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The Synthesis of the Vitamers of Biotin

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An efficient synthetic pathway for the preparation of the vitamers of biotin which provides easy access to optically pure KAPA as well as diastereomeric mixtures of DAPA, DTB, and analogs thereof has been developed.

INTRODUCTION

Biotin is a water-soluble vitamin that functions as a coenzyme in certain carboxylation and transcarboxylation reactions (*1*). Perhaps the most important of these, the carboxylation of acetyl-CoA to form malonyl-CoA, is catalyzed by acetyl-CoA carboxylase and is an initial step in fatty acid and polyketide biosynthesis. Since the proper function of at least one of the reactions in which biotin participates is a prerequisite for the growth and development of almost all organisms, biotin is an essential nutrient. Many organisms, including the majority of plants and microbes, synthesize their own biotin; others, including animals and certain microbes such as yeast and *Lactobacillus*, must obtain the vitamin from external sources. The later intermediates in the biosynthetic path, the so-called vitamers 7-keto-8-amino pelargonic acid (KAPA), 7,8-diamino pelargonic acid (DAPA), and desthiobiotin (DTB) (Scheme 1), have been identified.

Earlier reports (*2*) described the synthesis of racemic KAPA and DAPA without reporting any spectral data. Moreover, while a very large number of publications (*3*) have described the partial or total synthesis of biotin, none of these synthetic routes have been based on the natural biosynthetic path.

The final stage in the biosynthesis, at which DTB is converted into biotin, involves insertion of a S atom between two nonactivated positions (methyl and methylene groups) to form the tetrahydrothiophene ring and is of particular interest. This reaction is exceedingly difficult from a chemical standpoint. The mechanism of the enzymatic reaction is unknown. Although the enzymatic reaction has been observed *in vitro* (*4*), the sulfur donor has not been established (*5*). There is evidence that the reaction catalyzed by biotin synthase can be the rate-limiting step in the biotin biosynthetic pathway.

We were interested in developing convenient syntheses of the natural vitamers

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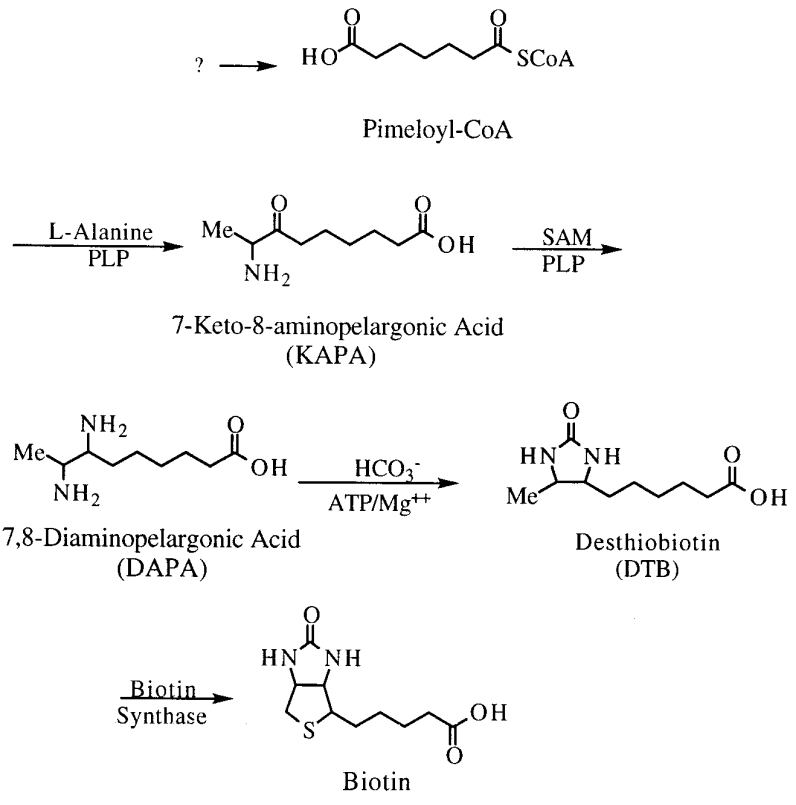


SCHEME 1. Biosynthetic pathway of biotin.

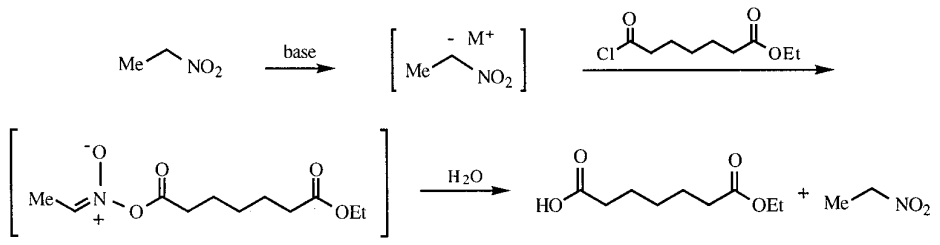
and in extending the methodology developed, for the synthesis of analogs that may be used to study the mechanism of this last biosynthetic step. We describe herein a new total synthesis of the three vitamers.

RESULTS AND DISCUSSION

Synthesis of KAPA. Our initial approach involved acylation of the anion of 2-nitroethane with a suitable derivative of pimelic acid. However, hydrolytic workup of the reaction gave only the monoester of pimelic acid (Scheme 2).

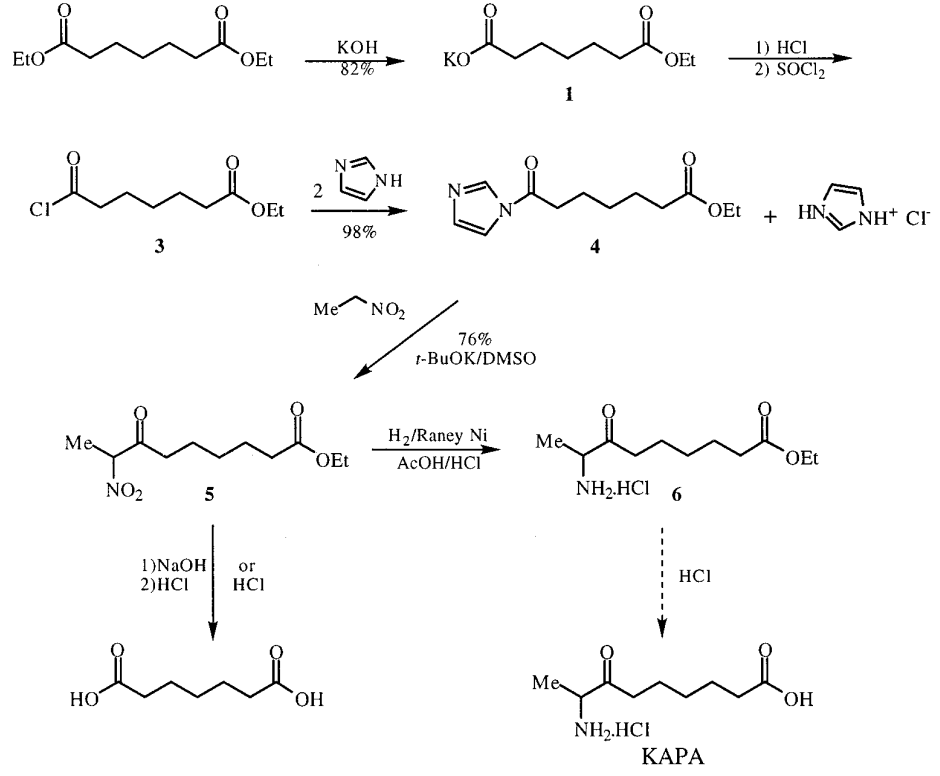
This problem was overcome by initial conversion of the acyl chloride **3** to the corresponding acyl imidazole **4** that led to the desired 7-keto-8-nitro-pelargonate **5.** Reduction of the latter with Raney Ni gave the KAPA ethyl ester **6** in poor yield (Scheme 3).

This approach suffered from several deficiencies: (a) The products obtained were racemic, (b) the yield in the reduction step was very low, and (c) pimelic acid, as

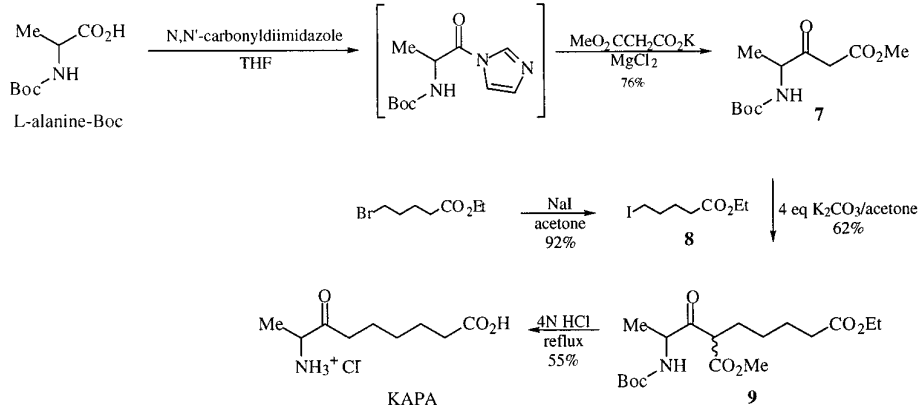


SCHEME 2

well as other 7-carbon chain unit derivatives, is expensive. To overcome these problems a new approach was taken whereby the N-containing moiety was derived from L-alanine. This approach provided the desired chirality and enabled condensation with readily available derivatives having shorter carbon chains. The most suitable L-alanine derivative in this approach was Mansour's β-keto ester **7** (*6*)



SCHEME 3

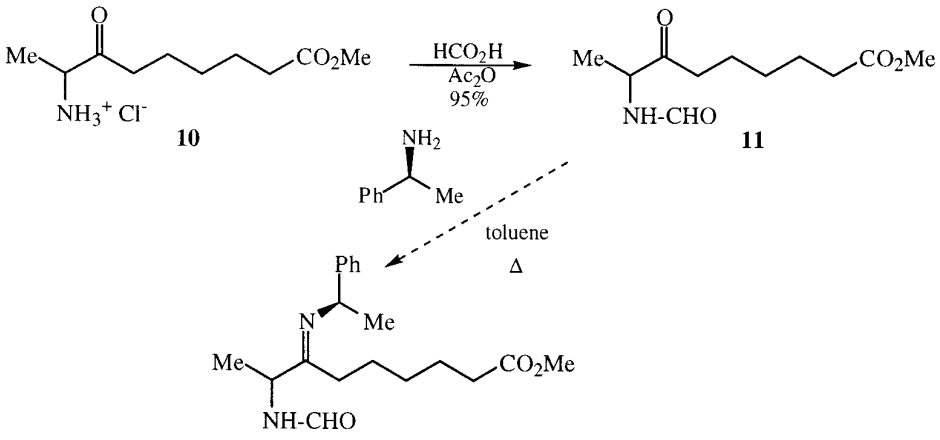


SCHEME 4

protected by an *N*-Boc group (Scheme 4). Our earlier experiments, when using the corresponding *N*-phthalimido derivatives, led to various rearrangement products (*7*). Alkylation of **7** with ethyl 5-iodovalerate **8** in the presence of 4 equivalents of K_2CO_3 in acetone (conditions found to be optimal, other bases and solvents resulting in much lower yields) gave **9.** Complete deprotection by acid hydrolysis gave optically pure KAPA, found to be identical to the natural vitamer (Scheme 4).

Synthesis of DAPA. The reductive amination of KAPA to DAPA in the biosynthetic pathway involves PLP and SAM (Scheme 1). Our initial approaches were to carry out this reaction by nonenzymatic methods taking into consideration the stereospecificity of the reductive step. Ketones have been converted to chiral amines by reduction of their corresponding chiral imines, such as those obtained by condensation with optically pure α-methylbenzyl amine, where the imine formation takes place under basic conditions (*8*). In our case, since KAPA is an α-amino ketone unstable in basic media, an initial N-protection step was required. N-protection reactions are commonly conducted in basic media; however, in this case the protective step required nonbasic conditions in order to prevent the formation of α-amino ketone intermolecular condensation products. Such an N-protection was carried out by N-formylation in the presence of formic acid/acetic anhydride (*9*) (Scheme 5). Unfortunately the subsequent ketimine formation under a variety of conditions failed.

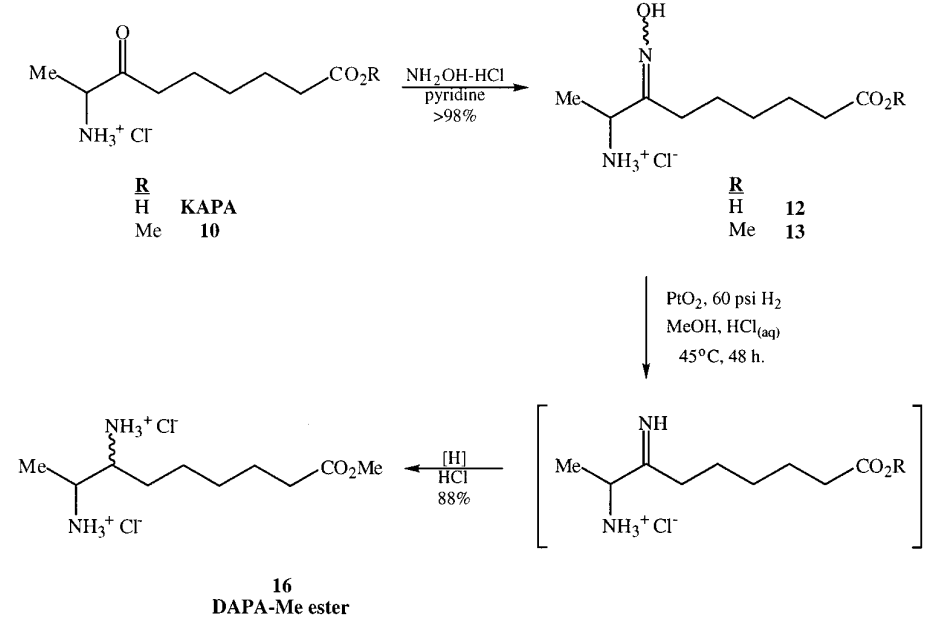
The next approach involved reduction of the readily formed oxime derivative of KAPA. Oximation of KAPA or its methyl ester **10** gave a mixture of *syn*- and *anti*-oximes (**12** or **13,** respectively) consisting mainly of the *syn* isomers. This suggested that the chiral center at the C-8 position induced stereoselectivity and it was thus hoped that a similar induction would be obtained in the course of the following reduction step. However, upon reduction an approximately equimolar mixture of diastereomers was obtained. The loss of stereospecific induction may be attributed to initial reduction of the oxime to an intermediate imine, combined with a weakening of the H bond between the imine and the neighboring ammonium ion under



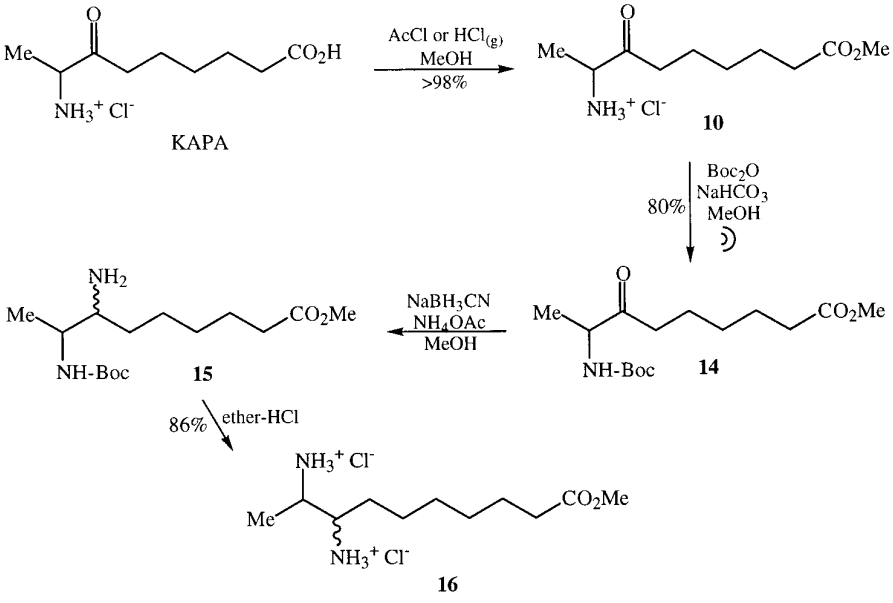
SCHEME 5

the acidic conditions of the reaction. Furthermore, under the methanolic–acidic conditions of the reaction, the corresponding DAPA methyl ester **16** was isolated (Scheme 6).

An alternative reductive amination was carried out in the presence of $NaBH_3CN$



SCHEME 6

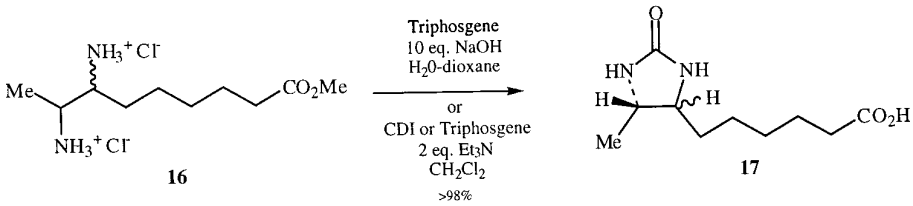


SCHEME 7

that gave **16** also as an approximately equimolar mixture of diastereomers (Scheme 7). This approach required double protection, both of the acid as its Me ester **10,** conveniently carried out in the presence of anhydrous HCl/MeOH (*10*) and of the N as the *N*-Boc, **14.** The latter was conducted by sonication under mild basic conditions in order to avoid standard harsh basic media that would result in decomposition and/or dimerization of the free α-amino ketone.

Synthesis of DTB. The formation of the imidazolidone ring of DTB was carried out by reaction of DAPA with either triphosgene or carbonyl diimidazole (CDI). In the former case the reaction proceeded in aqueous media in the presence of base using the DAPA-free carboxylic acid or its ester **16.** In the course of the workup, the ester readily hydrolyzed. Alternatively the reaction was performed in organic media from **16** and either triphosgene or CDI, from which the yields obtained using triphosgene were quantitative (Scheme 8).

Summary. An efficient synthetic pathway for the synthesis of the vitamers of biotin, which provides easy access to optically pure KAPA as well as diastereomeric mixtures of DAPA, DTB, and analogs thereof, has been developed. As indicated, the reduction of the oximes **12** and **13** did not proceed stereospecifically. No further attempts were made at this point to separate the diastereomers since our ultimate goal is to develop a stereospecific synthesis for the vitamers. Further investigations are in progress dealing with stereospecific reductive amination of KAPA to DAPA. If this goal is attained, it should be possible to proceed to DTB maintaining complete stereospecificity using the reactions described in this paper.



SCHEME 8

EXPERIMENTAL

^{1}H NMR spectra at 200 and 300 MHz were obtained on Bruker AC-200 and AM-300 spectrometers, respectively. When D_2O was used as NMR solvent, its own peak was used as a standard for ^{1}H NMR, while MeOH was used as an internal standard for ^{13}C NMR. Mass spectra were obtained on a Varian Mat 731 spectrometer (CI =chemical ionization). HRMS were obtained on a VG AutoSpec E spectrometer. Progress of the reactions was monitored by TLC on silica gel (Merck, Art. 5554) or alumina (Riedel-de Haen, Art. 37349). Flash chromatography was carried out on silica gel (Merck, Art. 9385). Most commercially available chemicals were purchased from Aldrich Co., Sigma Co., and Fluka Co. and were used without further purification.

Heptanedioic acid monoethyl ester K salt (***1***). To an ice-cold solution of diethyl heptanedioate (99.9 g, 0.46 mol) in absolute EtOH (15 ml) was added dropwise an ice-cold solution of KOH (30.5 g, 0.46 mol, 85%) in absolute EtOH. The mixture was stirred for 1 h, then ether was added until the sample began to crystallize. The solid (40 g) was filtered and to the filtrate more ether was added. An additional two crops of white crystals (combined total, 43 g) were collected. ^{1}H NMR (D_2O) δ 1.23 (t, J = 7.2 Hz, 3H, Me), 1.3 (m, 2H, $CH_2CH_2CH_2CO$), 1.48–1.64 (m, 4H, 2 CH_2CH_2CO), 2.15 (t, J = 7.7 Hz, 2H, CH_2COOK), 2.36 (t, J = 7.4 Hz, 2H, $EtOOCCH_2$), 4.13 (q, J = 7.2 Hz, 2H, OCH_2). The NMR spectrum of the first crop indicated it was contaminated with about 50% di-K heptanedioate.

Heptanedioic acid monoethyl ester (***2***). An ice-cold solution of K ethyl heptanedioate (16.5 g, 79 mmol) in H_2O (45 ml) was acidified with concd HCl to pH 5 and was rapidly extracted with ether (2 × 100 ml). The organic phase was dried and evaporated to give the acid as an oil (11.2 g, 75%). ^{1}H NMR ($CDCl_3$) δ 1.25 (t, J = 7.3 Hz, 3H, Me), 1.3–1.5 (m, 2H, $CH_2CH_2CH_2CO$), 1.6–1.75 (m, 4H, 2 CH_2CH_2CO), 2.25–2.40 (m, 4H, 2 CH_2CO), 4.15 (q, J = 6.9 Hz, 2H, OCH_2).

Chlorocarbonylhexanoic acid ethyl ester (***3***). A solution of monoethyl heptanedioate (40 g, 0.21 mol) in 50 ml of $SOCl_2$ was heated to reflux until no more gas evolution was evident. The excess $SOCl_2$ was evaporated and the residue was distilled.

7-Imidazol-1-yl-7-oxo-heptanoic acid ethyl ester (***4***). To a solution of ethyl heptanedioyl chloride (24.5 g, 0.12 mol) in THF (200 ml), cooled in an ice bath, was added a solution of imidazole (16.1 g, 0.24 mol) in ice-cold THF (100 ml). The

mixture was stirred for 1 h and the solid precipitate was filtered and washed with THF (150 ml), and the filtrate was evaporated at 60°C to give the product as an oil (27.9 g, 98%). ^{1}H NMR ($CDCl_3$) δ 1.25 (t, J = 7.2 Hz, 3H, Me), 1.40–1.50 (m, 2H, $CH_2CH_2CH_2CO$), 1.69 (quintet, J = 7.6 Hz, 2H, CH_2CH_2CO), 1.83 (quintet, J = 7.6 Hz, 2H, CH_2CO), 2.33 (t, J = 7.4 Hz, 2H, CH_2CON), 2.92 (t, J = 7.3 Hz, 2H, O_2CCH_2), 4.13 (q, J = 7.1 Hz, 2H, OCH_2), 7.08 (m, 1H, Ar), 7.50 (m, 1H, Ar), 8.20 (s, 1H, Ar).

8-Nitro-7-oxononanoic acid ethyl ester (***5***). To an ice-cold solution of nitroethane (57 mmol) in DMSO (50 ml) was added *tert*-BuOK (6.40 g, 57 mmol) followed by the addition of ethyl 1-imidazolyl heptanedioate (57 mmol). The mixture was stirred for 72 h, then poured into H_2O (300 ml), and washed with ether. The aqueous phase was acidified to pH 5.8 with concd HCl and was extracted with EtOAc (2 × 100 ml). The organic phase was dried, mixed with charcoal, filtered, and evaporated to give the ethyl 8-nitro-7-oxononanoate as an oil (76%). ^{1}H NMR (CD_3OD) δ 5.56 (q, J = 7.1 Hz, 1H, CH), 4.06 (q, J = 7.2 Hz, 2H, MeCH_2), 2.25–2.35 (m, 4H, 2 CH_2CO), 1.65 (d, J = 7.1 Hz, 3H, *Me*CH), 1.60 (quintet, J = 7.6 Hz, 4H, 2 CH_2CH_2CO), 1.23–1.40 (m, 2H, $CH_2CH_2CH_2CO$), 1.24 (t, J = 7.1 Hz, 3H, *Me*CH_2). MS (EI) $C_{11}H_{19}NO_5$ 200 (M–C_2H_5O), 171 (M–$C_2H_4NO_2$), 125 (M–$C_2H_4NO_2$–C_2H_6O).

8-Amino-7-oxononanoic acid ethyl ester hydrochloride (***6***). To solution of ethyl 8-nitro-7-oxononanoate (0.5 g, 2 mmol) in EtOH (5 ml) was added concd HCl (0.2 ml). The solution was hydrogenated over Raney Ni at 35°C, 1000 psi for 2 h. After 1 h no further consumption of H_2 was detected. The mixture was filtered and to the filtrate was added a solution of NaHS until no further formation of black NiS was evident. The mixture was filtered and flash evaporated. ^{1}H NMR (D_2O) δ 1.24 (t, J = 7.1 Hz, 3H, *Me*CH_2), 1.25–1.45 (m, 2H, $CH_2CH_2CH_2CO$), 1.55 (d, J = 7.4 Hz, 3H, *Me*CH), 1.61 (q, J = 7.6 Hz, 4H, 2 CH_2CH_2CO), 2.30–2.45 (m, 4H, 2 CH_2CO), 4.15 (q, J = 7.2 Hz, 2H, MeCH_2), 4.28 (q, J = 7.4 Hz, 1H, CH).

4-tert-Butoxycarbonylamino-3-oxopentanoic acid methyl ester (***7***). To a solution of *N*-Boc-alanine (1 mmol) in dry THF (15 ml) was added, portionwise, CDI (0.195 g, 1.2 mmol). The solution was stirred at room temperature (RT) under N_2 for 0.5 h, followed by the addition of $MgCl_2$ (0.1 g, 1 mmol) and monomethyl malonate potassium salt (0.16 g, 1 mmol), and the resulting slurry was stirred at 50°C overnight. The mixture was filtered and evaporated, and the residue was partitioned between 1 N HCl and EtOAc. The aqueous layer was extracted with EtOAc (3 × 15 ml). The combined organic layer was washed with 1 N HCl (15 ml), 5% $NaHCO_3$ (3 × 15 ml), brine (15 ml), dried ($MgSO_4$), and evaporated to give the desired product as white to light yellow crystals (76% yield). ^{1}H NMR ($CDCl_3$) δ 1.38 (d, J = 7 Hz, 3H, *Me*CH), 1.45 (s, 9H, Me_3C), 3.54 (ABq, J_{AB} = 17 Hz, 2H, CH_2), 3.71 (s, 3H, OMe), 4.55 (quintet, J = 7 Hz, 1H, CH), 5.10 (br d, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 15.00 (*Me*CH), 28.00 (Me_3C), 43.75 (CH_2), 51.78 (OMe), 53.82 (CH), 80.03 (Me_3C), 155.62 (CON), 169.73 (CO_2), 200.09 ($CHCOCH_2$). MS (CI, *i*-Bu) *m/e* 245 (MH^+). $[\alpha]_D^{25}$ 50.68° (*c* 0.74, MeOH).

5-Iodopentanoic acid ethyl ester (***8***). To a solution of NaI (1.1 mmol) in dry acetone (15 ml) was added ethyl 5-bromovalerate (1 mmol). A precipitance formed almost immediately and the resulting suspension was stirred at RT overnight. The

mixture was filtered, the salts were washed with acetone, and the filtrate was evaporated to give a mixture of oily product and unreacted NaI. This mixture was diluted with CH_2Cl_2 and filtered. Evaporation of the filtrate gave the product as an orange liquid (92% yield, ca. 95% conversion of alkyl bromide to the corresponding iodide). ^{1}H NMR ($CDCl_3$) δ 1.26 (t, J = 7 Hz, 3H, CH_2*Me*), 1.65–1.95 (m, 4H, CH_2*CH*$_2$*CH*$_2$$CH_2CO$), 2.33 (t, J = 7 Hz, 2H, CH_2CO), 3.20 (t, J = 7 Hz, 2H, ICH_2), 4.14 (q, J = 7 Hz, 2H, *CH*$_2$Me). ^{13}C NMR ($CDCl_3$) δ 5.70 (CH_2I), 14.24 (Me), 25.82 (CH_2CH_2CO), 32.78 (CH_2CO), 33.13 (CH_2CH_2I), 60.37 (OCH_2), 173.01 (CO). MS (EI) *m/e* 256 (M^+).

2-(2-tert-Butoxycarbonylaminopropionyl]heptanedioic acid 7-ethyl ester 1-methyl ester (***9***). To a solution of **7** (1 mmol) in dry acetone (15 ml), was added anhydrous K_2CO_3 (4 mmol), followed by the addition of **8** (1 mmol). The resulting suspension was refluxed under N_2 for 6 h. The reaction mixture was cooled and filtered, and the salts were washed with acetone. The filtrate was concd and the residue was purified by flash chromatography on a silica gel column using hexane:EtOAc (usually 2 : 1 or 3 : 1) as eluent, to give the desired *C*-alkylated product as a light yellow oil, containing both diastereomers (62% yield). ^{1}H NMR ($CDCl_3$) δ 1.25–1.40 (superimposed, m, 8H, CH_2*Me*, CH*Me*, CH*CH*$_2$), 1.45 (s, 9H, Me_3C), 1.60–1.95 (m, 4H, $CHCH_2$*CH*$_2$*CH*$_2$), 2.30 (t, J = 7.5 Hz, 2H, CH_2CO_2), 3.70–3.80 (superimposed, m, 4H, *CH*CO_2*Me*), 4.12 (q, J = 7 Hz, 2H, *CH*$_2$Me), 4.41 (quintet, J = 7 Hz, 1H, *CH*Me), 5.22 (br d, J = 7 Hz, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 14.23 (CH_2*Me*), 16.95 and 17.45 (2 CH*Me*), 24.57 and 24.65 (2 *C*H_2CH_2COCH), 26.87 and 26.96 (2 $CHCH_2$*C*H_2) 27.64 ($CHCH_2$), 28.33 (*Me*$_3$C), 33.93 (*C*H_2CO_2), 52.35 and 52.48 (2 OMe), 54.60 (*C*HMe), 55.07 (*C*HCH_2), 60.22 (O*C*H_2), 79.93 (Me_3C), 155.07 (CON), 169.60 (*C*O_2Me), 173.30 (*C*O_2Et), 204.48 and 204.63 (2 CH*C*OCH). MS (CI, *i*-Bu) *m/e* 374 (MH^+, 13), 318 (MH^+–C_4H_8, 22). HRMS (DCI, CH_4) calcd for $C_{18}H_{32}NO_7$ (MH^+): 374.2178. Found: 374.2143.

8-Amino-7-oxononanoic acid·hydrochloride (*KAPA·HCl*). A suspension of **9** (1 mmol) in 4 N HCl (5 ml) was refluxed at 110°C for 2 to 4 h. Gas evolution was observed. Heating was continued until the mixture became homogeneous. The resulting dark yellow solution was evaporated and dried under high vacuum. When the color became darker, the solution was filtered through charcoal, prior to the evaporation. The residue usually crystallized under high vacuum. Recrystallization was carried out by dissolving in absolute EtOH and crystallizing upon addition of dry ether, or ether–HCl, to give a derivative of KAPA·HCl as off-white powder (55% yield, after recrystallization). ^{1}H NMR (D_2O) δ 1.24–1.42 (m, 2H, *CH*$_2CH_2CH_2CO_2H$), 1.55 (d, J = 7.5 Hz, 3H, Me), 1.5–1.7 (m, 4H, *CH*$_2CH_2$*CH*$_2CH_2CO_2H$), 2.38 (t, J = 7.5 Hz, 2H, *CH*$_2CO_2H$), 2.68 (ABq of t, J_{AB} = 18 Hz, J_{AX} = J_{BX} = 7 Hz, 2H, *CH*$_2$COCH), 4.26 (q, J = 7.5 Hz, 1H, CH). ^{13}C NMR (D_2O) δ 15.27 (Me), 22.86 (*C*$H_2CH_2CO_2H$), 24.57 (*C*H_2CH_2COCH), 28.16 (*C*$H_2CH_2CH_2CO_2$), 34.28 (*C*H_2CO_2H), 38.46 (*C*H_2COCH), 55.38 (CH), 179.64 (CO_2H), 209.83 (CH_2*C*OCH). MS (CI, NH_3) *m/e* 188 (MH^+–HCl); mp 133–134°C [lit.[2c] 134–136°C]; $[\alpha]_D^{25}$ −4.46° (*c* 0.23, MeOH).

8-Amino-7-oxononanoic acid methyl ester hydrochloride (*KAPA·HCl methyl ester,* ***10***). To an ice-cold solution of KAPA·HCl (1 mmol) in dry MeOH (10 ml) was dropwise added acetyl chloride, in order to generate $HCl_{(g)}$ *in situ*. The resulting

mixture was allowed to warm to RT and was stirred overnight. Evaporation yielded the desired methyl ester as an off-white powder (quantitative yield). ^{1}H NMR (D_2O) δ 1.33 (m, 2H, $CH_2CH_2CH_2CO_2$), 1.58 (superimposed, m and d, J_d = 7.5 Hz, 7H, CH*Me*, $CH_2CH_2CH_2CH_2CO_2$), 2.40 (t, J = 7 Hz, 2H, CH_2CO_2), 2.68 (ABq of t, J_{AB} = 18 Hz, J_{AX} = J_{BX} = 7.5 Hz, 2H, CH_2COCH), 3.69 (s, 3H, OMe), 4.26 (q, J = 7.5 Hz, 1H, CH). ^{13}C NMR (D_2O) δ 15.27 (Me), 22.82 ($CH_2CH_2CO_2$), 24.50 (CH_2CH_2COCH), 28.16 ($CH_2CH_2CH_2CO_2$), 34.03 (CH_2CO_2), 38.44 (CH_2COCH), 52.66 (OMe), 55.36 (CH), 178.04 (CO_2), 209.80 (CH_2COCH). MS (CI, *i*-Bu) *m/e* 202 (MH^+–HCl).

General procedure for the preparation of KAPA · HCl ketoxime derivatives. To a solution of a KAPA · HCl derivative (1 mmol) in dry pyridine (1.5 ml) was added a solution of $NH_2OH \cdot HCl$ (3 mmol) in dry pyridine (2.5 ml). The mixture was stirred at RT overnight and was then evaporated to dryness under high vacuum. The residue was dissolved in distilled water (2.5 ml) and was made basic by adding 0.5 N NaOH (until pH =8). This solution was extracted with CH_2Cl_2 (4 × 2.5 ml) to remove the remaining pyridine. The aqueous phase was evaporated under high vacuum yielding the desired product as a solid. TLC plates used to monitor the reaction were sprayed with a solution of 0.5% $CuCl_2$, to give strong blue spots for oximes. The oximes were obtained as mixtures of two diastereomers (*ca.* 3:1) derived from *syn*- and *anti*-oximes.

8-Amino-7-hydroxyiminononanonic acid hydrochloride (*KAPA · HCl oxime,* ***12***). Obtained as a white powder, recrystallized from MeOH (quantitative yield). ^{1}H NMR (D_2O) δ 1.37 (m, 2H, $CH_2CH_2CH_2CO_2$), 1.49 (d, J = 7 Hz, 3H, Me), 1.57 (m, 4H, $CH_2CH_2CH_2CH_2CO_2$), 2.21 (superimposed, t and m, J_t = 7 Hz, 2H, CH_2CO_2, and 1H, $CNCH_2$), 2.59 (m, 1H, $CNCH_2$), 4.12 (q, J = 7 Hz, 1H, CH). ^{13}C NMR (D_2O) δ 17.09 (Me), 24.92 ($CH_2CH_2CO_2$), 25.65 (CH_2CH_2CN), 29.09 ($CH_2CH_2CH_2CN$), 37.34 (CH_2CO_2), 49.35 (CH), 159.43 (CN), 183.55 (CO_2). MS (CI, NH_3) *m/e* 203 (MH^+–HCl).

8-Amino-7-hydroxyiminononanoic acid methyl ester hydrochloride (*KAPA · HCl oxime methyl ester,* ***13***). Obtained as a white powder (quantitative yield). ^{1}H NMR (D_2O) δ 1.37 (m, 2H, $CH_2CH_2CH_2CO_2$), 1.48 (d, J = 7 Hz, 3H, Me), 1.60 (m, 4H, $CH_2CH_2CH_2CH_2CO_2$), 2.22 (ddd, J = 14, 9, 5.5 Hz, 1H, $CNCH_2$), 2.40 (t, J = 7.5 Hz, 2H, CH_2CO_2), 2.56 (ddd, J = 14, 9.5, 6.5 Hz, 1H, $CNCH_2$), 3.69 (s, 3H, OMe), 4.10 (q, J = 7 Hz, 1H, CH). ^{13}C NMR (D_2O) δ 17.07 (Me), 24.28 ($CH_2CH_2CO_2$), 24.78 (CH_2CH_2CN), 25.67 (CH_2CN), 28.71 ($CH_2CH_2CH_2CN$), 33.93 (CH_2CO_2), 49.38 (CH), 52.54 (OMe), 159.23 (CN), 178.03 (CO_2). MS (CI, NH_3) *m/e* 217 (MH^+–HCl).

8-Formylamino-7-oxononanoic acid methyl ester (***11***). Ac_2O (0.75 ml) was added dropwise to an ice-cooled solution of KAPA · HCl methyl ester (0.23 g, 1 mmol) in HCOOH (2.25 ml). The mixture was stirred at RT for 1 h. A small amount of ice was added and the mixture was evaporated to dryness to give the desired *N*-formylated product as a white solid (95% yield). ^{1}H NMR ($CDCl_3$) δ 1.37 (m, 2H, $CH_2CH_2CH_2CO_2$), 1.64 (superimposed, m, 7H, *Me*CH and $CH_2CH_2CH_2CH_2CO_2$), 2.31 (t, J = 7.4 Hz, 2H, CH_2CO_2), 2.60 (t, J = 7.3 Hz, CH_2COCH), 3.66 (s, 3H, OMe), 4.42 (m, 1H, CH), 8.29 (m, 2H, NH and CHO). ^{13}C NMR ($CDCl_3$) δ 15.56 (CH*Me*), 22.66 ($CH_2CH_2CO_2$), 24.50

($CH_2CH_2CH_2CH_2CO_2$), 28.34 ($CH_2CH_2CH_2CO_2$), 33.66 (CH_2CO_2), 38.31 (CH_2COCH), 51.42 (OMe), 55.23 (CH), 163.23 (COH), 174.00 (CO_2), 206.31 (CH_2COCH). MS (CI, *i*-Bu) *m/e* 230 (MH^+–HCl).

8-tert-Butoxycarbonylamino-7-oxononanoic acid methyl ester (***14***). A suspension of KAPA · HCl methyl ester (1 mmol), di-*tert*-butyl dicarbonate (0.218 g, 1 mmol), and $NaHCO_3$ (0.25 g) in dry MeOH (5 ml) was sonicated at RT for 6 h. The resulting mixture was filtered and the filtrate was evaporated to dryness. The residue was dissolved in ether, filtered, and evaporated to give the desired product as a light yellow oil (80% yield). ^{1}H NMR ($CDCl_3$) δ 1.31 (d, J = 7.2 Hz, 3H, CH*Me*), 1.32 (m, 2H, $CH_2CH_2CH_2CO_2$), 1.44 (s, 9H, Me_3C), 1.63 (2 m, 4H, $CH_2CH_2CH_2CH_2CO_2$), 2.31 (t, J = 7.4 Hz, 2H, CH_2CO_2), 2.50 (ABq of t, J_{AB} = 17.3 Hz, J_{AX} = J_{BX} = 7.3 Hz, 2H, CH_2COCH), 3.67 (s, 3H, OMe), 4.30 (quintet, J = 7.1 Hz, 1H, *CH*Me), 5.26 (br d, J = 5.3 Hz, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 17.86 (CH*Me*), 23.18 ($CH_2CH_2CO_2$), 24.67 ($CHCOCH_2CH_2$), 28.39 (Me_3C), 28.68 ($CH_2CH_2CH_2CO_2$), 33.83 ($CHCOCH_2$), 33.84 (CH_2CO_2), 51.41 (OMe), 55.13 (*C*HMe), 79.73 (Me_3C), 155.09 (CON), 173.88 (CO_2), 209.27 (CH*C*OCH). MS (CI, NH_3) *m/e* 319 (MNH_4^+, 37), 302 (MH^+, 9), 263 (MNH_4^+–C_4H_8, 52), 246 (MH^+–C_4H_8, 5), 202 (MH^+–$C_5H_8O_2$, 100).

7,8-Diaminononanoic acid methyl ester dihydrochloride (*DAPA · 2HCl methyl ester,* ***16***). *Method A*. The oxime **12** or **13** (1 mmol) and 4N HCl (2 ml) in absolute MeOH (10 ml) was hydrogenated at 60 psi/45°C/48 h over 10% PtO_2 (0.1 g). The mixture was filtered through celite and washed with MeOH, and the filtrate was evaporated to dryness to give the product as yellow crystals (88% yield, consisting of a mixture of two diastereomers). *Method B*. A solution of *N*-Boc KAPA methyl ester **14** (1 mmol), $NaBH_3CN$ (63 mg, 0.7 mmol), and NH_4OAc (0.77 g, 10 mmol) in dry MeOH (10 ml) was stirred at RT for 48 h. The resulting mixture was acidified (HCl conc) to pH 1 and evaporated to dryness. The residue was dissolved in distilled H_2O and brought to pH 10, using solid KOH. The aqueous solution was extracted with EtOAc (4×) and the combined organic layers were washed with brine, dried ($MgSO_4$), filtered, and evaporated to give the mono-*N*-Boc diamine ester. Removal of the Boc group was carried out under anhydrous conditions, by stirring in ether–HCl at RT until precipitation of the di-HCl salt. The desired diamine was obtained as a mixture of two diastereomers (86% yield). ^{1}H-NMR (D_2O) δ 1.43 (m, 7H, Me, $CH_2CH_2CH_2CH_2CO_2$), 1.70 (m, 4H, $CH_2CH_2CH_2CH_2CH_2CO_2$), 2.44 (t, J = 7.5 Hz, 2H, CH_2CO_2), 3.62 (brdt, 1H, $CHCH_2$), 3.71 (s, 3H, OMe), 3.81 (brdq, 1H, C*H*Me). ^{13}C-NMR (D_2O) major diastereomer δ 13.17 (CH*Me*), 24.31 ($CH_2CH_2CO_2$), 24.62 (CH_2CH_2CHN), 27.00 (CH_2CHN), 28.23 ($CH_2CH_2CH_2CO_2$), 33.99 (CH_2CO_2), 48.88 (*C*HMe), 52.64 (OMe), 53.37 ($CHCH_2$), 177.85 (CO_2). Minor diastereomer δ 13.83 (CH*Me*), 24.31 ($CH_2CH_2CO_2$), 24.56 (CH_2CH_2CHN), 28.15 ($CH_2CH_2CH_2CO_2$), 29.24 (CH_2CHN), 34.09 (CH_2CO_2), 48.95 (*C*HMe), 51.78 (OMe), 54.16 ($CHCH_2$), 179.16 (CO_2). MS (CI/NH_3) *m/e* 217 (MH^+–2HCl).

6-(5-Methyl-2-oxoimidazolidin-4-yl)hexanoic acid (*DTB,* ***17***) *and methyl ester. Method A*. A mixture of a DAPA · 2HCl methyl ester (1 mmol) and Et_3N (0.2 g, 2 mmol) in dry CH_2Cl_2 (10 ml) was stirred at *ca*. 0°C for 30 min. A solution of CDI (0.2 g, 1.2 mmol) in dry CH_2Cl_2 (5 ml) was added and the mixture was stirred at RT overnight. The mixture was evaporated to dryness and the residue was partitioned

between 1 N HCl and EtOAc. The aqueous layer was extracted with EtOAc (4×) and the combined organic layers were washed with 5% $NaHCO_3$ (2×) and brine, dried ($MgSO_4$), filtered, and evaporated to give the desired DTB methyl ester, in the form of two diastereomers. *Method B*. A solution of NaOH (0.4 g, 10 mmol) and a DAPA · 2HCl derivative (1 mmol) in distilled H_2O (10 ml) was stirred at RT for 10 min. A solution of triphosgene (0.3 g, 1 mmol) in dioxane (10 ml) was added. The mixture became hot and the pH dropped from ~10 to ~8. The resulting mixture was stirred at RT for 2 d, after which it was evaporated to dryness. Trituration of the residue with MeOH, filtration, and evaporation afforded the desired product. *Note*. Both the free acid and the ester could be used as substrates. The product was either a mixture of both or pure acid. In the case of a mixture, the product was dissolved in 4 N HCl and stirred at RT overnight, to afford, after evaporation, the desired acid as white crystals (quantitative yield, method B). ^{1}H NMR (D_2O) δ 1.09 and 1.19 (2 d, J = 6.2 Hz, 3H, Me), 1.33 (m, 4H, $CH_2CH_2CH_2CH_2CO_2$), 1.52 (m, 4H, $CH_2CH_2CH_2CH_2CH_2CO_2$), 2.40 (t, J = 7 Hz, 2H, CH_2CO_2), 3.35 and 3.70 (2 m, 1H, $CHCH_2$), 3.53 and 3.85 (2 m, 1H, *CH*Me). MS (CI, NH_3) *m/e* 215 (MH^+, 100). HRMS (CI, CH_4) calcd for $C_{10}H_{19}N_2O_3$ (MH^+): 215.1396. Found: 215.0874. (M^+): 214.1317. Found: 214.0920.

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