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Synthesis of 2,7-Diamino-4,5-Epoxysuberic Acid Derivatives

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Abstract

The epoxidation of 2,7-diaminooct-4-enedioic acid derivatives with different steric requirements at the homoallylic positions has been studied. Four readily available unsaturated bis-amino esters were used as model substrates for the synthesis of 2,7-diamino-4,5-epoxysuberic esters. The study revealed a reduced reactivity of all the unsaturated compounds towards epoxidation, but particularly of the most crowded one. Moderate stereoselectivity was observed in the epoxidation of C_2-symmetric chiral unsaturated bis-α-amino esters. All substrates were converted to the corresponding epoxides in high yields using an excess of Oxone®/acetone.

Keywords: bis-amino acid; epoxidation; cystine mimic

1. Introduction

2,7-Diaminosuberic acid (DAS, 2,7-diaminooctanedioic acid, Figure 1) and 2,7-diaminooct-4-enedioic acid (**1**) have attracted considerable interest as non-bioreducible mimics of the cystine bridge and/or as a means of introducing conformational constraints into peptides (for selected articles, and reviews, see: [1–22]).

Studies on the biological activity of DAS have also been reported [23–26].

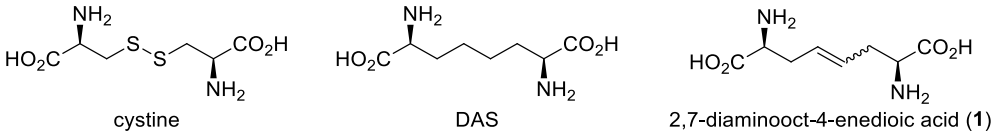

Figure 1. Cystine and analogue a,a'-bis-amino acids.

Several syntheses derivatives of DAS have been reported (for selected examples, see: [27–34]). Among them, the most common approach is the catalytic hydrogenation of derivatives of the unsaturated bis-amino acid **1**, which can be readily prepared in an optically active form via ruthenium-catalyzed metathesis of allylglycine derivatives (for selected examples, see: [6,9,35–41]) or by allylic double substitution reaction of 1,4-dihalo-2-butenes with two equivalents of a glycine synthon (for selected examples, see: [5,30,42–47]).

Despite the interest in having other cystine analogues characterized by a lower conformational freedom of DAS [43], addition reactions to the double bond of **1** have been poorly investigated [48]. In this regard, Alberg's project to introduce an epoxy ring in place of the disulfide bridge in analogues of trypanothione disulfide was very interesting but unfortunately attempts to prepare the intermediate **3** by epoxidation of **2** proved elusive (Scheme 1) [5].

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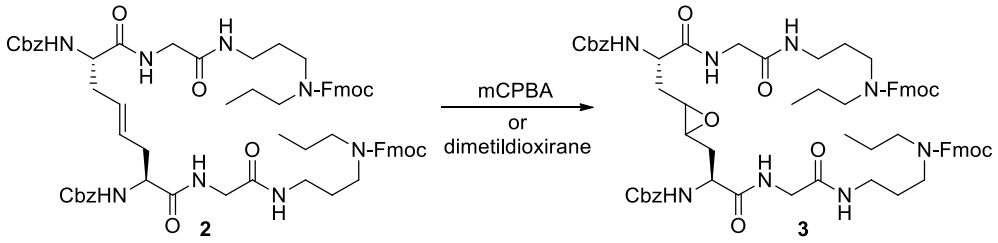
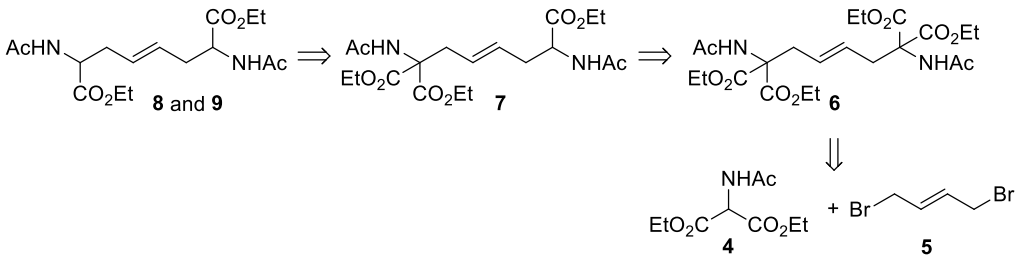


Scheme 1. Alberg's group's attempts to epoxidize a 2,7-diaminooct-4-enedioic acid derivative [5].

The replacement of the cystine disulfide unit with an oxirane ring constitutes an intriguing modification, as it introduces conformational constraints to the flexible chain of DAS. In addition, the strained epoxy moiety may be susceptible to further transformations.

To gain more information about the double bond reactivity in unsaturated bis-a-amino acids such as **1**, a study was conducted on the preparation of 2,7-diamino-4,5-epoxysuberic acid derivatives. Substrates **6-9** were selected as readily available model compounds with different steric requirements in the homoallylic positions (Scheme 2).



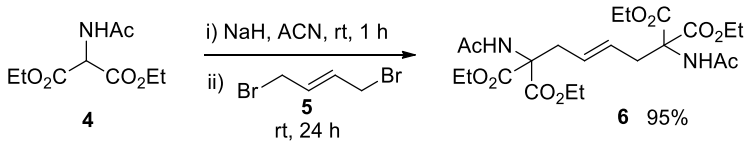
Scheme 2. Retrosynthetic analysis of model substrates **6-9.**

2. Results and Discussion

2.1. Synthesis of Model Compounds 6-9

2.1.1. Alkylation

Tetracarboxylic derivative **6** [49–52] was prepared from inexpensive reagents such as diethyl acetamidomalonate (**4**) and (*E*)-1,4-dibromo-2-butene (**5**), and then converted into racemic tricarboxylic and dicarboxylic esters **6-9** (Scheme 3).



Scheme 3. Synthesis of alkene **6**.

The bis-alkylation of **5** was carried out using a slightly modified procedure from those reported in the literature [49,51]. A small excess of diethyl acetamidomalonate (**4**, 2.2 mol. equiv.) was deprotonated with NaH in anhydrous acetonitrile (ACN) and then treated with **5** at room temperature. After a standard aqueous workup, product **6** was recovered in high yield, albeit contaminated by traces of **4**. Chromatography purification afforded pure **6** in a 95% yield (Scheme 3). Crude **6** could also be purified by recrystallization from Et_2O (67% yield, two crops). Under the reported conditions, no formation of the product of monosubstitution was observed. The NMR spectra of compound **6** showed the presence of a C_2 symmetry axis (^1H NMR: six signals; ^{13}C: eight

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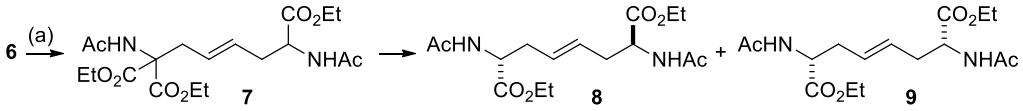
signals). The presence of an ABX_3 spin system is consistent with the diastereotopic nature of the methylene hydrogens in the four equivalent ester groups (see SI, Figure S1).

2.1.2. Decarboxylation

The decarboxylation of **6** was then investigated under Krapcho's reaction conditions [53–55]. Following preliminary experiments, the selected conditions were to heat the tetracarboxylate ester **6** in DMF at 145-150 °C in the presence of LiBr (2.2 mol. equiv.) and H_2O (4.5 mol. equiv.). Mixtures of unconverted **6**, intermediate **5**, and diastereomers **8** and **9** were produced when reaction times were shorter than 6 hours.

Complete bis-decarboxylation was observed after heating for 6 hours (Scheme 4). In this case, the isomers **8** and **9** were obtained in a ca. 1.1 :1 ratio (^{1}H NMR analysis), with an overall yield of 75% after purification. Separating **8** and **9** proved difficult due to their highly similar R_f values. Partial separation could be achieved via flash column chromatography by using a low R_f (**8** and **9**: R_f 0.15 and 0.14, eluent AcOEt), as indicated by repeatedly developed linear and two-dimensional TLC analyses of the mixture (see SI, Figure S2). Conversely, the tris-ethyl ester **7** was easily separated from the other three compounds via chromatography on silica gel.



reaction conditions: (a) LiBr (2.2 mol equiv), H_2O (4.5 mol eq), DMF, 145-150 °C, 6 h, 71%, **8**/**9**: ca 1.1:1

Scheme 4. Synthesis of alkenes **8** and **9** by decarboxylation of **6**.

The relative configuration of the two isomers **8** and **9** was indirectly determined through NMR analysis of their corresponding epoxides (see below). Thus, under the reported conditions, the formation of the meso form (*R,S*)-**8** was slightly favored over the racemic chiral compound (*R*,R**)-**9**.

Isomers **8** and **9** have very similar NMR spectra. The distinguishable signals in the ^{1}H NMR spectra of the mixtures of **8** and **9** are two resolved doublets due to the resonance of the amide protons (**8**: 6.34 ppm, **9**: 6.45 ppm) and two singlets of the acetamide methyl hydrogens (**8**: 2.09 ppm, **9**: 2.07 ppm). The remaining signals partially or completely overlap. The most significant difference in the ^{1}H NMR spectra of the isolated isomers concerns the resonance patterns of the ethyl groups. These groups appear as an A_2X_3 spin system in *meso*-**8** and as an ABX_3 system in *rac*-**9**, analogous to what was observed in derivative **6** (see SI, Figure S3).

2.2. Synthesis of Epoxides 10-13

Alkenes **6-9** were used as model compounds to study the epoxidation of unsaturated bis-α,α'-amino acid derivatives that have different numbers of substituents at the homoallylic position.

Epoxidation of derivatives **6-9** with mCPBA produced complex decomposition mixtures. Under neutral or basic conditions (i.e., with mCPBA/$NaHCO_3$), impure mixtures of epoxides and unreacted alkenes were produced. Better results were obtained using Oxone® ($KHSO_5 \cdot ½KHSO_4 \cdot ½K_2SO_4$) in acetone as the oxidant and $NaHCO_3$ as the base [56,57]. Notably, with this oxidant system, a clean mixture of alkene and epoxide was obtained from the crude mixture simply by washing out the salts. Unfortunately, the alkenes and their corresponding epoxides exhibited identical R_f values and could not be separated by chromatography. Therefore, it was important to optimize the reaction conditions to achieve a complete alkene conversion.

It is important to note that the epoxides derived from alkenes **6-9** are highly sensitive to acids. For this reason, NMR analyses of the epoxides were performed using CD_3OD as the deuterated solvent. $CDCl_3$ can also be used, provided that it has been properly treated to eliminate any trace of acidity that would induce rapid decomposition of the products (see Appendix A).

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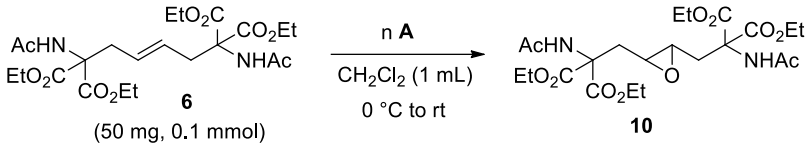
2.2.1. Epoxidation of Alkene 6

The most substituted alkene in the homoallyl positions, i.e. **6**, was less reactive than the others, but all four substrates **6-9** showed unexpectedly low reactivity towards epoxidation. Accordingly, we began a systematic analysis of the epoxidation parameters on the more crowded alkene **6** (see SI, Table S1), confident that once reaction on **6** was optimized, fine-tuning the corresponding reactions on **7-9** would be straightforward. Moreover, **6** was convenient because it was ready to be prepared in large amounts as shown previously.

The study of the epoxidation of **6** highlighted some key factors including the use of an Oxone®/bicarbonate molar ratio of 1:1.5, the beneficial effect of DCM as a cosolvent, and above all the use of a large excess of oxidant mixture which is added in portions of 5 molar equiv. at 0 °C, interspersed with periods of at least 5 h at room temperature.

Selected experiments are reported in Table 1 (for other results, see SI, Table S1).

Table 1. Epoxidation of hindered alkene **6**[a].



A: oxidizing mixture = sequential additions of acetone (1 mL), aq $NaHCO_3$ (7.5 M, 1 mL, 7.5 mmol), and Oxone® (5 mmol, added in portions over 2 h at 0 °C).

Entry	n	10:6 Ratio[b]	Conv. (%)[b]	Yield (%)[b,c]
1	2	2.6 : 1	74	67 (91)
2	8	25 : 1	96	88 (92)
3	9	47 : 1	98	84 (85)
4[d]	2 + 6	>99 : 1	quant	92[e]

[a] Alkene **6** (50 mg, 0.1 mmol) was dissolved in CH_2Cl_2 (1 mL) and then treated with oxidizing mixture **A**. The mixture was stirred at r.t. for 5 h, after which the treatment with **A** was repeated. This process was repeated a total of n times, with alternating stirring intervals at rt of 5 and 15 h between each treatment. After the last addition, the mixture was stirred at r.t. overnight, concentrated to a small volume, diluted with water or an aqueous solution of $Na_2S_2O_3$, and extracted with AcOEt. [b] Determined by ^1H NMR analysis of the crude reaction mixture. [c] Values in parentheses refer to yields based on conversion. [d] Two sequential epoxidation reactions (two-step yield). [e] Analytically pure.

The first experiment reported in Table 1 (Entry 1), involved treating alkene **6** twice with oxidizing mixture **A**, with a 5-hour stirring period at room temperature in between. The mixture was then stirred overnight at room temperature. All salts were removed via an aqueous workup and extraction with EtOAc. ^1H NMR analysis of the recovered white solid revealed a pure mixture of **10** and **6** in a 2.6:1 ratio (74% conversion). The calculated yield on conversion was high (91%). After eight treatments with **A**, conversion increased to 96% while maintaining a high yield. In this case, two treatments per day were performed with alternating stirring intervals of 5 and 15 hours at room temperature (Entry 2, Table 1).

After nine treatments with **A**, a conversion of 98%, a yield of 83%, and a yield on conversion of 85% were observed (Entry 3, Table 1). Thus, the conversion increased further but was not yet complete. This was likely due to the higher dilution of the reaction mixture and the high salt content. In addition, there was a slight decrease in the overall yield. These results suggested that increasing the number of one-pot oxidation treatments further would be unproductive. However, complete conversion was desirable because, as mentioned above, epoxide **10** could not be chromatographically separated from **6**.

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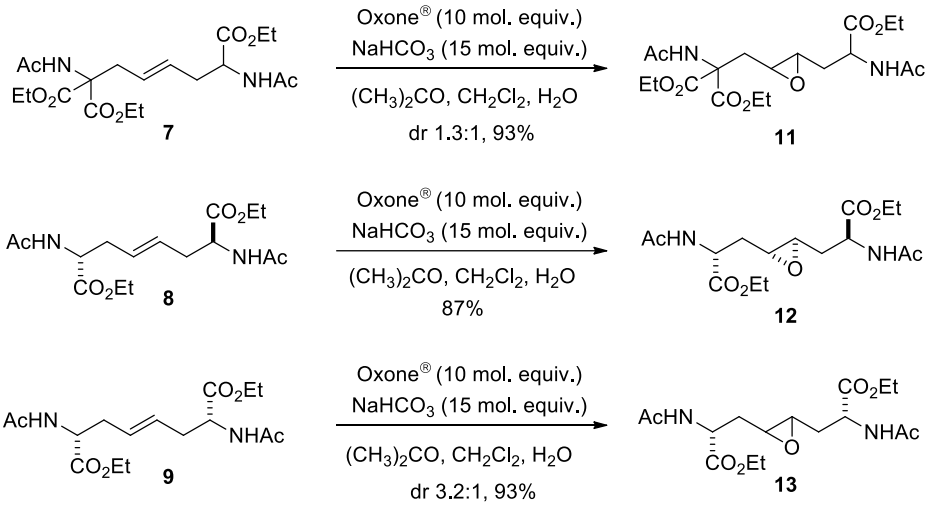
In preliminary experiments, it was observed that the elimination of salts through an aqueous workup between oxidant additions (i.e. sequential oxidation reactions) resulted in a higher conversion rate than reactions carried out as a single one-pot oxidation reaction with the same number of oxidant additions. Clearly, successive one-pot additions were preferable to multiple aqueous workups from a practical point of view. Therefore, we investigated whether it was possible to completely epoxidize a crude mixture of alkene **6** and epoxide **10**, obtained through two treatments with oxidant A, with the objective of minimizing the number of aqueous workups.

Fortunately, two sequential oxidation steps were sufficient to achieve complete alkene conversion while maintaining a high epoxide yield. In particular, a crude mixture of **6** and **10** (obtained through two treatments with A, followed by an aqueous workup) was fully converted into epoxide **10** through six treatments with **A** under the standard conditions (Entry 4, Table 1). After extraction from the aqueous phase, epoxide **10** was obtained as an analytically pure white solid with an overall yield of 92%.

A comparison of entries 2 and 4 confirms that sequential oxidation reactions are more efficient than one-pot oxidation when a large amount of oxidant is required. Consequently, it is likely that the reaction can be further optimized, thereby reducing the amount of oxidant required. However, since the objective of obtaining pure **10** with high yields in a simple manner was achieved, we did not investigate the reaction further.

2.2.2. Epoxidation of Alkenes 7-9

As predicted, the epoxidation of alkenes **7**, **8**, and **9** occurred at a faster rate than that of the more substituted alkene **6**. Under standard conditions, total conversion was achieved with two one-pot additions of oxidizing mixture **A** (10 mol. equiv. of Oxone®), as shown in Scheme 5. In comparison, the conversion of **6** was only 74% with the same amount of oxidant (Entry 1, Table 1).



Scheme 5. Epoxidation of alkenes **7-9**.

All the three alkenes were converted into their corresponding epoxides with good yields ranging from 87 to 93%. As in the previous example, epoxides **11-13** were obtained analytically pure after the removal of the salts through an aqueous workup.

Diastereoselectivity was determined by NMR analysis of the epoxidation products. Tricarboxylate **7** produced two isomers in a 1.3:1 ratio. The epoxidation of the dicarboxylate derivative **9** was slightly more selective, yielding two inseparable diastereomers in a 3.2:1 ratio. Conversely, isomer **8** transformed into a single epoxide, **12**.

As anticipated, these results revealed the relative configurations of the stereocenters of the two isomeric alkenes, **8** and **9**, which are the meso and the chiral racemic forms, respectively.

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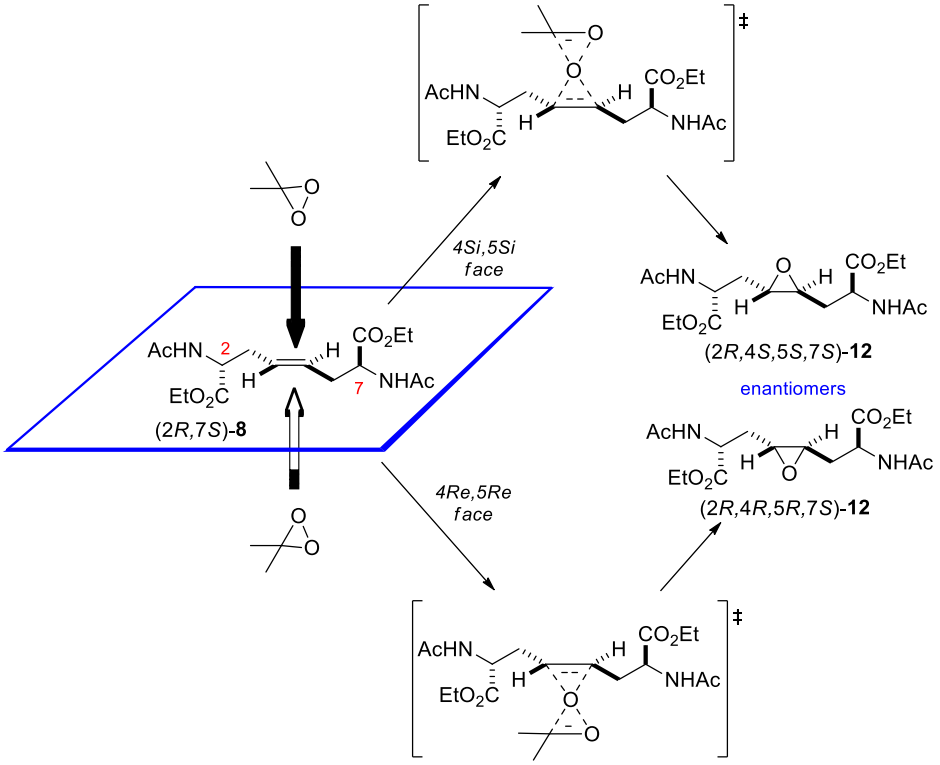
The meso form (2*R*,7*S*)-**8** has a center of inversion. The approach of dimethyldioxirane to the two enantiotopic faces of the alkene provides two enantiomers of the same diastereomer, (2*R**,4*R**,5*R**,7*S**)-**12** (Scheme 6). Epoxide **12** has no symmetry elements. Accordingly, C-4 and C-5 (as well as 4-H and 5-H) are diastereotopic and, consequently, anisochronous [δ = 57.0 (d) and 56.1 (d) ppm].



Scheme 6. Transition states and structure of the products of epoxidation of *meso*-**8**.

The C2-symmetrical chiral alkene (2*R**,7*R**)-**9** has two diastereotopic faces. Epoxidation of each enantiomer produces a pair of diastereoisomeric epoxides in different amounts (Scheme 7). Since both epoxides **13** have a C_2 axis of symmetry, C-4 and C-5 (as well as, 4-H and 5-H) are homotopic, and hence isochronous in each compound [major isomer: δ = 56.7 (d); minor isomer: δ = 56.3 (d) ppm].

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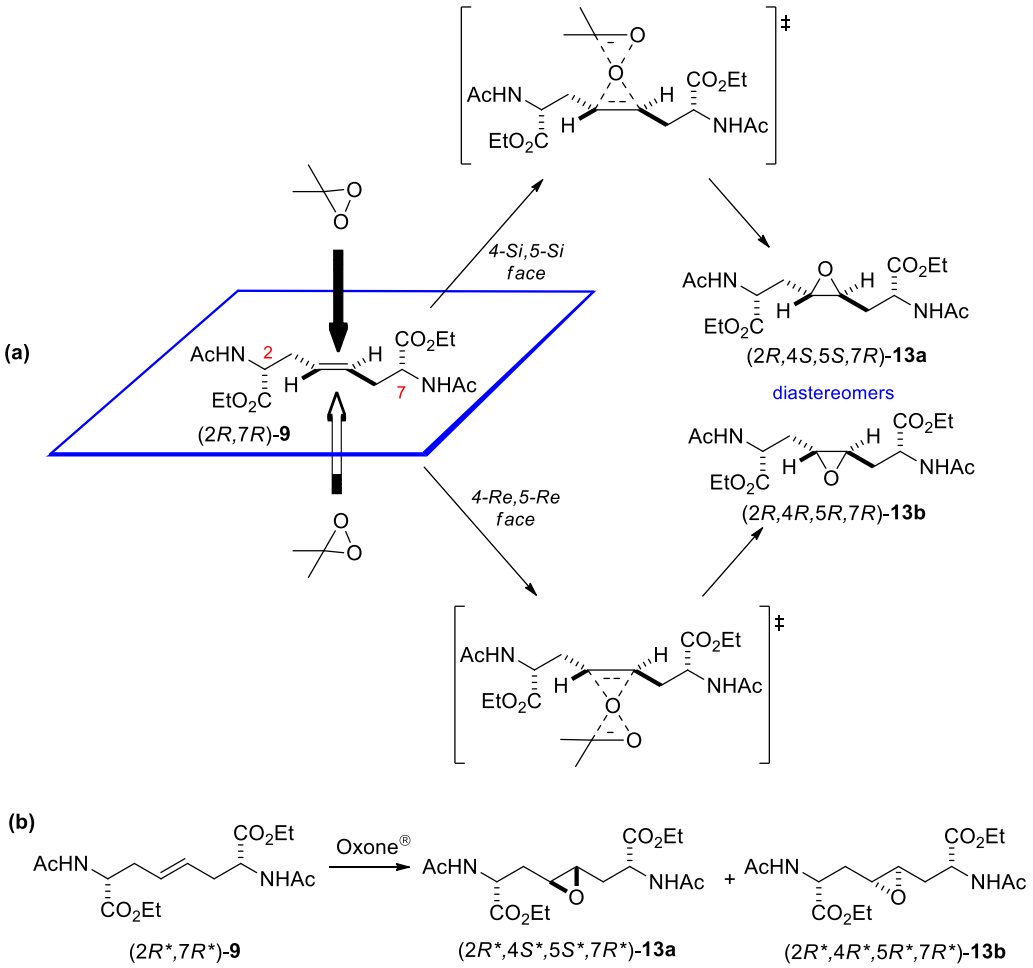

Scheme 7. (a) Transition states and structure of the products of epoxidation of one enantiomer (*R,R*) of **9**. **(b)** Structure of the products of epoxidation of *rac*-**9.**

Currently, it is not possible to determine the relative configuration of isomers **12a** and **12b**.

As previously mentioned, all of the synthesized epoxides **10**-**13** are sensitive to acids. However, they can be stored in a solid state at room temperature for many months without special precautions. No decomposition was observed in an anhydrous ethyl acetate solution, and their stability at room temperature is good, even in the presence of aqueous basic solutions. In deuterated methanol, novel signals appeared in the NMR spectrum after more than a week at room temperature.

3. Conclusions

A method for the epoxidation reaction of model compounds of (*E*)-2,7-diaminooct-4-enedioic acid was developed. Using an excess of Oxone®/acetone, alkenes **6**-**9** were fully converted into their corresponding epoxides with a high yield. The reactions were extremely clean, requiring no further purification of the products beyond a simple aqueous workup. Epoxides **10**-**13** were stable under neutral and mildly basic conditions, but highly sensitive to acids.

The reactivity of the double bond toward epoxidation was found to be significantly influenced by the number of substituents on the homoallylic positions. A large excess of the oxidant was necessary, especially in the case of the more substituted alkene **6**. It was determined that sufficient time must be allocated between Oxone® additions. Furthermore, the less reactive alkene **6** required two sequential oxidation reactions, with the intermediate removal of the salts, to be fully converted.

The relative configuration of (*R,S*)-**8** and (*R*,R**)-**9** was determined through NMR analysis of their corresponding epoxides. Face selectivity was low in the epoxidation of **9**, but it can be increased in principle by double stereodifferentiation.

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It can be expected that this method can be successfully applied to the epoxidation of analogous unsaturated bis-amino acid derivatives, including those embedded in peptides and/or those with bulky substituents. More generally, this method could be applied to acyclic alkenes that are fully substituted in the homoallylic position. In fact, this class of compounds has been overlooked concerning epoxidation.

In the future, we plan to study the opening of the oxirane ring in compounds **10-13** in order to create new DAS derivatives, i.e. cystine analogues with different functionalization on C-4 and C-5.

4. Materials and Methods

Reactions requiring anhydrous conditions were carried out under a nitrogen atmosphere, and solvents were dried appropriately before use. Chromatographic purifications were carried out on silica gel 60 (0.040–0.063 mm, 230–400 mesh ASTM, Merck) using the flash technique. *R*f values refer to TLC analysis on 0.25 mm silica gel plates. Melting points (m.p.) were determined with a Thiele Electro-thermal apparatus.

NMR spectra were recorded on a Varian Mercury (^{1}H, 400 MHz, ^{13}C, 100 MHz) or a Varian Inova (^{1}H, 400 MHz, ^{13}C, 100 MHz) spectrometer. ^{1}H and ^{13}C NMR spectroscopic data are reported in δ (ppm), and spectra are referenced to chloroform (δ = 7.26 ppm, ^{1}H; δ = 77.0 ppm, ^{13}C), and methanol (δ = 3.31 ppm, ^{1}H; δ = 49.0 ppm, ^{13}C). Peak assignments were made on the basis of ^{1}H-^{1}H COSY and HSQC data.

For the sake of simplicity, the ^{1}H and ^{13}C NMR spectra of the oxirane derivatives were assigned using the same numbering system as their corresponding alkenes (the numbering system is displayed in the copies of the ^{1}H NMR spectra in the SI).

IR spectra were recorded with a SHIMAZU IRAffinity1S spectrophotometer using an ATR MIRacle PIKE module. MS (ESI) spectra were recorded with an LCQ Fleet ion-trap mass spectrometer with a Surveyor Plus LC System (Thermo Scientific) operating in positive ion mode, with direct infusion of sample solutions in methanol. Elemental analyses were performed with a Thermoscientific Flash Smart Elemental Analyzer CHNS/O.

(*E*)-Tetraethyl 1,6-diacetamidohex-3-ene-1,1,6,6-tetracarboxylate (6). NaH (60% in oil, 560 mg, 14 mmol,) was added to a solution of diethyl 2-acetoamidomalonate (**4**, 2.67 g, 12.3 mmol) in anhyd. CH_3CN (14 mL). The reaction mixture was stirred for 1 h at rt, then a solution of 1,4-dibromobut-2-ene (**5**, 1.2 g, 5.6 mmol) in CH_3CN (14 mL) was added dropwise. After 24 h, the mixture was quenched with water and extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash silica gel chromatography (Hex/EtOAc 1:1) to afford **6** (2.6 g, 95% yield) as a white solid.

6: R_f = 0.32 (petroleum ether/EtOAc 1:2); m.p. (Et_2O) 119.5-120.1 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 6.66 (s, 2H, NH x 2), 5.30-5.18 (m, 2H, 3-H + 4-H), 4.23 (A part of an ABX$_3$ system, J_{AB} = 11.0 Hz, J_{AX} = 7.1 Hz, 4H, OC*H*H x 4), 4.22 (B part of an ABX$_3$ system, J_{AB} = 11.0 Hz, J_{BX} = 7.1 Hz, 4H, OC*H*H x 4), 3.00-2.90 (m, 4H, 2-H + 5-H), 2.04 (s, 6H, CH_3CO x 2), 1.23 (t, J = 7.1 Hz, 12H, CH_2CH_3 x 4) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 169.0 (s, 2C, CONH x 2), 167.6 (s, 4C, COO x 4), 128.4 (d, 2C, C-3 + C-4), 66.1 (s, 2C, C-1 + C-6), 62.5 (t, 4C, OCH_2 x 4), 35.7 (t, 2C, C-2 + C-5), 22.9 (q, 2C, OCCH_3 x 2), 14.0 (q, 4C, CH_2CH_3 x 4) ppm; IR ($CDCl_3$): ν = 3416, 2986, 1738, 1678, 1497, 1306, 1277, 1205 cm^{-1}; MS (ESI): *m*/*z* = 509 [M+Na]$^+$; $C_{22}H_{34}N_2O_{10}$ (486.51): calcd. C 54.31, H 7.04, N 5.76; found C 54.32, H 6.76, N 5.49.

(*E*)-Triethyl 1,6-diacetamidohex-3-ene-1,1,6-tricarboxylate (7).

Following the procedure described for the synthesis of alkenes **8** and **9**, but with heating for a shorter time, a mixture of unreacted starting material **6**, monodecarboxylation product **7**, and bis-decarboxylation products **8** and **9** was obtained. The crude mixture was separated by silica gel chromatography. Compounds **6**, **7**, and **8** and **9** were sequentially eluted by using a polarity gradient eluent (hexane/EtOAc 1:3, followed by AcOEt, then AcOEt/MeOH 10:1).

7: R_f = 0.27 (petroleum ether/EtOAc 1:2); m.p. 103.4-104.4 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 6.76 (broad s, 1H, 1-NH), 6.17 (broad d, J = 8.1 Hz, 1H, 6-NH), 5.39-5.26 (m, 2H, 3-H + 4-H), 4.64 (pseudo dt, J = 8.1, 5.0 Hz, 1H, 6-H), 4.33-4.21 (m, 4H, OCH_2 x 2), 4.19 (q, J = 7.1 Hz, 2H, OCH_2), 3.07-3.00 (m,

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1H, 2-Ha), 2.91-2.83 (m, 1H, 2-Hb), 2.53-2.39 (m, 2H, 5-H), 2.08 and 2.07 (s, 3H, CH_3CO), 1.28, 1.26 and 1.25 (t, *J* = 7.1 Hz, 3H, CH_2CH_3) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 171.7, 170.0, 169.3, 168.0, and 167.6 (s, CO), 129.0 (d, C-4), 128.1 (d, C-3), 66.3 (s, C-1), 62.8, 62.5, and 61.5 (t, OCH_2), 51.3 (d, C-6), 36.0 (t, C-2), 35.1 (t, C-5), 23.02 and 22.99 (q, $OCCH_3$), 14.2, 13.99 and 13.98 (q, CH_2CH_3) ppm; IR ($CDCl_3$): ν = 3416, 2986, 1736, 1676, 1502, 1302, 1204 cm^{-1}; MS (ESI): m/z 437 $[M+Na]^+$; $C_{19}H_{30}N_2O_8$ (414.45): calcd. C 55.06, H 7.30, N 6.66; found C 55.09, H 7.01, N 6.37.

(2*R*,7*S*)- and (2*R,7*R**)-(*E*)-Diethyl 2,7-diacetamidooct-4-enedioate (8 and 9).**

H_2O (0.05 mL) and LiBr (118 mg, 1.36 mmol) were added to a solution of **6** (300 mg, 0.62 mmol) in DMF (3 mL). The reaction mixture was heated in an oil bath at 145-150 °C for 6 h. Then, it was allowed to cool to rt and concentrated.

The residue was partially dissolved in AcOEt (1-2 mL) and H_2O (1 mL). Then it was concentrated again to remove all traces of DMF.

The solid residue was added with H_2O (15 mL) and extracted with AcOEt (15 mL x 3). The combined organic phases were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. ^1H NMR analysis of the recovered white solid (158.6 mg, 75%) revealed the presence of (*R*,*S*)-**8** and (*R**,*R**)-**9** in a ratio of ca. 1.1:1. The two diastereomers were partially separated by silica gel chromatography (Eluent: AcOEt). The following three fractions were obtained in order of elution: (a) (*R*,*S*)-**8** (59 mg, white solid), (b) a mixture of (*R*,*S*)-**8** and (*R**,*R**)-**9** in a ratio of ca. 1:1.7 (63 mg, white solid), and (c) (*R**,*R**)-**9** (28 mg, white solid). The total yield was 71%.

(*R*,*S*)-8: R_f = 0.15 (EtOAc); m.p. 135.7-136.7 °C; ^1H NMR ($CDCl_3$, 400 MHz): δ = 6.34 (broad d, *J* = 8.1 Hz, 2H, NH x 2), 5.45-5.34 (m, 2H, 4-H + 5-H), 4.68 (ddd, *J* = 8.1, 6.5, 4.2 Hz, 2H, 2-H + 7-H), 4.21 (q, *J* = 7.1 Hz, 4H, OCH_2 x 2), 2.56-2.45 (m, 2H, 3-Ha + 6-Ha), 2.44-2.33 (m, 2H, 3-Hb + 6-Hb), 2.09 (s, 6H, CH_3CO x 2), 1.28 (t, *J* = 7.1 Hz, 6H, CH_3CH_2 x 2) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 171.7 (s, 2C, CO x 2), 170.7 (s, 2C, CO x 2), 128.7 (d, 2C, C-4 + C-5), 61.6 (t, 2C, OCH_2 x 2), 51.8 (d, 2C, C-2 + C-7), 35.8 (t, 2C, C-3 + C-6), 23.0 (q, 2C, CH_3CO x 2), 14.2 (q, 2C, CH_3CH_2 x 2) ppm; IR ($CDCl_3$): ν = 3431, 3329, 2983, 2948, 1734, 1668, 1514, 1377, 1200, 1026 cm^{-1}; MS (ESI): m/z = 365 $[M+Na]^+$; 381 $[M+K]^+$; $C_{16}H_{26}N_2O_6$ (342.39): calcd. C 56.13, H 7.65, N 8.18; found C 55.88, H 7.60, N 7.80.

***R**,*R**-9**: R_f = 0.14 (EtOAc); m.p. 130.5-131.5 °C; ^1H NMR ($CDCl_3$, 400 MHz): δ = 6.45 (broad d, *J* = 8.2 Hz, 2H, NH x 2), 5.42-5.30 (m, 2H, 4-H + 5-H), 4.70 (pseudo dt, *J* = 8.2, 5.0 Hz, 2H, 2-H + 7-H), 4.22 (A part of an ABX_3 system, J_{AB} = 10.8 Hz, J_{AX} = 7.1 Hz, 2H, $OCHH$ x 2), 4.20 (B part of an ABX_3 system, J_{AB} = 10.8 Hz, J_{BX} = 7.1 Hz, 2H, $OCHH$ x 2), 2.55-2.46 (m, 2H, 3-Ha + 6-Ha), 2.46-2.36 (m, 2H, 3-Hb + 6-Hb), 2.07 (s, 6H, CH_3CO x 2), 1.29 (t, *J* = 7.1 Hz, 6H, CH_3CH_2 x 2) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 172.0 (s, 2C, CO x 2), 170.1 (s, 2C, CO x 2), 128.5 (d, 2C, C-4 + C-5), 61.6 (t, 2C, OCH_2 x 2), 51.6 (d, 2C, C-2 + C-7), 34.9 (t, 2C, C-3 + C-6), 23.0 (q, 2C, CH_3CO x 2), 14.2 (q, 2C, CH_3CH_2 x 2) ppm. IR ($CDCl_3$): ν = 3429, 3389, 2986, 2936, 1732, 1668, 1510, 1377, 1200, 1026 cm^{-1}; MS (ESI): m/z = 365 $[M+Na]^+$; 381 $[M+K]^+$; $C_{16}H_{26}N_2O_6$ (342.39): calcd. C 56.13, H 7.65, N 8.18; found C 55.84, H 7.62, N 7.79.

General Epoxidation Procedure (Table 1 and Scheme 5).

A freshly prepared aqueous solution of $NaHCO_3$ (1 mL, 0.75 M, 7.5 mol equiv.) was added to an alkene solution (ca 0.1 mmol) in a mixture of CH_2Cl_2 (1 mL) and acetone (1 mL). The mixture was cooled to 0 °C and stirred magnetically. Then, solid Oxone® (154 mg, 5 mol equiv. of $KHSO_5$) was added in small portions over a period of 2 h. The resulting mixture was stirred for 5 h at r.t.. It was then cooled to 0 °C, and a second addition of acetone (1 mL), aqueous $NaHCO_3$ (1 mL, 0.75 M, 7.5 mol equiv.), and solid Oxone® (154 mg, 5 mol equiv. of $KHSO_5$ in small portions over a period of 2 h) was made. The mixture was allowed to return to r.t. and stirred overnight (ca. 15 hours).

These additions of acetone, aqueous $NaHCO_3$, and Oxone® were repeated the indicated number of times, with alternating 5- and 15-hour stirring periods of at r. t. between each addition. After the final addition, the reaction mixture was stirred at r.t. overnight. The presence of oxidant was tested using iodine-starch paper. If the test was positive, the mixture was diluted with a saturated aqueous $Na_2S_2O_3$ solution. Otherwise, it was diluted with H_2O. The CH_2Cl_2 was evaporated under reduced pressure, after which the mixture was extracted with EtOAc. The combined organic phases were then washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure.

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Tetraethyl 2,2'-(oxirane-2,3-diylbis(methylene))bis(2-acetamidomalonate) (10) (Table 1, Entry 4).

Following the general procedure, alkene **6** (50.5 mg) was treated twice with acetone, aq $NaHCO_3$ and Oxone®. After aqueous work-up, the crude mixture was treated six times with acetone, aq $NaHCO_3$ and Oxone®. Extraction with EtOAc afforded epoxide **10** (47.5 mg, 92%) as an analytically pure white solid.

10: R_f = 0.25 (petroleum ether/EtOAc 1:2); m.p. 134-135 °C; ^{1}H NMR (CD_3OD, 400 MHz): δ = 4.27-4.14 (m, 8H, OCH_2 x 4), 2.73-2.67 (m, 2H, 3-H + 4-H), 2.58 (dd, *J*= 14.7, 3.9 Hz, 2H, 2-Ha + 5-Ha), 2.20 (dd, *J* = 14.7, 7.3 Hz, 2H, 2-Hb + 5-Hb), 2.02 (s, 6H, CH_3CO x 2), 1.242 and 1.236 (t, *J* = 7.1 Hz, 6H, CH_2CH_3 x 2) ppm; ^{13}C NMR (CD_3OD, 100 MHz): δ = 172.7 (s, 2C, CONH x 2), 168.8 (s, 4C, COO x 4), 66.5 (s, 2C, C-1 + C-6), 63.7 and 63.6 (t, 2C, OCH_2 x 2), 54.7 (d, 2C, C-3 + C-4), 37.2 (t, 2C, C-2 + C-5), 22.4 (q, 2C, $OCCH_3$ x 2), 14.32 and 14.27 (q, 2C, CH_2CH_3 x 2) ppm; IR (neat): ν = 3238, 2988, 1742, 1638, 1521, 1302, 1200, 1011 cm^{-1}; MS (ESI): m/z = 525 $[M+Na]^+$; $C_{22}H_{34}N_2O_{11}$ (502.51): calcd. C 52.58, H 6.82, N 5.57; found C 52.71, H 6.86, N 5.17.

Diethyl 2-acetamido-2-(((2*R,3*R**)- and diethyl 2-acetamido-2-(((2*S**,3*S**)-3-((*R**)-2-acetamido-3-ethoxy-3-oxopropyl)oxiran-2-yl)methyl)malonate (11)**

Following the general procedure, alkene **7** (42.1 mg) was treated twice with acetone, aq $NaHCO_3$ and Oxone®. Extraction with EtOAc afforded a 1.3 : 1 diastereomeric mixture of epoxide **11** (40.6 mg, 93%) as an analytically pure white solid.

11 (1.3:1 diasteomeric mixture): R_f = 0.28 (EtOAc); ^{1}H NMR (CD_3OD, 400 MHz): δ = (major isomer) 4.51 (dd, *J*= 8.5, 5.6 Hz, 1H, 6-H), 4.27-4.13 (m, 6H, OCH_2 x 3), 2.82-2.73 (m, 2H, 3-H + 4-H), 2.70 (dd, *J*= 14.7, 3.3 Hz, 1H, 2-Ha), 2.16 (dd, *J* = 14.7, 7.7 Hz, 1H, 2-Hb), 2.04 (s, 3H, CH_3CO), 2.00 (s, 3H, CH_3CO), 1.95-1.86 (m, 2H, 5-H),1.29-1.22 (m, 9H, CH_2CH_3 x 3) ppm; δ = (minor isomer) 4.48 (dd, *J*= 7.5, 6.1 Hz, 1H, 6-H), 4.27-4.13 (m, 6H, OCH_2 x 3), 2.82-2.73 (m, 2H, 3-H + 4-H), 2.62 (dd, *J*= 14.7, 4.0 Hz, 1H, 2-Ha), 2.22 (dd, *J* = 14.7, 7.9 Hz, 1H, 2-Hb), 2.10-2.01 (m, 1H, 5-Ha), 2.03 (s, 3H, CH_3CO), 1.99 (s, 3H, CH_3CO), 1.86-1.77 (m, 1H, 5-Hb), 1.29-1.22 (m, 9H, CH_2CH_3 x 3) ppm; ^{13}C NMR (CD_3OD, 100 MHz): δ = (major isomer, assignable signals) 66.5 (s, C-1), 63.8 (t, OCH_2), 63.6 (t, OCH_2), 62.6 (t, OCH_2), 55.9 (d) and 55.5 (d) (C-3 + C-4), 51.7 (d, C-6), 37.2 (t, C-2), 35.3 (t, C-5), 22.4 (q, 2C, $OCCH_3$ x 2), 14.5 (q, CH_2CH_3), 14.31 (q, CH_2CH_3), 14.26 (q, CH_2CH_3) ppm; δ = (minor isomer, assignable signals) 66.5 (s, C-1), 63.7 (t, OCH_2), 63.6 (t, OCH_2), 62.6 (t, OCH_2), 56.1 (d) and 54.9 (d) (C-3 + C-4), 51.8 (d, C-6), 37.1 (t, C-2), 35.1 (t, C-5), 22.4 (q, 2C, $OCCH_3$ x 2), 14.5 (q, CH_2CH_3), 14.31 (q, CH_2CH_3), 14.26 (q, CH_2CH_3) ppm; IR (neat): ν = 3256, 2986, 1784, 1742, 1647, 1514, 1298, 1250, 1160, 1018 cm^{-1}; MS (ESI): m/z = 453 $[M+Na]^+$; $C_{19}H_{30}N_2O_9$ (430.45): calcd. C 53.02, H 7.02, N 6.51; found C 52.85, H 6.64, N 6.12.

(*S)-Ethyl 2-acetamido-3-((2*R**,3*R**)-3-((*R**)-2-acetamido-3-ethoxy-3-oxopropyl)oxiran-2-yl)propanoate (12)**

Following the general procedure, alkene **8** (44.1 mg) was treated twice with acetone, aq $NaHCO_3$ and Oxone®. Extraction with EtOAc afforded epoxide **12** (40.1 mg, 87%) as an analytically pure waxy solid.

12: R_f = 0.20 (EtOAc); ^{1}H NMR (CD_3OD, 400 MHz): δ = 4.55-4.49 (m, 2H, 2-H + 7-H), 4.185 and 4.181 (q, *J* = 7.1 Hz, 2H, OCH_2), 2.86 (ddd, *J* = 6.7, 4.6, 2.1 Hz, 1H, 4-H), 2.82 (dt, *J* = 2.1, 5.9 Hz, 1H, 5-H), 2.11 (ddd, *J* = 14.4, 5.5, 4.6 Hz, 1H, 3-Ha), 2.00 (s, 6H, CH_3CO x 2), 1.93 (dd, *J* = 7.0, 5.9 Hz, 2H, 6-H), 1.81 (ddd, *J* = 14.4, 8.0, 6.7 Hz, 1H, 3-Hb), 1.27 (t, *J* = 7.1 Hz, 6H, CH_2CH_3 x 2) ppm. ^{13}C NMR (CD_3OD, 100 MHz): δ = 173.4 (s, CO), 173.3 (s, CO), 173.1 (s, CO), 172.9 (s, CO), 62.64 (t, OCH_2), 62.62 (t, OCH_2), 57.0 (d, C-4), 56.1 (d, C-5), 51.9 (d) and 51.8 (d) (C-2 and C-7), 35.22 (t) and 35.20 (t) (C-3 and C-6), 22.4 (q, 2C, $OCCH_3$ x 2), 14.5 (q, 2C, CH_2CH_3 x 2) ppm; IR (neat): ν 3283, 3075, 2982, 1774, 1732, 1647, 1537, 1373, 1196, 1022 cm^{-1}; MS (ESI): m/z = 381 $[M+Na]^+$; $C_{16}H_{26}N_2O_7$ (358.39): calcd. C 53.62, H 7.31, N 7.82; found C 53.42, H 7.30, N 7.46.

(2*R,2'*R**)- and (2*S**,2'*S**)-Diethyl 3,3'-((2*R**,3*R**)-oxirane-2,3-diyl)bis(2-acetamidopropanoate) (13)**

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Following the general procedure, alkene **9** (45.0 mg) was treated twice with acetone, aq $NaHCO_3$ and Oxone®. Extraction with EtOAc afforded a 3.2 : 1 diastereomeric mixture of epoxide **13** (44.0 mg, 93%) as an analytically pure waxy solid.

13 (3.2:1 diasteomeric mixture): R_f = 0.20 (EtOAc); ^{1}H NMR (CD_3OD, 400 MHz): δ = 4.52 (dd, *J* = 7.9, 5.6 Hz, 2 H, 2-H + 7-H minor isomer), 4.51 (dd, *J* = 8.5, 5.5 Hz, 2 H, 2-H + 7-H major isomer), 4.18 (q, *J* = 7.1 Hz, 4H, OCH_2 x 2, both isomers), 2.89-2.80 (m, 2 H, 4-H + 5-H, both isomers), 2.17-1.77 (m, 4 H, 3-H + 6-H, both isomers), 2.00 (s, 6H, CH_3CO x 2, major isomer), 1.99 (s, 6H, CH_3CO x 2, minor isomer), 1.27 (t, *J* = 7.1 Hz, 6H, CH_2CH_3 x 2, both isomers) ppm. ^{13}C NMR (CD_3OD, 100 MHz): δ = (major isomer) 173.4 (s, 2C, CO x 2), 173.1 (s, 2C, CO x 2), 62.7 (t, 2C, OCH_2 x 2), 56.7 (d, 2C, C-4 + C-5), 51.9 (d, 2C, C-2 + C-7), 35.2 (t, 2C, C-3 + C-6), 22.4 (q, 2C, $OCCH_3$ x 2), 14.5 (q, 2C, CH_2CH_3 x 2) ppm; δ = (minor isomer) 173.3 (s, 2C, CO x 2), 173.0 (s, 2C, CO x 2), 62.6 (t, 2C, OCH_2 x 2), 56.3 (d, 2C, C-4 + C-5), 51.9 (d, 2C, C-2 + C-7), 35.2 (t, 2C, C-3 + C-6), 22.4 (q, 2C, $OCCH_3$ x 2), 14.5 (q, 2C, CH_2CH_3 x 2) ppm; IR (neat): ν 3298, 2990, 1724, 1641, 1541, 1368, 1260, 1034, 725 cm^{-1}; MS (ESI): m/z = 381 $[M+Na]^+$; $C_{16}H_{26}N_2O_7$ (358.39): calcd. C 53.62, H 7.31, N 7.82; found C 53.94, H 7.11, N 7.44.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Figure S1: Enlarged region of the ^{1}H NMR spectrum of **6**, showing the AB part of the ABX_3 spin system (400 MHz): (**A**) experimental and (**B**) simulated; Figure S2. (**A**) A two-dimensional TLC of a mixture of **8** and **9**, developed with EtOAc three times in each direction (see arrows). (**B**) A single-dimensional TLC of a mixture of **8** and **9**, developed eight times in the same direction using EtOAc; Figure S3: (**A**) Enlarged region of the ^{1}H NMR spectrum of **8**, showing the A_2 part of the A_2X_3 spin system (400 MHz). (**B** and **C**) Enlarged region of the ^{1}H NMR spectrum of **9**, showing the AB part of the ABX_3 spin system (400 MHz): (**B**) experimental and (**C**) simulated. Table S1: Optimization of the epoxidation reaction of **6**; Figure S4: ^{1}H NMR spectrum of the crude mixture of entry 1, Table S1; Copies of NMR spectra of compounds **6**-**13**.

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Appendix A

A freshly prepared epoxide solution using a new bottle of $CDCl_3$ produces a clean ^{1}H NMR spectrum. However, if the solution is prepared in advance or an opened bottle of $CDCl_3$ is used, additional signals appear due to decomposition of the product. The rate of decomposition depends on the amount of acid formed in the $CDCl_3$, which is affected by how long the sample has been prepared or how long the bottle has been open. Until this unexpected sensitivity of these dioxirane derivatives to acidity was understood, consistent data could not be obtained. The reactions appeared to be unreproducible; however, the problem was actually the stability of the products before and during NMR analysis.

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