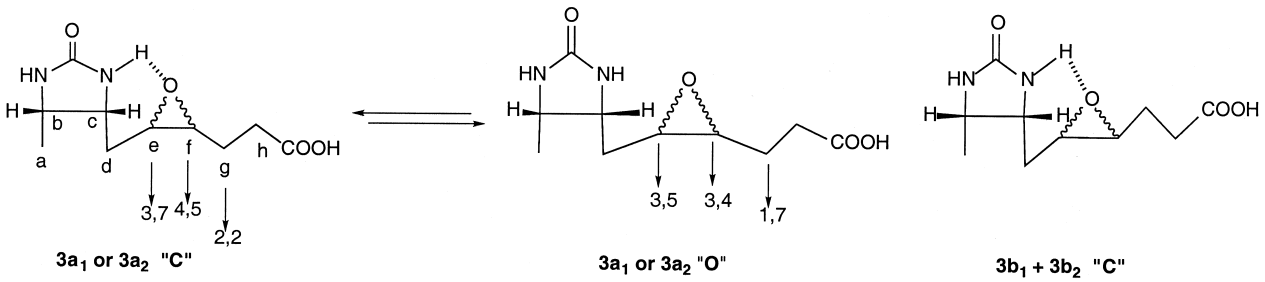
The regioisomeric 5,6-dehydrodethiobiotin **5a** was also obtained from **12**. **12** was oxidized to the aldehyde **14** which was submitted to a Wittig reaction. The best yield of **15** was obtained in THF at −70°C. The yield was lower (≈30%) in THF or THF/HMPT at 0°C. Under all conditions, a *Z*/*E* ratio of 90:10 was observed. Surprisingly, this ratio did not change when we used the Schlosser modification[9] under the conditions used for the homologous aldehyde **13**.[5] In this latter case, the Schlosser reaction gave, as expected, a higher proportion of the *E* isomer.[5] In the case of **14**, the strong brown color that appeared after the addition of the second butyl lithium equivalent indicated that the betaine ylide was formed but the composition did not change for unknown reasons. Either they did not equilibrate or the proportions correspond to the equilibrium mixture. **15** was debenzylated and its major component, the *Z* isomer **7a**, was then purified by crystallization. The *E* isomer was obtained by HPLC purification of the residue. The major product **7a** was finally converted into *Z*-5,6-dehydrodethiobiotin **5a** through the reaction sequence already reported.[5]

## Synthesis of the epoxides 3a and 3b

The *Z*-4,5-dehydrodethiobiotin **2a** was readily epoxidized with mCPBA into a 50:50 mixture of the *cis* epoxides **3a** (Scheme 5). Under the same conditions, a very slow reaction was observed with the *trans* isomer **2b** and if larger amounts of mCPBA were used, only degradation products were obtained.




**Scheme 5.** (a) mCPBA, $CH_2Cl_2$, RT, 90%; (b) $H_2O_2$, MTO, $H_2O$, RT, 74%.




**Scheme 6.**

Reaction with dimethyloxirane, a more powerful reagent[10] also more suitable for the oxidation of small amounts of radioactive compounds, was also investigated. With **2b** a complete degradation was observed and with **2a** the yield in epoxides **3a** was lower that with mCPBA.

Finally, the best results were obtained with $H_2O_2$ in the presence of methyltrioxorhenium,[11] which allowed the epoxidation of **2b** in 74% yield leading also to a 50:50 mixture of the two epoxides **3b**.

The *cis* epoxides $\mathbf{3a_1}$ and $\mathbf{3a_2}$ were separated by HPLC. Interestingly, the NMR spectrum of each of them, recorded in methanol and in DMSO as well, revealed the presence of two forms in equilibrium. The first one, unique just after dissolution of the dried compound is slowly transformed into a second one until a stable 60:40 composition is obtained. The phenomenon is reversible since after drying this mixture again, only the first form is observed just after dissolution. Relying on the strong deshielding of protons e and especially f in that form (Scheme 6) we assume that we are observing an equilibrium between an hydrogen-bonded 'closed' form (C) and an 'opened' form (O).

The *trans* epoxides $\mathbf{3b_1}$ and $\mathbf{3b_2}$ could not be separated by HPLC. The NMR spectrum of the mixture shows that each of them is present under only one form in which $\delta$ $H_f$ is around 4.5 ppm. They probably exist exclusively in the (C) form.

Attempts to synthesize the cyclopropyl derivatives **4** were performed on the Z protected derivative because the solubility of the deprotected form **2a** was incompatible with the solvents required for the cyclopropanation (Fig. 2).

$CH_2I_2/Zn$[12] at reflux of dimethoxyethane (DME) or dichloroethane (DCE) with sonication or $ClCH_2I/Et_2Zn$[13] in DCE or benzene at room temperature left the starting material unchanged. Steric hindrance by the *N*-benzyl group is probably responsible for this lack of reactivity.

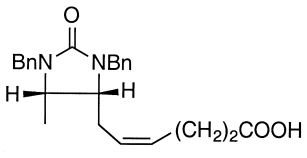



**Figure 2.** Protected **2a**.

## Preparation of the [$^{14}$C] labeled compounds

The synthesis of [$^{14}$C]**2a** and [$^{14}$C]**2b** has already been described.[5] [$^{14}$C]**5a** was obtained from **7a** following the same procedure.

The epoxides [$^{14}$C]**3a** and [$^{14}$C]**3b** were obtained by oxidation of [$^{14}$C]**2a** and [$^{14}$C]**2b**, respectively, with $H_2O_2$ in the presence of MTO. [$^{14}$C]$\mathbf{3a_1}$ and [$^{14}$C]$\mathbf{3a_2}$ were separated by HPLC. [$^{14}$C]$\mathbf{3b_1}$ and [$^{14}$C]$\mathbf{3b_2}$ could not be separated and were recovered as a mixture.

## Preliminary biological evaluation

The ethylenic compounds **2a**, **2b**, **5a** and the epoxides $\mathbf{3a_1}$, $\mathbf{3a_2}$ and $\mathbf{3b_1}+\mathbf{3b_2}$ were evaluated under different conditions with purified *E. coli* biotin synthase, in the in vitro system described by our group containing besides the enzyme, S-Adenosyl Methionine, an electron transfer system (NADPH, flavodoxin, flavodoxin reductase) and different cofactors.[2a]

They were first tested as substrates: the supernatants of the incubation media were assayed for their growth promoting activity of *Lactobacillus plantarum*, a biotin auxotroph commonly used for biotin microbiological determination.[14] The epoxides **3a** and **3b** did not promote the growth of *L. plantarum*. On the other hand, the ethylenic compounds did, with the order of efficiency $\mathbf{2a}>\mathbf{5a}\gg\mathbf{2b}$.

TLC was then performed on these supernatants. Revelation of the plates with *L. plantarum* showed that the compounds which promoted the growth of the microorganism had the same $R_F$ as biotin.

The analogs may have been transformed into biotin analogs with unsaturated side-chains, supporting the growth of *L. plantarum*.

When the radioactive compounds were incubated under the same conditions and the supernatant analyzed by TLC, no new radioactive compounds could be detected. Due to the very low conversion yield, the amount of radioactivity expected for these compounds lies in the range of the noise.

The in vitro system that we are using is presently not catalytic. With the natural substrate, DTB, less than one mole of biotin is produced per mole of enzyme. With the analogs, which are worse substrates, we expect very tiny amounts and we cannot consider determination of the

structures of the products. This should become possible once a more efficient in vitro system is available.

## Experimental

All reactions using nonaqueous reagents were run under a dry argon atmosphere. Organic layers were dried on magnesium sulfate ($MgSO_4$). Flash chromatographies were performed on Kieselgel 60 Merck (230–400 mesh). Reaction progress was monitored by analytical TLC on silica gel 60 F-254 from Merck. Visualization of TLC was done by phosphomolybdic acid or by UV light or paradimethylamino cinnamaldehyde for the amido groups. $^1H$ and $^{13}C$ NMR spectra were recorded on a Bruker AR X 400 (400 and 100 MHz, respectively). Solutions in chloroform-*d* ($CDCl_3$) were used unless otherwise stated, coupling constants have been obtained by spin decoupling. Mass spectra (CI) were carried out on a Nermag R10-10C spectrometer using $NH_3$ as reactant gas. Melting points were measured on a Kofler apparatus. Elemental analyses were performed at the Service Régional de Microanalyse (SIAR-Jussieu). All chemicals were purchased from Aldrich or Acros. [$^{14}C$] KCN was from NEN. Analytical HPLC was performed with a Perkin Elmer pump, a PE 785 A absorbance detector and a PE Model 1022 recorder. Counting was performed with a LKB 1214 Rackbeta scintillation counter, using Aquasol-2 (Packard) or Optiphase 'Hisafe' (Wallac) as scintillation liquid. Radioactive TLC plates were scanned with an automatic TLC linear analyzer Berthold LB 2821.

***trans*-1,3-Dibenzyl-5-hydroxymethyl-4-methoxycarbonyl-imidazolidin-2-one 9.** At room temperature, **8** (15 g, 47 mmol) and then sodium methoxide (0,38 g, 7 mmol) were successively dissolved in anhydrous methanol (30 mL). The mixture was refluxed for 4 h. After neutralization with 12N HCl, evaporation and purification on a silica gel column ($C_6H_{12}$:$CH_3CO_2C_2H_5$, 9:1 to 7:3), **9** was obtained as a white solid (11.5 g, 69%) which crystallized from $C_6H_{12}$:$CH_3CO_2C_2H_5$, mp: 105–107°C. Starting material **8** was also recovered 2.7 g (25%). $^1H$ NMR: $\delta$ 1.83 (s, 1H, O*H*); 3.43–3.48 (m, 1H, C*H*H); 3.50–3.53 (m, 1H, C*H*N); 3.58–3.62 (m, 1H, C*H*H); 3.60 (s, 3H, COOC*H*$_3$); 3.91 (d, 1H, *J*=6.1 Hz, C*H*N); 4.22; 4.32; 4.65; 4.93 (4d, 4H, $J_{Gem}$=15.2 Hz, 2NC*HH*Ph); 7.23–7.35 (m, 10H, arom.). $^{13}C$ NMR: $\delta$(46.49 et 46.89 (2N*C*H$_2$Ph); 52.35 (COO*C*H$_3$); 56.52 (C*H*N); 57.97 (*C*HN); 60.90 (*C*H$_2$); 127.57–137.16 (arom.); 159.95 (C=O); 170.91 (*C*OOCH$_3$). Anal. calcd for $C_{20}H_{22}N_2O_4$: C, 67.82; H, 6.21; N, 7.91. Found: C, 67.70; H, 6.25; N, 7.91.

***trans*-1,3-Dibenzyl-4-hydroxymethyl-5-methyl-imidazolidin-2-one 10. 9** (7.68 g, 21.7 mmol), 4-fluorophenylchlorothionoformate (4-FPTCl) (3.35 mL, 23.9 mmol) and pyridine (2.28 mL, 28.2 mmol) were dissolved in anhydrous $CH_2Cl_2$ (20 mL). After 15 min stirring at room temperature $CH_2Cl_2$ (300 mL) was added. The organic solvents were washed with HCl (2×20 mL) then with saturated NaCl (10 mL) and evaporated under vacuum. After crystallization ($C_5H_{12}$:$CH_3COOC_2H_5$) the FTP derivative was obtained as a white solid (10.4 g, 94%). mp 108–110°C. $^1H$ NMR: $\delta$ 3.65 (s, 3H, COOC*H*$_3$); 3.80–3.83 (m, 1H, C*H*N); 3.85 (d, 1H, *J*=5.6 Hz, C*H*N); 4.18; 4.24; 4.92; 5.08 (4d, 4H, $J_{Gem}$=15.2 Hz, 2NC*HH*Ph); 4.32 (dd, 1H, *J*=4.1 Hz, $J_{Gem}$=11.7 Hz, C*H*H); 4.54 (dd, 1H, *J*=3.6 Hz, $J_{Gem}$=11.7 Hz, CH*H*); 6.99–7.03 (m, 2H, 2 C*H*CO); 7.07–7.14 (m, 2H, 2C*H*CF); 7.23–7.36 (m, 10H, 2 NCH$_2$*Ph*). $^{13}C$ NMR: $\delta$ 42.26–46.61 (2N*C*H$_2$Ph); 52.51 (COO*C*H$_3$); 54.27 (*C*N); 56.56 (*C*N); 71.62 (*C*H$_2$); 116.26 (d, $^2J_{C-F}$=24.42 Hz, *C*CF); 123.20 (d, $^3J_{C-F}$=8.13 Hz, *C*CCF); 127.57–128.67; 135.92; 136.39 (2CH$_2$*Ph*); 149.07 (O*C*CCCF); 159.08 (*C*=O); 160.62 (d, $J_{C-F}$=246 Hz, *C*F), 170.16 (*C*OOCH$_3$); 194.54 (*C*=S). Anal. calcd for $C_{27}H_{25}FN_2O_5S$: C, 63.76; H, 4.97; N, 5.51. Found C, 63.59; H, 4.98; N, 5.53.

The FTP derivative (4.25 g, 8.37 mmol), AIBN (687 mg, 4.18 mmol) and tributyltin hydride ($Bu_3SnH$ (3.6 mL, 13.4 mmol) were dissolved in anhydrous toluene (85 mL) under argon. The solution was deoxygenated with argon during 40 min and heated under reflux for 1.5 h with continuous bubbling of argon. $Bu_3SnH$ (0.9 mL, 3.35 mmol) and AIBN (172 mg, 1.04 mmol) were added and reflux was continued for 30 min. After evaporation and purification on a flash silica gel column ($C_6H_{12}$:$CH_3COOC_2H_5$ 9:1 to 7:3) 2.24 g of dehydroxylated compound were obtained (containing stannous impurities) and dissolved at 0°C in anhydrous THF (15 mL), 1M $LiAlH_4$ in THF (3.6 mL, 3.6 mmol) was added. After 10 min stirring, the mixture was hydrolyzed with 1N HCl and the product extracted by $CHCl_3$. The organic phase was dried and concentrated under vacuum. A flash silica gel column ($C_6H_{12}$:$CH_3COOC_2H_5$ 7:3 to 2:8) yielded **10** (1.23 g, 47% from FTP derivative) as a white powder which crystallized from $C_5H_{12}$:$CH_2Cl_2$. mp 111–113°C. $^1H$ NMR: $\delta$ 1.6 (d, 3H, *J*=6.1 Hz, C*H*$_3$); 1.78–1.82 (m, 1H, –O*H*); 2.96–3.00 (m, 1H, C*H*N); 3.32–3.37 (m, 1H, *J*=7.5 Hz, C*H*N); 3.30–3.42 (m, 1H, *J*=11.7 Hz, C*H*HOH); 3.54–3.59 (m, 1H, *J*=11.7 Hz, CH*H*OH); 4.14; 4.33; 4.62; 4.75 (4d, 4H, $J_{Gem}$=15.3 Hz; 2NC*HH*Ph); 7.22–7.35 (m, 10H, *arom.*). $^{13}C$ NMR: $\delta$ 18.42 (CH$_3$); 45.56; 46.59 (2N*C*H$_2$Ph); 50.51 (*C*HN); 60.52 (*C*H$_2$OH); 62.4 (*C*HN); 127.29–128.77; 137.26–137.70 (*arom.*); 160.76 (*C*=O). Anal. calcd for $C_{19}H_{22}N_2O_2$: C, 73.56; H, 7.09; N, 9.03. Found: C, 73.46; H, 7.22; N, 9.09.

***cis*-1,3-Dibenzyl-4,5-dihydroxymethyl-imidazolidin-2-one 11. 8** (40 g, 124 mmol) was dissolved in THF (250 mL) and 1 M $LiAlH_4$ in THF (93 mL, 93 mmol) was added at 0°C. The mixture was stirred 30 min at 0°C and hydrolyzed with 1N HCl (300 mL). The product was extracted with $CH_2Cl_2$ (3×100 mL) and the organic layers washed with brine, dried ($MgSO_4$) and evaporated under vacuum. Crystallization from $CH_2Cl_2$ gave **11** (39.1 g, 98%) as white crystals. mp: 131–133°C. $^1H$ NMR: $\delta$ 3.49–3.56 (m, 2H, 2C*H*N); 3.73–3.87 (m, 6H, 2C*H*$_2$OH); 4.15; 4.91 (2d, 4H, $J_{Gem}$=15.2 Hz, 2NC*HH*Ph); 7.28–7.37 (m, 10H, *arom.*). $^{13}C$ NMR: $\delta$ 45.60 (2N*C*H$_2$Ph); 56.45 (2*C*HN); 57.71 (2*C*H$_2$OH); 127.59; 127.90; 128.75; 137.06 (*arom.*) 161.40 (*C*=O). Anal. calcd for $C_{19}H_{22}N_2O_3$: C, 69.92; H, 6.79; N, 8.58. Found: C, 69.75; H, 6.78; N, 8.49.

***cis*-1,3-Dibenzyl-4-hydroxymethyl-5-methyl imidazolidin-2-one 12.** In a solution of **11** (11.8 g, 34 mmol) in anhydrous $CH_2Cl_2$ (200 mL) were added anhydrous pyridine (4.23 mL, 52.4 mmol) and then at −30°C, under vigorous stirring and

dropwise, 4-FPTCl (5 g, 26 mmol) dissolved in anhydrous $CH_2Cl_2$ (100 mL). After 30 min stirring at −30°C, solvents were evaporated under vacuum and the residue purified on a flash silica gel column ($C_6H_{12}$:$CH_3COOC_2H_5$ 1:0 to 1:1 and then ethanol) the FTP derivative was obtained as a white solid (8.03 g, 49%) which crystallized from $CH_2Cl_2$. mp: 104–106°C $^1$H NMR: $\delta$ 2.51 (s, 1H, O*H*); 3.59–3.63 (m, 1H, *J*=9.1 Hz, C*H*N): 3.76–3.84 (2dd, 2H, $J_{Gem}$=12.7 Hz, *J*=2.5 Hz, *J*=5.1 Hz, C*HH*OH); 3.87–3.92 (m, 1H, C*H*N); 4.77; 4.91 (2dd, 2H, $J_{Gem}$=11.7 Hz, *J*=6.1 Hz, *J*=4.6 Hz, C*HH*OCS); 4.30; 4.31; 4.80; 4.96 (4d, 4H, $J_{Gem}$=15.7 Hz, 2NC*HH*Ph); 7.01–7.05 (m, 2H, 2C*H*CO); 7.08–7.14 (m, 2H, 2C*H*CF); 7.28–7.40 (m, 10H, 2NC$H_2$*Ph*). $^{13}$C NMR: $\delta$ 46.10 and 46.57 (2N*C*$H_2$Ph); 53.68 (*C*HN); 56.31 (*C*HN); 56.80 (*C*$H_2$OH); 71.60 (*C*$H_2$OCS); 116.28 (d, $^2J_{C-F}$=22.4 Hz, *C*CF); 123.29 (d, $^3J_{C-F}$= 8 Hz, *C*CCF); 127.51–128.77; 136.96; 137.19 (2C$H_2$*Ph*); 149.09 (O–*C* arom.); 160.66 (d, $J_{C-F}$=246.2 Hz, *C*–F); 161.00 (*C*=O); 194.54 (*C*=S). Anal. calcd for $C_{26}H_{25}FN_2O_4S$: C, 64.99; H, 5.24; N, 5.83. Found: C, 64.85; H, 5.32; N, 5.77.

FTP derivative (4.21 g, 8.77 mmol), AIBN (720 mg, 4.38 mmol) and freshly distilled $Bu_3SnH$ (3.77 mL, 14 mmol) were dissolved in anhydrous toluene (150 mL). Argon was bubbled in the mixture during 45 min. After heating with reflux for 1 h with continuous bubbling of argon, toluene was evaporated under vacuum and the residue was purified on flash silica gel column ($C_6H_{12}$:$CH_3COOC_2H_5$ 1:0 to 4:6). **12** (1.51 g, 57%) was obtained as a colorless oil. $^1$H NMR: $\delta$(1.21 (d, 3H, *J*=6.6 Hz, C*H*$_3$); 2.02 (s, 1H, O*H*); 3.39–3.43 (m, 1H, C*H*N); 3.55–3.62 (m, 1H, *J*=8.7 Hz, C*H*N); 3.69; 3.74 (2dd, 2 H, $J_{Gem}$=12.2 Hz, *J*=4.1 Hz, *J*=5.1 Hz, C*HH*OH); 4.09; 4.30; 4.78; 4.86 (4d, 4H, $J_{Gem}$=15.2 Hz, 2NC*HH*Ph); 7.27–7.37 (m, 10H, *arom.*). $^{13}$C NMR: $\delta$ 12.70 (*C*$H_3$); 45–25; 46.30 (2N*C*$H_2$Ph); 51.02 (*C*HN); 57.34 (*C*HN); 59.39 (*C*$H_2$OH); 127.31; 127.43; 127.84; 128.00; 128.52; 128.64; 137.28; 137.84 (*arom.*); 161.12 (*C*=O). Anal. calcd $C_{19}H_{22}N_2O_2$: C, 73.52; H, 7.14; N, 9.02. Found: C, 73.44; H, 7.19; N, 9.07.

***cis*-1,3-Dibenzyl-5-methyl-4-(2-oxyethyl) imidazolidin-2-one 13. 12** (1.97 g, 6.35 mmol) was dissolved into anhydrous $CH_2Cl_2$ (20 mL), mesyl chloride (0.64 g, 8.2 mmol) and $NEt_3$ (1.32 mL; 9.52 mmol) were added. After stirring 15 min at room temperature, 1N HCl (10 mL) was added, the product was extracted with $CH_2Cl_2$. The organic phase was washed with brine, dried ($MgSO_4$) and evaporated. Purification was performed on a flash silica gel column ($C_6H_{12}$:$CH_3COOC_2H_5$, 7:3 to 1:1) giving the mesylate (2 g, 80%) as a white solid recrystallized from $CH_2Cl_2$:$C_5H_{12}$, mp: 124–126°C. To a solution of the mesylate (1.74 g, 4.48 mmol) in anhydrous DMSO (10 mL) was added KCN (583 mg, 8.96 mmol). After heating at 70°C during 20 h, DMSO was evaporated under vacuum at 50°C. The crude product was dissolved in $CH_2Cl_2$ (300 mL) and $CH_2Cl_2$ was washed successively with water (15 mL) and brine (15 mL) then dried and evaporated under vacuum. Purification on a flash silica gel column ($C_6H_{12}$:$CH_3COOC_2H_5$, 8:2 to 1:1) yielded the cyano derivative as a white solid (1.28 g, 80%) recrystallized from $CH_2Cl_2$:$C_5H_{12}$. mp 125–127°C. $^1$H NMR: $\delta$ 1.24 (d, 3H, *J*=6.1 Hz, C*H*$_3$); 2.43–2.54 (m, 2H, C*H*$_2$CN); 3.58–3.68 (m, 2H, 2C*H*N); 4.12: 4.24; 4.88; 4.91 (4d, 4H, $J_{Gem}$=15.2 Hz, 2NC*HH*Ph); 7.29–7.40 (m, 10H, *arom.*). $^{13}$C NMR: $\delta$ 12.57 (*C*$H_3$); 16.50 (*C*$H_2$CN); 45.27; 45.88 (2N*C*$H_2$Ph); 51.18; 53.10 (2*C*HN); 116.92 (*C*N); 127.51; 128.81; 136.57; 136.75 (*arom.*); 159.87 (*C*=O). Anal. calcd for $C_{20}H_{21}N_3O$: C, 75.21; H, 6.63; N, 13.15. Found: C, 75.04; H, 6.68; N, 13.18.

The cyano derivative (1.15 g, 3.60 mmol) was dissolved in anhydrous toluene (15 mL) and at −70°C, 1.5 M diisobutylaluminium hydride (DIBAH) in toluene (6 mL, 9 mmol) was added. After 30 min stirring at −70°C, the mixture was hydrolyzed by 1N HCl (0.5 mL) and was allowed to warm to room temperature, then 1N HCl (75 mL) was added. The product was extracted with $CH_2Cl_2$. The organic phase was washed with brine, dried and evaporated under vacuum. Purification performed on a flash silica gel column ($C_6H_{12}$:$CH_3COOC_2H_5$, 7:3 to 1:1) yielded **13** as a colorless oil (800 mg, 70%). $^1$H NMR: $\delta$ 1.00 (d, 3H, *J*=6.6 Hz, C*H*$_3$); 2,64 (ddd, 1H, *J*=8.2 Hz, $J_{Gem}$=18.3 Hz, *J*=0.6 Hz, C*H*HCHO); 2.70 (ddd, 1H, *J*=5.1 Hz, *J*=18.3 Hz, $J_{Gem}$=0.6 Hz, CH*H*CHO); 3.64 (dq, 1H, *J*=7.6 Hz, *J*=6.6 Hz, C*H*N); 3.88–3.94 (m, 1H, C*H*N); 4.05; 4.23; 4.71; 4.91 (4d, 4H, $J_{Gem}$=15.2, 2NC*HH*Ph); 7.27–7.37 (m, 10H, *arom.*); 9.66 (s, 1H, C*H*O). $^{13}$C NMR: $\delta$ 12.61 (*C*$H_3$); 42.17 (*C*$H_2$CHO); 45.12; 46.00 (2N*C*$H_2$Ph); 51.03 (*C*HN); 52.13 (*C*HN); 127.35–128.62; 137.18; 137.27 (*arom.*); 160.33 (C=O); 199.42 (*C*HO).

***cis*-1,3-Dibenzyl-4-formyl-5-methyl imidazolidin-2-one 14.** DMSO (273 μL, 385 mmol) was dissolved in anhydrous $CH_2Cl_2$ (10 mL) at −78°C and oxalylchloride (169 μL, 1.93 mmol) was added dropwise. After 10 min stirring at −78°C, **12** (500 mg, 1.61 mmol) in $CH_2Cl_2$ (5 mL) was added slowly. The mixture was stirred 30 min, then $NEt_3$ (673 μL, 4.84 mmol) was added and after 40 min stirring at −78°C, $H_2O$ (2 mL). The mixture was extracted with $CH_2Cl_2$ (4×15 mL). The organic phase was washed with brine, dried, evaporated under vacuum. The crude aldehyde **14** was obtained as a colorless oil (496 mg) and was not purified. $^1$H NMR: $\delta$ 1.15 (d, 3H, *J*=6.1 Hz, C*H*$_3$); 3.70–3.77 (m, 2H, *J*=9.1 Hz, 2C*H*N); 4.11; 4.31; 4.82; 4.88 (4d, 4H $J_{Gem}$=15.2 Hz, 2NC*HH*Ph); 7.26–7.37 (m, 10, *arom.*); 9.51 (d, 1H, *J*=4.1 Hz, C*H*O). $^{13}$C NMR: $\delta$ 14.07 (*C*$H_3$); 45.47; 47.09 (2N*C*$H_2$Ph); 50.87; 64.20 (2*C*HN); 127.57–128.75; 136.37; 136.47 (*arom.*); 160.15 (*C*=O); 200.59 (*C*HO).

***cis*-4-[(*Z* and *E*)-5-Hydroxypent-1-enyl]-5-methyl imidazolidin-2-one 7a, 7b.** 1.6N *n*-butyllithium in hexane (5.63 mL, 9 mmol) was added at 0°C to a solution of $Ph_3P^+$ $(CH_2)_4OBn$, $Br^{-15}$ (4.8 g, 9.75 mmol, dried for 30 min at 80°C under vacuum) in anhydrous THF (15 mL). The brick red solution was stirred 10 min at 0°C and cooled at −60°C. **14** (2 g, 6.5 mmol) dissolved in anhydrous THF (15 mL) was then added, followed after 25 min stirring by 1.6N *n*-butyllithium (4.38 mL, 7 mmol). The mixture was allowed to warm to −30°C, kept at −30°C for 30 min and then cooled at −60°C, treated with absolute ethanol (550 μL, 9.5 mmol) with strong agitation, then warmed to room temperature. *t*-BuOK (1.43 g, 12.7 mmol) was added and after 2 h stirring at room temperature,

$CH_2Cl_2$ (200 mL) was added. The organic phase was successively washed with 1N HCl (60 mL) and brine and dried. The solvents were evaporated under vacuum and the residue purified on silica gel column ($C_6H_{12}$:$CH_3COOC_2H_5$, 1:0 to 1:1) yielding **15** (1.59 g, 54%) as a mixture of *Z* and *E* isomers (9:1 from $^1$H NMR).

In a three-necked round-bottom flask, at −78°C, $NH_3$ (70 mL) was condensed and absolute ethanol (3 mL) was added. The solution was allowed to warm to −30°C. Then **15** (1.59 g, 3.6 mmol) in THF (8 mL) was added, followed by small sodium pieces (850 mg, 31.5 mat), waiting for decolorization between the two additions. The addition was stopped when the blue color persisted for 1 min. $NH_3$ was then evaporated at room temperature. After addition of water and then 12N HCl up to neutralization, the solvents were evaporated under vacuum at 50°C. The residue was purified by $C_{18}$ reverse phase chromatography ($CH_3CN$:$H_2O$; 0:100 to 20:80) yielding **7** (260 mg, 40%) as a colorless oil (mixture of *Z* and *E* isomers). **7a** was obtained as white crystals by crystallization from MeOH:$CH_3CN$ and **7b** as an oil after HPLC of the residue (Lichrosorb $C_{18}$ reverse phase column, $H_2O$:TFA, 0.1%; $CH_3CN$, 92:8).

**7a**: mp: 115–117°C. $^1$H NMR ($CD_3OD$): 1.07 (d, 3H, *J*=6.1 Hz, $CH_3$); 1.56–1.63 (m, 2H, C*H*$_2$CH$_2$OH); 2.11–2.23 (m, 2H, C*H*$_2$CH$_2$CH$_2$OH); 3.55 (t, 2H, *J*=6.6 Hz, C*H*$_2$OH); 3.86–3.92 (m, 1H, *J*=8.2 Hz, C*H*N); 4.60–4.64 (m, 1H, C*H*N); 5.42–5.47 (m, 1H, *J*=10.7 Hz, CHC*H*=CH); 5.65 (dt, 1H, *J*=10.7 Hz, *J*=7.6 Hz, CH=C*H*CH$_2$). $^{13}$C NMR: *δ* 16.67 (*C*H$_3$); 24.8 (*C*H$_2$CH$_2$CH$_2$OH); 33.33 (*C*H$_2$CH$_2$OH); 53.10 (*C*HN); 54.32 (*C*HN); 62.04 (*C*H$_2$OH); 127.74 (CH*C*H=CH); 134.72 (CH=*C*HCH$_2$); 166.16 (*C*=O). Anal. calcd for $C_9H_{16}N_2O_2$: C, 58.67; H, 8.75; N, 15.20. Found: C, 58.78; H, 8.77; N, 15.16.

**7b**: $^1$H NMR ($CD_3OD$): *δ* 1.06 (d, 3H, *J*=6.1 Hz, C*H*$_3$); 1.58–1.64 (m, 2H, C*H*$_2$CH$_2$OH); 2.12–2.17 (m, 2H, C*H*$_2$CH$_2$CH$_2$OH); 3.55 (t, 2H, *J*=6.6 Hz, C*H*$_2$OH); 3.84–3.91 (m, 1H, *J*=8.2 Hz, C*H*N); 4.17–4.21 (m, 1H, C*H*N); 5.49 (dd, 1H, *J*=15.3 Hz, *J*=8.1 Hz, CHC*H*=CH); 5.71 (dt, 1H, *J*=15.3 Hz, *J*=6.6 Hz, CH=C*H*CH$_2$). $^{13}$C NMR: *δ* 16.87 (*C*H$_3$); 29.57 (*C*H$_2$CH$_2$CH$_2$OH); 33.06 (*C*H$_2$CH$_2$OH); 53.31 (*C*HN); 59.89 (*C*HN); 62.22 (*C*H$_2$OH); 127.80 (CH*C*H=CH); 134.92 (CH=*C*HCH$_2$); 165.98 (*C*=O).

***cis*-4-(*Z*-5-Carboxypent-1-enyl)-5-methyl imidazolidin-2-one 5a.** At 0°C, **7a** (143 mg, 0.78 mmol) was dissolved in anhydrous acetonitrile (3 mL) then anhydrous pyridine (250 μL, 3.10 mmol) and triphenylphosphine bromide (1.15 g, 2.73 mmol) were added. After stirring 1 h at room temperature, quick filtration on silica gel and evaporation of the filtrate yielded crude compound **16a** (decomposition at room temperature and on silica gel, conservation at −20°C), which was directly dissolved in DMSO (3 mL). KCN (152 mg, 2.34 mmol) was added; after sonication at room temperature during 2 h, DMSO was evaporated under vacuum at 50°C. The residue (nitrile **17a**) was dissolved in 1N NaOH (6 mL) and heated under reflux 90 min. Then 12N HCl was added to obtain pH≈1. The mixture was purified on a $C_{18}$ reverse phase column ($CH_3CN$:$H_2O$; 1:9). After evaporation **5a** was obtained (69 mg, 41% from **7a**) as a white solid crystallized from $H_2O$.

mp: 145–147°C. $^1$H NMR ($CD_3OD$): *δ* 1.07 (d, 3H, *J*=6.6 Hz, C*H*$_3$); 1.64–1.71 (m, 2H, C*H*$_2$CH$_2$COOH); 2.07–2.22 (m, 2H, C*H*$_2$(CH$_2$)$_2$COOH); 2.30 (t, 2H, *J*=7.1 Hz, C*H*$_2$COOH); 3.85–3.92 (m, 1H, *J*=8.3 Hz, C*H*N); 4.57–4.62 (m, 1H, C*H*N); 5.44–5.49 (m, 1H, C*H*=CH–CH$_2$); 5.63 (dt, 1H, *J*=11.2 Hz, *J*=7.6 Hz, CH=C*H*CH$_2$). $^{13}$C NMR ($CD_3OD$): *δ* 16.67 (*C*H$_3$); 25.85 (*C*H$_2$CH$_2$COOH); 27.71 (*C*H$_2$(CH$_2$)$_2$COOH); 34.12 (*C*H$_2$COOH); 53.10 (*C*HN); 54.32 (*C*HN); 128.19 (*C*H=CH); 134.35 (*C*H=CH); 166.14 (*C*=O); 177.28 (*C*OOH). Anal. calcd for $C_{10}H_{16}N_2O_3$: C, 56.59; H, 7.60; N, 13.20. Found: C, 56.66; H, 7.46; N, 13.24.

***cis*-4-(5-Carboxy-*cis*-2,3-epoxy-pentyl)-5-methyl imidazolidin-2-one 3a.** 2.50 mL of a 70% metachloroperbenzoic acid solution in $CH_2Cl_2$ (244 mg, 0.99 mmol) was added to **2a** (70 mg, 0.33 mmol) at −70°C. After 3 h stirring, $CH_2Cl_2$ was evaporated, the residue dissolved in $H_2O$ (10 mL) and purified on a $C_{18}$ silica gel reverse phase column ($CH_2CN$:$H_2O$; 1:9). **3a** (67 mg, 90%) was obtained as a mixture of two diastereoisomers, which were separated by HPLC (Lichrosorb $C_{18}$, $H_2O$, TFA 0.1%:$CH_3CN$, 97.6:2.4).

**3a$_1$(C)**: white solid, mp: 217–219°C. $^1$H NMR ($CD_3OD$: *δ* 1.13 (d, 3H, *J*=6.6 Hz, C*H*$_3$); 1.56–1.75 (m, 2H, NCHC*H*$_2$); 2.08–2.17 (m, 1H, C*H*HCH$_2$COOH); 2.25–2.34 (m, 1H, CH*H*CH$_2$COOH); 2.53–2.58 (m, 2H, C*H*N); 3.65–3.71 (m, 1H, CH$_2$C*H*O); 3.85–3.92 (dq, 1H, *J*=8.2 Hz, *J*=6.6 Hz, C*H*N); 3.94–4.00 (m, 1H, C*H*N); 4.46–4.50 (dt, 1H, *J*=4.1 Hz, *J*=7.6 Hz, OC*H*CH$_2$). $^{13}$C NMR ($CD_3OD$): *δ* 15.77 (*C*H$_3$); 24.74 (*C*H$_2$CH$_2$COOH); 29.40 (*C*H$_2$COOH); 33.67 (NCH*C*H$_2$); 52.75 (*C*HN); 55.78 (*C*HN): 72.93 (CH$_2$*C*O): 84.51 (O*C*CH$_2$); 165.95 (*C*=O); 180.19 (*C*OOH). Anal. calcd for $C_{10}H_{16}N_2O_4$: C, 52.62; H, 7.06; N, 12.27. Found: C, 52.58; H, 7.04; N, 12.26. I.C. ($NH_3$) $MH^+$=229.

**3a$_1$(O)**: $^1$H NMR ($CD_3OD$): *δ* 1.13 (d, 3H, *J*=6.6 Hz, C*H*$_3$); 1.57–1.88 (m, 4H, NCH–C*H*$_2$, C*H*$_2$CH$_2$COOH); 2.39–2.58 (m, 2H, C*H*$_2$COOH); 3.39–3.43 (m, 1H, O–C*H*–CH$_2$); 3.48–3.54 (m, 1H, CH$_2$C*H*O); 3.68–3.93 (m, 1H, C*H*N); 3.95–4.00 (m, 1H, C*H*N). $^{13}$C NMR ($CD_3OD$): *δ* 15.92 (*C*H$_3$); 29.20 (*C*H$_2$CH$_2$COOH); +31.39 (*C*H$_2$COOH) 34.26 (*C*H$_2$–*C*O); 53.09 (*C*HN); 54.36 (*C*HN); 72.03 (*C*H$_2$CO); 74.78 (O*C*CH$_2$); 166.04 (*C*=O); 180.26 (*C*OOH).

**3a$_2$(C)**: $^1$H NMR ($CD_3OD$): *δ* 1.12 (d, 3H, *J*=6.6 Hz, C*H*$_3$); 1.68–1.80 (m, 2H, NCHC*H*$_2$); 2.08–2.18 (m, 1H, C*H*HCH$_2$COOH); 2.23–2.33 (m, 1H, CH*H*CH$_2$COOH); 2.52–2.57 (m, 2H, C*H*$_2$COOH); 3.66–3.70 (m, 1H, CH$_2$C*H*O); 3.82–3.88 (m, 1H, C*H*N); 3.92 (dt, 1H, *J*=8.2 Hz, C*H*N); 4.51 (m, 1H, *J*=3.6 Hz, *J*=7.1 Hz, OC*H*CH$_2$. $^{13}$C NMR ($CD_3OD$): *δ* 15.78 (*C*H$_3$); 24.75 (*C*H$_2$CH$_2$COOH); 29.40 (*C*H$_2$COOH); 33.69 (NCH*C*H$_2$); 52.76 (*C*HN); 57.79 (*C*HN); 72.94 (CH$_2$*C*O); 84.51 (O*C*CH$_2$); 165.96 (*C*=O); 180.19 (*C*OOH).

**$3a_2$(O)**: $^1$H NMR ($CD_3OD$); $\delta$ 1.11 (d, 3H, $J$=6.1 Hz, C$H_3$); 1.59–1.87 (m, 4H, C$H_2$CHO, C$H_2$C$H_2$COOH); 2.38–2.52 (m, 2H, C$H_2$COOH); 3.41 (dt, 1H, $J$=3.6 Hz, $J$=10.2 Hz, OC$H$C$H_2$); 3.52 (dt, 1H, $J$=3.6 Hz, $J$=9.7 Hz, C$H_2$C$H$O); 3.81–3.97 (m, 1H, 2C$H$N).

***cis*-4-(5-Carboxy-*trans*-2,3-epoxy-pentyl)-5-methylimidazolidin-2-one 3b.** To a solution of **2b** (20 mg, 0.094 mmol) in $H_2O$ (2 mL) were added methyltrioxorhenium (MTO, 7 mg, 0.028 mmol) and a 35% $H_2O_2$ solution into $H_2O$ (16 μL, 1.88 mmol). After 15 min sonication and 3 h stirring at room temperature, $H_2O_2$ (16 μL, 1.88 mmol) and MTO (7 mg, 0.028 mmol) were added once more. The mixture was stirred for 30 min and immediately laid down on a $C_{18}$ silica gel reverse phase column ($CH_3CN$:$H_2O$; 5:95) yielding **3b** (16 mg, 76%) as a colorless oily (50:50) mixture of two diastereoisomers. $^1$H NMR ($CD_3OD$): $\delta$ 1.11; 1.13 (2d, 3H, $J$=6.1 Hz, C$H_3$); 1.48–1.56; 1.58–1.68; 1.72–1.78 (3m, 2H, NCHC$H_2$); 2.14–2.27 (m, 2H, C$H_2$C$H_2$COOH); 2.52–2.57 (m, 2H, $J$=7.6 Hz, C$H_2$COOH); 3.82–4.01 (m, 3H, $J$=8.1 Hz, $J$=7.6 Hz, 2C$H$N, C$H_2$C$H$O); 4.45 (dt, 1H, $J$=4.1 Hz, $J$=7.6 Hz, OC$H$C$H_2$). $^{13}$C NMR ($CD_3OD$): $\delta$ 15.78; 15.96 (*C*$H_3$); 22.81; 22.97 (*C*$H_2$C$H_2$COOH); 29.36 (*C*$H_2$COOH); 32.37; 33.63 (NCH*C*$H_2$); 52.70; 52.86 (*C*HN); 53.91; 56.08 (*C*HN); 69.87; 71.97 (C$H_2$*C*HO); 84.61; 84.87 (O*C*HC$H_2$); 165.92; 166.38 (*C*=O); 180.09 (*C*OOH). I.C. ($NH_3$) $MH^+$=229.

***cis*-4-(*Z*-5[$^{14}$C]-Carboxypent-1-enyl)-5-methyl imidazolidin-2-one [$^{14}$C]5a.** [$^{14}$C]KCN (SA 46 Ci mol$^{-1}$) into $H_2O$ (300 μL, 300 μCi, 6.51 μmol) was diluted with $CH_3OH$ (1 mL) and evaporated under vacuum, anhydrous DMSO (100 μL) and **16a** (1.1 mg, 5.21 μmol) were added. After stirring for 2 h at 40°C, DMSO was evaporated under vacuum at 50°C, 1N NaOH (0.5 mL) was added, following by heating under reflux for 2 h, addition of 12N HCl (150 μL) and purification by HPLC (Lichrospher $C_8$, $H_2O$, TFA, 0.1%: $CH_3CN$, 87:13) yielded **[$^{14}$C]5a** (27.6 μCi, 0.6 μmol).

***cis*-4-(5-[$^{14}$C]-Carboxy-*cis*-2,3-epoxy-pentyl)-5-methyl imidazolidin-2-one [$^{14}$C]3a.** To **[$^{14}$C]2a**[5] (SA: 53 Ci mol$^{-1}$) into $H_2O$ (1 mL, 0.322 μmol, 17.1 μCi) MTO (traces) and a 0.35% $H_2O_2$ solution (286 μL) were added. After 30 min stirring at room temperature, MTO and $H_2O_2$ (67 μL) were added once more. The mixture was stirred for 1 h at room temperature and purified by HPLC (Lichrosopher $C_8$, $H_2O$, TFA 0.1%:$CH_3CN$, 95:5) giving **[$^{14}$C]$3a_1$** (110 nmol, 5.84 μCi) and **[$^{14}$C]$3a_2$** (107 nmol, 5.65 μCi).

***cis*-4-(5-[$^{14}$C]-Carboxy-*trans*-2,3-epoxy-pentyl)-5-methyl imidazolidin-2-one [$^{14}$C]3b.** A solution of **[$^{14}$C]2b**[5] (SA: 53 Ci mol$^{-1}$) into $H_2O$ (1.37 mL, 0.629 μmol, 34 μCi) was treated as above for **3a** yielding after HPLC purification ($H_2O$, TFA 0.1%:$CH_3CN$, 96:4) **3b** (89 nmol, 4.8 μCi) as a mixture of epoxides **[$^{14}$C]$3b_1$** and **[$^{14}$C]$3b_2$**.

## Biological experiments

**Assays with biotin synthase of *E. coli*.** Two mixtures were separately prepared and left under argon for 1 h at room temperature after which they were mixed and incubated for 3 h at 37°C. Mixture A consisted of 6.3 μM pure biotin synthase, 50–500 μM substrates. 2 mM NADPH, 0.2 mM S-Adenosyl-L-methionine, 0.5 mM L-cysteine, 5 mM dithiothreitol, 2 mM 1,6-fructose biphosphate, 10 mM KCl, 40 mM Tris–HCl buffer pH=8. Mixture B consisted of 5 μM flavodoxin, 1 M flavodoxin reductase, 1 mM $Fe(NH_4)_2(SO_4)_2$ in $H_2O$. The final volume of the assay was 100 μL. The reaction was stopped by precipitation with trichloroacetic acid. Precipitated proteins were removed by centrifugation and the amount of biotin formed was determined in the supernatant by the paper disc plate method using *L. plantarum*.[15]

**Analytical TLC.** Supernatants of the assays with the different analogs were chromatographed on TLC (silica gel 60 Merk 5748, 0.2 mm, eluent: $CHCl_3$; $CH_3OH$; $CH_3COOH$, 90:10:5). After migration and drying, the plate was transferred on an agar plate cultivated with *L. plantarum*. After one night at 37°C, the $R_F$ value of the spot, which appeared on each lane, was compared to that of pure biotin taken as reference.

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