Synthesis and Antioxidative Properties of 1,2,3,4-Tetrahydropyridine Derivatives with Different Substituents in 4-Position

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1. Introduction

Natural colors in fruits and plants are essential for photosynthesis, pollination, and seed dissemination [1,2]. Colors in plants are caused by three chemically distinct pigment types, anthocyanins (1), betalains (2), and carotenoids (3) (Figure 1). Anthocyanins are water-soluble pigments that give blue, red, and purple hues. The chemistry, production, and distribution of these compounds have been extensively investigated in the past [3–7].

In the last fifty years, there has been a growing interest in betalains. With few exceptions, plants and fruits of the order Caryophyllales exhibit a range of colors from red/purple to orange/yellow, due to the presence of these hydrophilic pigments. Initially, betalains were classified as anthocyanins. However, it was later discovered that the main enzymes required for the formation of anthocyanins are not present in betalain-producing plants [8,9].

Betalains are nitrogen-containing water-soluble pigments. Their biosynthesis in plants starts with L-tyrosine (4), which is converted into L-3,4-dihydroxyphenylalanine L-DOPA (5). The enzyme tyrosinase was thought to be responsible for the hydroxylation of L-tyrosine [10]. Recently, it has been observed that cytochrome P-450 monooxygenases are also able to catalyze this reaction [11]. Through the action of the enzyme 4,5-DOPA-extradiol dioxygenase, L-DOPA is converted into 4,5-seco-DOPA (7). Spontaneous cyclization of 4,5-seco-DOPA leads to betalamic acid (9), the key intermediate in the biosynthesis of all betalains. Moreover, tyrosinase is also involved in the oxidation
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natural pigments present in various plants; anthocyanins (1), betalains (2) and carotenoids (3).

Betaxanthins are yellow, regardless of the amino acid or amine condensed with betalamic acid. Betaxanthins have a maximum absorption wavelength of 480 nm, while betacyanins show a maximum absorption wavelength of 536 nm. Additionally, a sugar moiety is linked to one of the phenolic OH moieties in betacyanin’s cyclo-DOPA portion [10,12,13].

The food industry has demonstrated a growing interest in these pigments as food colorants [14,15]. Moreover, betalains exhibit antioxidant and radical scavenging activity [16–25]. Numerous investigations have demonstrated that indicaxanthin (12) possesses both antiproliferative and chemoprotective properties [26–28].

The majority of betalains employed in biological research are extracted directly from plants by solid–liquid extraction. Maceration of vegetables facilitates the diffusion of the substances. Additional cellular components are released after tissue breakdown, which makes further purification necessary. Although betalains are typically extracted with H2O, other solvents such as MeOH and EtOH are frequently added to aid the extraction process. Unfortunately, this approach requires longer extraction time, additional purification procedures, and provides limited yields. As a result, innovative extraction methods were used to increase the efficiency of the isolation process of betalains, such as diffusion extraction, ultrafiltration, reverse osmosis, and cryogenic freezing [25,29–32]. Another significant issue encountered during the extraction and purification of these pigments is their chemical instability when exposed to oxygen, acids, bases, light, and heat. These parameters have a considerable impact on the extraction and purification procedures’ efficiency [33]. Several strategies for increasing the stability of betalains have been implemented, most notably in the food industry [34].

Betalamic acid (9) is a critical intermediary in the formation of both kinds of betalains. Although two syntheses of this compound have already been reported [35–37], the first synthesis developed by Dreiding et al. [35,37] started with chelidamic acid (I). Hydrogenation of 19 in the presence of rhodium on activated alumina afforded an all-cis-configured piperidine derivative, which was converted into the dimethyl ester II upon treatment with
methanol and HCl. Oxidation of the secondary alcohol led to the formation of piperidin-4-one III. To avoid overoxidation to the corresponding pyridine derivative, a polymeric carbodiimide was used for the Pfitzner–Moffatt oxidation and the transformation was carefully monitored. For the introduction of the side chain, a fully methyl-protected semicarbazide was employed as the Horner–Wittig reagent. This reagent led to the formation of hydrazone IV as a pure ε-configured diastereomer (C=N bond). Dehydrogenation of IV with t-butyl hypochlorite and triethylamine (NEt₃) provided dihydropyridine V as a 7:3 mixture of (E)- and (Z)-configured diastereomers. In this case, (E) and (Z) configuration refers to the exocyclic C=C double bond, whereas the C=N double bond is still (E)-configured. Recrystallization from t-butanol provided the pure (E,E)-configured betalamic acid derivative (E,E)-23 (Scheme 1).

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\text{L-tyrosine (4)} \\
\text{tyrosinase or} \ \ \ \ \ \ \ \ \ \text{CYP-450} \\
\text{tyrosinase} \\
\text{4,5-DOPA-extradiol dioxygenase} \\
\text{4,5-seco-DOPA (7)} \\
\text{α-DOPA-quinone (6)} \\
\text{cyclo-DOPA (8)} \\
\text{Betalamic acid (9)} \\
\text{proline (10)} \\
\text{Betanidin (11)} \\
\text{Indicaxanthin (12)} \\
\text{Enzymatic reaction} \\
\text{Spontaneous}
\]

Figure 2. Biosynthetic pathway of betalains 11 and 12.
Scheme 1. Synthesis of betalamic acid derivative 23 according to Dreiding et al. [35,37]. Reagents and reaction conditions were as follows: (a) 1. 5% Rh/Al₂O₃, H₂O, H₂ (4 atm), 75 °C; 2. HCl, MeOH, 42%. (b) Polimeric carbodiimide, pyridinum trifluoroacetate, DMSO, r.t., 33 h, 90%. (c) (EtO)₂OPCH₂CH=NN(Me)CONMe₂, NaH, DME, r.t., 15 h, 44%. (d) t-BuOCl/t-BuOH, Et₃N, C₆H₆, r.t., 1 h, 16%.

In Scheme 2, the second strategy for the synthesis of betalamic acid (9), developed by Bixhi et al. [36], is displayed. This approach started from benzylnorteleoidine VI obtained by Robinson–Schöpf condensation. The first reaction includes the protection of the diol by formation of a cyclic ortho ester. Hydrogenolytic cleavage of the N-benzyl protective group provided the secondary amine VII. Reaction of the aminoketone VII with allyl magnesium chloride yielded the tertiary alcohol VIII with high diastereoselectivity. The secondary amine VIII was then converted into O-benzoylhydroxylamine IX. First, amine IX was neutralized with K₂CO₃ and reacted with dibenzoyl peroxide in DME, leading to formation of the protected amine. Subsequently, acetylation of the alcohol provided O-benzoylhydroxylamine IX. Next, the ortho ester was cleaved with oxalic acid in water to obtain diol X. This latter compound was then oxidized with N-chlorosuccinimide (NCS) and dimethylsulfide to achieve the diketone XI. Ozonolysis of XI led to the formation of aldehyde XII. Treatment of XII with lead tetraacetate in benzene and methanol converted the diketone moiety into an unstable dicarboxylic acid, which, upon loss of HOAc and BzOH, yielded (±)-betalamic acid (9) as a mixture of (E)- and (Z)-configured diastereomers after silica gel chromatography [36].

Despite the fact that two methods for the synthesis of betalamic acid (9) and its derivatives have been reported in the literature, the majority of betalamic acid (9) is produced through extraction from pigments, followed by basic hydrolysis.

To investigate relationships between the chemical structure and biological properties of indicaxanthin derivatives in further detail, analogs 13 of 12 that lack the two carboxy moieties in positions C-2- and C-6 were considered first. Herein, we describe the design and synthesis of betalamic acid analog 13 that is devoid of carboxy groups in positions C-2 and C-6. Additionally, experiments were conducted to synthesize the betalamic acid derivative 14 in a simpler and more cost-effective manner and to evaluate its reactivity toward oxygen (Scheme 3).
The plan for the synthesis of 13, the analog of betalamic acid without carboxy groups in positions C-2- and C-6, is outlined in Scheme 4.

We planned to synthesize 13 from the α,β-unsaturated ester 15 that bears a Boc-protective group at the piperidine ring. At first, the ester must be reduced to afford an aldehyde and finally, the Boc-protective group must be removed. The α,β-unsaturated ester 15 can be obtained by a Wittig reaction of α-bromoketone 17 and the subsequent
β-elimination of 16. The α-bromoketone should be available by α-bromination of an appropriate piperidone derivative, e.g., 18.

The synthesis started with piperidine 19 (Scheme 5), which was protected with (Boc)2O to afford Boc-protected piperidine 18. In order to introduce a double bond in positions C-5 and C-6 of the piperidine ring, piperidine 18 was brominated in the α-position using Br2 and AlCl3 to generate the α-bromoketone 17 in a 46% yield [38]. The conjugated double bond system is a characteristic feature of the class of betalains. Thus, the first double bond was introduced by a Wittig reaction of the α-bromoketone 17 with Ph3P=CHCO2Et to give the α,β-unsaturated ester 16 in a 95% yield [39]. Although the formation of (E)/(Z)-diastereomers was expected, the 1H and 13C NMR spectra reveal only one set of signals, indicating a single diastereomer, presumably (E)-16. LiBr and Li2CO3 were used to induce dehydrobromination (β-elimination), resulting in the formation of completely conjugated compound 15, which was isolated in a 88% yield [40]. The 1H NMR spectrum of 15 reveals two distinct sets of signals, indicating the presence of (E)- and (Z)-configured esters 15 in the ratio 9:1. Since diastereomeric (E)- and (Z)-configured esters 15 could not be separated by flash column chromatography, the mixture was used to prepare the aldehyde 21. According to the first theory, aldehyde 21 should be obtained directly by the reduction of the ester 15 with DIBAL-H. However, even at −78 °C in toluene, only the primary alcohol 20 was formed and isolated in a 94% yield. Alternatively, the primary alcohol 20 was synthesized by the reduction of the ester 15 with LiAlH4. Several methods have been reported in the literature for the oxidation of primary alcohols to aldehydes [41]. A method with broad applicability and high yields is the Dess–Martin periodinane (DMP) oxidation method. Unexpectedly, the oxidation of allyl alcohol 20 with DMP resulted in low yields of the product, which was difficult to purify. Therefore, the alcohol 20 was oxidized via radical oxidation with TEMPO [41] and CuCl to provide the aldehyde 21 in a 76% yield. To obtain the aldehyde 13 as an analog of betalamic acid (9), the Boc-protective group of 21 was removed. Unfortunately, removing the Boc-protective group under typical conditions with F3CCO2H did not result in the desired aldehyde 13. Several methods were investigated to remove the Boc-protective group from 21 to achieve 13. In the end, a rather unusual method, i.e., heating the Boc-protected compound 21 in a mixture of water and dioxane under neutral conditions [42], was successful. Due to the instability of the secondary amine 13, the isolated yield of 13 was rather low. In particular, condensation and polymerization reactions, as well as oxidation processes, were observed during the purification process. Despite the instability, 1H and 13C NMR spectra could be recorded to identify and characterize 13.

Scheme 5. Synthesis of the key intermediate 13. Reagents and reaction conditions were as follows: (a) Boc2O, NaHCO3, THF/H2O, r.t., 16 h 96%. (b) Br2, AlCl3, THF/Et2O, 0 °C, 16 h, 46%. (c) Ph3P=CHCO2Et, CH2Cl2, 40 °C, 2 h, 95%. (d) Li2CO3, LiBr, DMF, 75 °C, 3 h, 88%. (e) DIBAL-H, toluene, −78 °C, 1 h, 94% or LiAlH4, THF, −10 °C, 1 h, 70%. (f) TEMPO, CuCl, DMF, r.t, 16 h, 90%. (g) H2O/dioxane, 90 °C, 2 h, 5%.
In addition to betalamic acid analog 13, 1,2,3,4-tetrahydropyridine derivatives 22 and 23 were designed and synthesized (Scheme 6). The reactivity of these 1,2,3,4-tetrahydropyridines 22 and 23 and further analogs towards oxygen should be investigated. The key intermediate for the synthesis of 22 and 23 is 4-methylenepepiperidine 24, which can be obtained by double allylation of iminodiacetic acid diester 25 with dichloride 26, as reported by Einhorn et al. [43]. Transformation of the methylene moiety of 24 into a ketone and subsequent introduction of a double bond in the ring result in the formation of 23. The α,β-unsaturated ester 22 can prepared by an additional Wittig reaction of a ketone intermediate.

For the synthesis of methylenepepiperidine 24, the diester 25 and the diiodide 29 were prepared (Scheme 7). The diester HCl salt 28 was obtained by esterification of iminodiacetic acid (27) with SOCl₂ in refluxing ethanol. The secondary amine of 27 was protected with Boc₂O to afford the carbamate 25 in a 76% yield. The diester 25 was initially treated with dichloride 26, which, however, did not lead to the desired 4-methylenepepiperidine 24. To obtain the desired methylenepepiperidine 24, the more reactive diiodide 29 should be employed instead of the dichloride 26. Allyl diiodide 29 was freshly prepared by a Finkelstein reaction of commercially available 3-chloro-2-(chloromethyl)prop-1-ene (26) with NaI in acetone [44]. After a reaction time of 16 h in refluxing acetone, the diiodide 29 was obtained in a 99% yield.

For the double allylation of diester 25, LDA was generated in situ from n-BuLi and i-Pr₂NH. Deprotonation of diester 25 with freshly prepared LDA and subsequent treatment with diiodide 29 provided the methylenepepiperidine 24 in a 77% yield. The IR and ¹H NMR spectra of piperidine 24 demonstrate the successful synthesis of the piperidine ring. A band at 1655 cm⁻¹ in the IR spectrum originates from the C=C stretching vibration. Two sets of signals can be found in the ¹H NMR spectrum, as illustrated by two singlets for...
the protons of the exocyclic methylene moiety (\(R_2C=CH_2\)) at 4.83 and 4.92 ppm and two singlets for the Boc group at 1.42 and 1.47 ppm. These signal pairs confirm the formation of trans- and cis-configured diastereomers trans-24 and cis-24, which are present in the ratio 9:1. Lemieux–Johnson oxidation using catalytic amounts of OsO₄ and an excess of NaIO₄ transformed the 4-methylenepiperidine 24 into piperidinone 30 [45]. Despite the fact that compound 24 was used as a mixture of diastereomers, only one diastereomer could be observed for compound 30. The subsequent Wittig reaction of ketone 30 provided the \(\alpha,\beta\)-unsaturated ester 31, which shows an even higher structural similarity to betalamic acid than methylenepiperidine 24 and piperidinone 30 (Scheme 8).

![Scheme 8. Synthesis of \(\alpha,\beta\)-unsaturated ester 31. Reagents and reaction conditions were as follows: (a) 1. LDA, THF, \(-78\) °C, 1 h, 2. Allyl diiodide 29, THF, \(-78\) °C to r.t., 16 h, 77%; (b) OsO₄ (0.05 M in H₂SO₄), NaIO₄, pyridine, H₂O, t-BuOH, r.t., 48 h, 73%; (c) Ph₃P=CHCO₂Et, toluene, reflux, 48 h, 75%.](image)

Since the piperidines 24, 30, and 31 do not contain a halogen atom for elimination, another method for the introduction of a double bond into the piperidine ring was required. For this purpose, the Boc-protective group was removed, yielding the secondary amines 32, 33 and 34. The secondary amines 32–34 were reacted with in situ prepared \(t\)-BuOCl followed by base-induced HCl elimination, according to the method reported by Zhong et al. [46] (Scheme 9). For compound 32, isolation of the expected product 35 was not possible due to the fast oxidation to its pyridine form 38, isolated in a 6% yield. For compound 33, the formation of the conjugate system was successful, leading to the desired product 36 in a 39% yield. For this product, we did not observe the formation of the pyridine form 39. With compound 34, the conjugate derivative 37 was obtained. Although, a slow conversion to the pyridine form 40 was observed.

2.2. Antioxidant Activity and Stability

Due to the instability of aldehyde 13, we decided to evaluate the total antioxidant activity (TAC) of the protected aldehyde 21. For this purpose, the Folin–Ciocalteu assay was employed [47]. This method can be classified among the protocols used to evaluate the TAC in the electron transfer (ET) group [48]. Reduction of the oxidant leads to a change in its properties, such as light absorption or fluorescence, which are measured using spectroscopy techniques [49]. In the Folin–Ciocalteu assay, a molybdotungstophosphate heteropolyanion (3H₂O·P₂O₅·14WO₃·4MoO₃·10H₂O) is used for the oxidation of phenolic compounds in basic solution (carbonate buffer). The reduction leads to a colored product with an absorption maximum (\(\lambda_{\text{max}}\)) at 765 nm. The molybdenum center in the complex is reduced from Mo(VI) to Mo(V) by an \(e^-\) donated from the antioxidant, leading to a blue solution [49].

Unfortunately, during the test of the protected aldehyde 21 in the Folin–Ciocalteu assay, a change in the color of the solution could not be recorded as reduction of the
molybdenum complex did not take place. All information were provided in Supplementary Materials (Page S2).

Scheme 9. Removal of the Boc-protective group and introduction of a double bond in the 5,6-position of the piperidine ring. Reagents and reaction conditions were as follows: (a) CF3COOH, CH2Cl2, r.t., 16 h, 11% (32), 71% (33), 90% (34); (b) 1. NaOCl (0.75 M in H2O), AcOH, t-BuOH, t-butyl methyl ether, r.t., 1 h; 2. ethyldiisopropylamine, r.t., 16 h, 6% (38), 39% (36), 30% (37).

The stability towards oxygen of the 1,2,3,4-tetrahydropyridines 35–37 was observed spectroscopically using ¹H NMR spectra. After oxidizing the 4-methylenepiperidine 32 with l-BuOCl, only pyridine 38 was detected, indicating the fast oxidation of intermediate tetrahydropyridine 35 by O2. In contrast to the fast oxidation of methylenetetrahydropyridine 35, tetrahydropyridone 36 did not show any potential to be further oxidized. The most promising properties were observed for the ester 37. Although it could be isolated in its pure form, recording of ¹H NMR spectra over a period of several days revealed the slow oxidation of ester 37 to pyridine 40 (Figure 3).

Figure 3. ¹H NMR spectra of 1,2,3,4-tetrahydropyridine 37 after different time intervals. Ratio of diastereomers of cis-37 and trans-37 is 55:45. The signals for the minor diastereomer are marked with an asterisk (*). Increasing amounts of aromatic pyridine 40 can be detected. Signals of 37 are marked with red boxes and signals for pyridine 40 with blue boxes.
3. Materials and Methods

3.1. Chemistry

Moisture and oxygen sensitive reactions were carried out under nitrogen, dried with molecular sieves (3 or 4 Å, 8 to 12 mesh, Acros Organics), in dry glassware (Schlenk flasks or Schlenk tubes, sealed with rubber septa). All solvents were of analytical grade quality. Flash chromatography (FC): silica gel 60, 40–63 μm (Machery Nagel); parentheses include: diameter of the column (Ø), length of the stationary phase (h), fraction size (V) and eluent. Melting point: melting point system MP50 (Mettler Toledo, gießen, Germany), open capillary, uncorrected. MS: MicroTOFQII mass spectrometer (Bruker Daltonics, Bremen, Germany); deviations of the found exact masses from the calculated exact masses were 5 ppm or less; the data were analyzed with DataAnalysis® (Bruker Daltonics). NMR: NMR spectra were recorded in deuterated solvents on Agilent DD2 400 MHz and 600 MHz NMR spectrometers (Agilent, Santa Clara, CA, USA); chemical shifts (δ) are reported in parts per million (ppm) against the reference substance tetramethylsilane and calculated using the solvent residual peak of the undeuterated solvent; coupling constants are given with 0.5 Hz resolution; assignment of \(^1\)H and \(^13\)C NMR signals was supported by 2D NMR techniques where necessary. IR: FT/IR IR Affinity®-1 spectrometer (Shimadzu, Düsseldorf, Germany) using ATR technique. Characterization data including \(^1\)H and \(^13\)C NMR spectra for synthesized compounds are reported in Supplementary Materials (Page S3).

3.1.1. Synthesis of Tert-Butyl 4-Oxopiperidine-1-Carboxylate (18)

Piperidin-4-one monohydrate hydrochloride 19 (5.0 g, 32.5 mmol, 1.0 eq.) was dissolved in a 1:1 mixture of THF:H₂O (100 mL) at room temperature. NaHCO₃ (5.47 g, 65 mmol, 2.0 eq.) was added and the mixture was stirred for 15 min at rt. Afterwards, Boc₂O (8.52 g, 39 mmol, 1.2 eq.) was added and the mixture was stirred for 16 h at room temperature. The mixture was diluted with Et₂O (50 mL) and washed with aqueous solution (KHSO₄ 5% (3 × 50 mL), H₂O (3 × 50 mL) and brine (3 × 50 mL). The combined organic layers were concentrated in vacuo. The residue was purified by flash column chromatography (petroleum ether/EtOAc 7:3). Colorless solid, mp 73–76 °C, yield 6.22 g (31 mmol, 96%). Exact mass (APCI): m/z = 200.1279 (calcd. 200.1287 for C₁₀H₁₈NO₃⁺ [M+H⁺]). \(^1\)H NMR (600 MHz, DMSO-d₆): δ (ppm) = 2.34 (t, J = 6.2 Hz, 2H, 2-(CH₂)₂), 3.60 (t, J = 6.2 Hz, 4H, 2-(CH₂)₂), 13C NMR (151 MHz, DMSO-d₆): δ (ppm) = 28.0 (3C, C(CH₃)₃), 40.0 (2C, C-3), 40.3 (2C, C-2), 79.2 (1C, OC(CH₃)₃), 153.8 (1C, C=O)OC(CH₃)₃, 207.4 (1C, R₂C=O). FT-IR (neat): ν (cm⁻¹) = 2985, 2870 (C-H, aliph.). 1724 (C=O carbamate), 1724 (C=O ketone), 1678 (C=O carbamate), 1674 (C=O ketone), 1161 (C=O).

3.1.2. Synthesis of Tert-Butyl 3-Bromo-4-Oxopiperidine-1-Carboxylate (17)

1-Boc-piperidin-4-one 18 (10 g, 50 mmol, 1.0 eq.) was dissolved in THF (30 mL) and Et₂O (30 mL). AlCl₃ (0.67 g, 5.0 mmol, 0.1 eq.) was added and at 0 °C, Br₂ (2.6 mL, 50 mmol, 1.0 eq.) was added slowly over a period of 30 min. Afterwards, the solution was stirred at 0 °C for 18 h. Afterwards, the formed solid was filtered off and washed with Et₂O. The organic layer was dried (Na₂SO₄), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (petroleum ether/EtOAc 9/1 → 5/5, Rf = 0.41 (cHex/EtOAc 7:3)). Colorless solid, mp 90–93 °C, yield 6.42 g (23 mmol, 46%). Exact mass (APCI): m/z = 278.0329 (calcd. 278.0392 for C₁₀H₁₇BrNO₃⁺ [M+H⁺]). \(^1\)H NMR (200 MHz, DMSO-d₆): δ (ppm) = 1.44 (s, 9H, C(CH₃)₃), 2.50–2.52 (m, 1H, 5-H), 2.70–2.78 (m, 1H, 5-H), 3.58–3.68 (m, 3H, 2 × 6-H, 2-H), 3.98–4.08 (m, 1H, 2-H), 4.77 (s, 1H, 3-H). 13C NMR (50 MHz, DMSO-d₆): δ (ppm) = 27.9 (3C, C(CH₃)₃), 35.8 (1IC, C-5), 42.7 (1IC, C-6), 47.7 (1IC, C-2), 49.0 (1IC, C-3), 79.8 (1IC, OC(CH₃)₃), 153.8 (1IC, C=O)OC(CH₃)₃, 199.7 (1IC, R₂C=O). FT-IR (neat): ν (cm⁻¹) = 2978, 2931 (C-H. aliph.), 1724 (C=O ketone), 1674 (C=O carbamate), 1157 (C=O), 648 (C-Br).
3.1.3. Synthesis of Tert-Butyl (E)-3-Bromo-4-(Ethoxycarbonylmethylene)Piperidine-1-Carboxylate (16)

(4-Ethoxycarbonylmethylene)triphenylphosphorane (6.9 g, 20 mmol, 1.1 eq.) was added to a solution of tert-butyl 3-bromo-4-oxopiperidine-1-carboxylate 17 (5.03 g, 18 mmol, 1.0 eq.) in CH₂Cl₂ (450 mL) and the reaction mixture was stirred at reflux for 2 h. Then, the mixture was cooled to room temperature and concentrated in vacuo. The residue was purified by flash column chromatography (petroleum ether/EtOAc 9/1 → 7/3, R₂ = 0.57 (cHex/EtOAc 7:3)). Colorless solid, mp 114–115 °C, yield 5.98 g (17 mmol, 95%). Exact mass (APCI): m/z = 348.0777 (calcld. 348.0810 for C₁₀H₁₇BrNO₃+ [M+H⁺]). ^1H NMR (200 MHz, DMSO-d₆): δ (ppm) = 1.21 (t, J = 7.1 Hz, 3H, OCH₂CH₃), 1.42 (s, 9H, C(CH₃)₃), 2.56–2.88 (m, 2H, 5-CH₂, 6-CH₂), 3.34–3.51 (m, 2H, 2-CH₂, 5-CH₂), 4.01–4.29 (m, 4H, OCH₂CH₃, 6-CH₂, 2-CH₂), 5.04–5.12 (m, 1H, 3-CH), 6.12 (s, 1H, R₂C=C=O). ^13C NMR (50 MHz, DMSO-d₆): δ (ppm) = 14.0 (1C, OCH₂CH₃), 24.1 (1C, C-5), 27.9 (3C, C(CH₃)₃), 42.5 (1C, C-6), 51.0 (1C, C-2), 53.3 (1C, C-3), 59.9 (1C, OCH₂CH₃), 79.2 (1C, C(CH₃)₃), 113.8 (1C, R₂C=C=CH), 153.7 (1C, C(=O)OC(CH₃)₃), 154.0 (1C, C-4), 165.1 (1C, CO₂Et). Only one set of signals can be observed in the spectra. FT-IR (neat): ν (cm⁻¹) = 2985, 2920 (C-H, aliph.), 1712 (C=O), 1670 (C=O), 1654 (C=C), 1161 (C-O), 641 (C-Br).

3.1.4. Synthesis of Tert-Butyl (E)- and (Z)-Ethoxycarbonylmethylene)-3,4-Dihydropyridine-1(2H)-Carboxylate (15)

Ester 16 (5.74 g, 16 mmol, 1.0 eq) was dissolved in dry DMF (165 mL). LiBr (8.6 g, 99 mmol, 6.0 eq) and Li₂CO₃ (7.31 g, 99 mmol, 6.0 eq) were added and the solution was stirred at 75 °C for 3 h. Then, the mixture was cooled to room temperature and extracted with EtOAc (3 × 100 mL). The combined organic layers were concentrated in vacuo. The residue was purified by flash column chromatography (petroleum ether/EtOAc 9/1 → 7/3, R₂ = 0.72 (cHex/EtOAc 7:3)). Yellow oil, yield 3.88 g (14 mmol, 88%). Exact mass (APCI): m/z = 268.1497 (calcld. 268.1549 for C₁₄H₂₂NO₄+ [M+H⁺]). Compound 15 was isolated as a mixture of (E):(Z) isomers. In the NMR spectra, a ratio of 9:1 is observed. ^1H NMR (600 MHz, DMSO-d₆): δ (ppm) = 1.19 (t, J = 7.1 Hz, 3H, OCH₂CH₃), 1.46 (s, 9H, C(CH₃)₃), 2.49–2.53 (m, 0.2H, 5-CH₂), 3.00–3.07 (m, 1.8H, 5-CH₂), 3.52–3.60 (m, 2H, 6-CH₂), 4.06 (q, J = 7.1 Hz, 2H, OCH₂CH₃), 5.32–5.34 (m, 0.1H, R₂C=C=CH), 5.47–5.53 (m, 0.9H, 3-CH), 5.55–5.59 (m, 0.9H, R₂C=C=CH), 6.54–6.61 (m, 0.1H, 3-CH) 7.00–7.13 (m, 0.9H, 2-CH), 7.14–7.16 (m, 0.1H, 2-CH). ^13C NMR (151 MHz, DMSO-d₆): δ (ppm) = 14.2 (1C, OCH₂CH₃), 24.8 (0.9C, C-5), 27.7 (3C, C(CH₃)₃), 30.0 (0.1C, C-5), 40.0 (1C, C-6), 59.0 (1C, OCH₂CH₃), 81.5 (1C, C(CH₃)₃), 103.4 (0.1C, C-3), 108.1 (0.9C, C-3), 109.6 (0.1C, R₂C=C=CH) 110.6 (0.9C, R₂C=C=CH), 132.5 (0.9C, C-2), 133.0 (0.1C, C-2), 147.5 (1C, C(=O)OC(CH₃)₃), 147.9 (0.1C, R₂C=C=CH) 149.1 (0.9C, R₂C=C=CH), 166.0 (1C, CO₂Et) FT-IR (neat): ν (cm⁻¹) = 2978, 2931, 2900 (C-H, aliph.), 1708 (C=O), 1700 (C=O), 1608 (C=C), 1145 (C-O), 1111 (C-O).

3.1.5. Synthesis of Tert-Butyl (E)- and (Z)-4-(2-Hydroxyethylidene)-3,4-Dihydropyridine-1(2H)-Carboxylate (20)

Procedure 1

Under N₂, 15 (2.80 g, 10.5 mmol, 1.0 eq.) was dissolved in dry toluene (25 mL). The solution was cooled to −78 °C, DIBAL-H (1 M solution in hexane, 31.5 mL, 31.5 mmol, 3.0 eq.) was added dropwise within 15 min, and the reaction mixture was stirred at −78 °C for 40 min. Then, at −78 °C, CH₃OH (2 mL) was added carefully, and the mixture was warmed to room temperature. The mixture was filtered, the solid was washed with EtOAc (3 × 30 mL) and the combined organic layers were concentrated in vacuo. Yellow oil, yield 2.22 g (9.9 mmol, 94%).

Procedure 2

Under N₂, 15 (570 mg, 2.1 mmol, 1.0 eq.) was dissolved in dry THF (5 mL) and at −10 °C, LiAlH₄ (160 mg, 4.3 mmol, 2.0 eq.) was added and the mixture was stirred for 40 min. A saturated solution of potassium sodium tartrate (5 mL) was added, and the
mixture was extracted with EtOAc (3 × 15 mL). The combined organic layers were dried (Na$_2$SO$_4$), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (cHex/EtOAc 9/1 → 6/4, $R_f$ = 0.45 (petroleum ether/EtOAc 7:3)). Yellow oil, yield 0.33 g (1.5 mmol, 70%). Exact mass (APCI): $m/z$ = 226.1383 (calc. 226.1443 for C$_{12}$H$_{20}$NO$_3^+$ [M+H$^+$]). Compound 20 was isolated as a mixture of ((E):(Z)) isomers. In the NMR spectra, a ratio of 8.5:1.5 is observed. Peaks of minor isomer are not all clearly visible in the spectra. $^1$H NMR (600 MHz, DMSO-$d_6$): δ (ppm) = 1.44 (s, 9H, C(CH$_3$)$_3$), 2.36–2.44 (m, 2H, 5-CH$_2$), 3.48–3.55 (m, 2H, 6-CH$_2$), 3.96–4.01 (m, 2H, 2H, 6-CH$_2$), 4.53–4.58 (m, 1H, 1H, OH), 5.13 (t, $J$ = 6.8 Hz, 0.15H, R$_2$C=CH), 5.35 (t, $J$ = 6.8 Hz, 0.85H, R$_2$C=CH), 5.40–5.46 (m, 1H, 3-CH), 6.65–6.78 (m, 1H, 2-CH). $^{13}$C NMR (151 MHz, DMSO-$d_6$): δ (ppm) = 26.9 (1C, C-5), 30.9 (3C, C(CH$_3$)$_3$), 43.1 (1C, C-6), 59.9 (1C, CH$_2$OH), 83.6 (1C, C(CH$_3$)$_3$), 112.7 (1C, C-3), 127.8 (1C, R$_2$C=CH), 128.4 (1C, C-2), 133.2 (1C, R$_2$C=CH), 154.3 (1C, C=O)OC(CH$_3$)$_3$). FT-IR (neat): $\tilde{\nu}$ (cm$^{-1}$) = 3398 (O-H), 2974, 2931, 2873 (C-H, aliph.), 1701 (C=O), 1643 (C=C), 1612 (C=C), 1161, 1141 (C-O).

3.1.6. Synthesis of Tert-Butyl ((E)- and (Z)-4-(Formylmethylene)-3,4-Dihydropyridine-1(2H)-Carboxylate (21))

CuCl (11 mg, 0.12 mmol, 0.1 eq.) and TEMPO (17 mg, 0.12 mmol, 0.1 eq.) were added to a solution of racemic mixture of allylic alcohol 20 (270 mg, 1.20 mmol, 1.0 eq.) in dry DMF (3 mL). The solution was stirred at room temperature for 16 h. Afterwards, the solution was poured into water/ice slowly and the mixture was warmed to room temperature. The solid was filtered, washed with H$_2$O and dried. Purification by flash column chromatography (petroleum ether/EtOAc 9/1 → 7/3 $R_{ta}$ = 0.38, $R_{rt}$ = 0.30 (cHex/EtOAc 7:3)). Yellow oil, yield 240 mg (1.07 mmol, 90%). Exact mass (APCI): $m/z$ = 224.2180 (calc. 224.2495 for C$_{24}$H$_{30}$N$_2$O$_6^+$ [M+H$^+$]). Compound 21 was isolated as a mixture of ((E):(Z)) isomers. In the NMR spectra, a ratio of 6.4 is observed. Peaks of minor isomer are not all clearly visible in the spectra. $^1$H NMR (600 MHz, CDCl$_3$): δ (ppm) = 1.52 (s, 3.6H, C(CH$_3$)$_3$), 1.53 (s, 5.4H, C(CH$_3$)$_3$), 2.64 (t, $J$ = 6.8 Hz, 0.8H, 5-CH$_2$), 2.91–3.14 (m, 1.2H, 5-CH$_2$), 3.74 (t, $J$ = 6.8 Hz, 2H, 6-CH$_2$), 5.44–5.53 (m, 0.6H, 3-CH), 5.57 (d, $J$ = 7.8 Hz, 0.4H, R$_2$C=CH), 5.78 (d, $J$ = 7.7 Hz, 0.6H, R$_2$C=CH), 6.23–6.36 (m, 0.4H, 3-CH), 7.13–7.19 (m, 0.6H, 2-CH), 7.27–7.41 (m, 0.4H, 2-CH), 9.94 (d, $J$ = 7.9 Hz, 0.6H, CHO), 10.05 (d, $J$ = 7.9 Hz, 0.4H, CHO). $^{13}$C NMR (151 MHz, CDCl$_3$): δ (ppm) = 24.7 (0.6C, C-5), 28.2 (3C, C(CH$_3$)$_3$), 30.9 (0.4C, C-5), 40.4 (1C, C-6), 82.8 (1C, C(CH$_3$)$_3$), 101.2 (0.4C, C-3), 108.1 (0.6C, C-3), 121.5 (1C, R$_2$C=CH), 134.0 (0.6C, C-2), 134.5 (0.4C, C-2), 151.1 (1C, R$_2$C=CH), 151.3 (1C, C=O)OC(CH$_3$)$_3$), 189.6 (0.4C, CHO), 190.0 (0.6C, CHO). FT-IR (neat): $\tilde{\nu}$ (cm$^{-1}$) = 3062, 2966, 2877 (C-H, aliph.), 1712 (C=O), 1647 (C=C), 1593 (C=C), 1141, 1122 (C-O).

3.1.7. Synthesis of (E)- and (Z)-2-[2,3-Dihydropyridin-4(1H)-Ylidene]Acetaldehyde (13)

A solution of 21 (110 mg, 0.49 mmol, 1.0 eq.) in water (9 mL) and 1,4-dioxane (1 mL) was heated to 85 °C for 2 h. Then, the solution was cooled to room temperature and the mixture was extracted with EtOAc (6 × 15 mL). The combined organic layers were dried (Na$_2$SO$_4$), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (petroleum ether/EtOAc 9/1 → 1/9 $R_{ta}$ = 0.15 (EtOAc)). Yellow/orange oil, yield 12 mg (0.10 mmol, 20%). The compound is highly unstable and quickly decomposed during purification. Aldehyde 13 was isolated as a mixture of ((E):(Z)) isomers. In the NMR spectra, a ratio of 6:4 is observed. Peaks of minor isomer are not all clearly visible in the spectra. $^1$H NMR (200 MHz, CDCl$_3$): δ (ppm) = 2.61 (t, $J$ = 7.0 Hz, 0.8H, 5-CH$_2$), 3.00–3.12 (m, 1.2H, 5-CH$_2$), 3.31–3.46 (m, 2H, 6-CH$_2$), 4.7–4.81 (m, 1H, NH), 5.19 (d, $J$ = 7.2 Hz, 0.6H, R$_2$C=CH), 5.29 (d, $J$ = 7.9 Hz, 0.4H, R$_2$C=CH), 5.60 (d, $J$ = 8.3 Hz, 0.6H, 3-CH), 6.00 (d, $J$ = 7.5 Hz, 0.4H, 3-CH), 6.66–6.77 (m, 1H, 2-CH), 9.79 (d, $J$ = 8.4 Hz, 0.6H, CHO), 9.93 (d, $J$ = 8.0 Hz, 0.4H, CHO). $^{13}$C NMR (50 MHz, CDCl$_3$): δ (ppm) = 25.3 (0.6C, C-5), 31.75 (0.4C, C-5), 40.5 (0.6C, C-6), 40.9 (0.4C, C-6), 93.9 (0.4C, C-3), 100.3 (0.6C, C-3), 116.2 (0.4C, R$_2$C=CH), 117.1 (0.6C, R$_2$C=CH), 142.6 (1C, C-2), 143.4 (1C, C-2), 155.3 (0.4C, R$_2$C=CH), 171.3 (0.6C, R$_2$C=CH), 189.2 (0.4C, CHO), 189.3 (0.6C, CHO).
3.1.1. Synthesis of Tert-Butyl 4-Oxopiperidine-1-Carboxylate (18)...

At 0 °C, SOCl₂ (20.0 mL, 275 mmol, 1.5 eq) was added dropwise to a suspension of iminodiacetic acid 27 (24.4 g, 184 mmol, 1.0 eq) in EtOH abs. (200 mL). Afterwards, the reaction mixture was heated to reflux for 16 h. The solution was cooled down to room temperature and concentrated in vacuo. Colorless solid, mp 88–89 °C, yield 40.6 g (98%). C₃H₅ClNO₄ (225.7 g/mol). ¹H NMR (600 MHz, DMSO-d₆): δ (ppm) = 1.24 (t, J = 7.1 Hz, 6H, 2 × OCH₂CH₃), 3.70 (brs, 2H, NH₂⁺), 3.99 (s, 4H, 2 × CH₂), 4.21 (q, J = 7.1 Hz, 4H, 2 × OCH₂CH₂). ¹³C NMR (151 MHz, DMSO-d₆): δ (ppm) = 13.9 (2C, 2 × OCH₂CH₂), 46.4 (2C, 2 × CH₂), 61.8 (2C, 2 × OCH₂CH₂), 166.4 (2C, 2 × O=COEt). IR (neat): ν (cm⁻¹) = 2936 (C-H), 1756 (C=O ester), 1204, 1076, 1015 (C-N, C-O). Exact mass (APCI): m/z = 348.0777 (calcd. 348.0810 for C₁₄H₂₃BrNO₄+ [M+H⁺]).

3.1.9. Synthesis of Diethyl 2,2'-[N-(Tert-Butoxycarbonyl)]Iminodiacetate (25)

NaHCO₃ (22.8 g, 271 mmol, 3.0 eq) was added to a solution of 28 (20.4 g, 90.4 mmol, 1.0 eq) in THF (80 mL) and H₂O (20 mL). The reaction mixture was stirred for 30 min at room temperature. After the addition of Boc₂O (19.3 mL, 90.4 mmol, 1.0 eq), the mixture was stirred at room temperature for 16 h. Then, it was extracted with EtOAc (3 × 50 mL) and the combined organic layers were dried (Na₂SO₄), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (cHex/EtOAc 3:1). Colorless oil, yield 19.9 g (76%). C₁₃H₂₅NO₆ (289.3 g/mol). TLC: Rf = 0.36 (cHex/EtOAc 8:2). ¹H NMR (400 MHz, DMSO-d₆): δ (ppm) = 1.19 (t, J = 7.1 Hz, 3H, OCH₂CH₃), 1.20 (t, J = 7.1 Hz, 3H, OCH₂CH₃), 1.35 (s, 9H, C(CH₃)₃), 3.98 (s, 2H, NCH₂), 4.01 (s, 2H, NCH₂), 4.10 (q, J = 7.1 Hz, 2H, OCH₂CH₃), 4.12 (q, J = 7.1 Hz, 2H, OCH₂CH₃). Ratio of rotamers is 1:1. ¹³C NMR (151 MHz, DMSO-d₆): δ (ppm) = 14.0 (1C, OCH₂CH₃), 14.1 (1C, OCH₂CH₃), 27.7 (3C, C(CH₃)₃), 49.2 (1C, CH₂), 49.7 (1C, CH₂), 60.4 (2C, OCH₂CH₃), 79.9 (1C, C(CH₃)₃), 154.6 (1C, N=C(O)O), 169.45 (1C, O=COEt), 169.53 (1C, O=COEt). Ratio of rotamers is 1:1. IR (neat): ν (cm⁻¹) = 2978 (CH₃aliph.), 1747 (C=Oester), 1700 (C=O carbamate), 1185, 1159, 1026 (C-N, C-O). Exact mass (APCI): m/z = 308.1598 (calcd. 308.1598 for C₁₃H₂₅NO₆ [M+H⁺]).

3.1.10. Synthesis of 3-Iodo-2-(Iodomethyl)Prop-1-Ene (29) [44]

NaI (17.8 g, 119 mmol, 2.5 eq) was added to a solution of 3-chloro-2-(chloromethyl)prop-1-ene 26 (5.50 mL, 47.5 mmol, 1.0 eq) in acetone (100 mL) and the mixture was stirred at reflux for 16 h. The suspension was cooled to room temperature and concentrated in vacuo. The residue was dissolved in H₂O (75 mL) and cHex (75 mL). After separation of the two layers, the organic layer was washed with Na₂SO₄ (2 × 50 mL) and H₂O (50 mL), dried (Na₂SO₄), filtered and concentrated in vacuo. Light green solid, mp 28–29 °C, yield 14.5 g (99%). C₂₂H₂₂I₂ (307.9 g/mol). ¹H NMR (600 MHz, CD₂OD): δ (ppm) = 4.21 (s, 4H, 2 × CH₂I), 5.40 (s, 2H, R₂C=CH₂). ¹³C NMR (151 MHz, CD₂OD): δ (ppm) = 6.6 (2C, 2 × CH₂I), 116.4 (1C, R₂C=CH₂), 146.0 (1C, R₂C=CH₂). Exact mass (APCI): m/z = 308.8367 (calcd. 308.8362 for C₂₂H₂₂I₂ [M+H⁺⁺]).

3.1.11. Synthesis of 1-Tert-Butyl 2,6-Diethyl Cis- and Trans-4-Methyleneepiperidine-1,2,6-Tricarboxylate (24)

At −78 °C, n-ButLi (1.6 M in n-hexane, 74.4 mL, 119 mmol, 2.1 eq) was added dropwise to a solution of n-Pr₂NH (16.7 mL, 119 mmol, 2.1 eq) in dry THF (170 mL). After the mixture was stirred for 1 h, a solution of 25 (16.4 g, 56.7 mmol, 1.0 eq) in dry THF (15 mL) was added and the mixture was stirred for 1 h at −78 °C. Then, a solution of 29 (22.1 g, 68.1 mmol, 1.2 eq) in dry THF (15 mL) was added and the reaction mixture was stirred for 30 min at −78 °C and warmed up to room temperature over 16 h. At 0 °C, H₂O (150 mL) was added and the mixture was extracted with EtOAc (3 × 80 mL). The combined organic layers were dried (Na₂SO₄), filtered and concentrated in vacuo. The residue was purified twice by flash column chromatography (1. φ = 8 cm, h = 25 cm, cHex/EtOAc 9:1 → 8:2, V = 80 mL, 2. φ = 8 cm, h = 25 cm, cHex/EtOAc 9:1 → 8:2, V = 80 mL). Yellow
3.1.1. Synthesis of Tert-Butyl 4-Oxopiperidine-1-Carboxylate (18)...

FT-IR (neat): $\nu$ (cm$^{-1}$) = 2985, 2920 (C-H, aliph.), 1712 (C=O), 1670 (C=O), 1654 (C=C), 1161 (C-O), 641 (C-Br).

3.1.2. Synthesis of...

DMSO-d$_6$): R$_2$C=Oketone). FT-IR (C=O carbamate), 1157 (C-O), 648 (C-Br).

The mixture was diluted with Et$_2$O (50 mL) and washed with aqueous... temperature. The mixture was diluted with Et$_2$O (30 mL). AlCl$_3$ (0.67 g, 5.0 mmol, 0.1 eq.) was added and at 0 °C, Br$_2$ (2.6 mL, 50 mmol, 3 eq.) was added to a solution of 17.40 (C=O, carbamate), 1655 (C=C), 1180, 1165, 1022 (C-N, C-O). Exact mass (APCI): m/z = 342.1913 (calcd. 342.1911 for C$_{17}$H$_{26}$NO$_6$ [M+H$^+$]).

3.1.12. Synthesis of 1-Tert-Butyl 2,6-Diethyl Trans-4-Oxopiperidine-1,2,6-Tricarboxylate (30)

OsO$_4$ (0.05 m in H$_2$SO$_4$, 3.60 mL, 0.18 mmol, 0.01 eq), pyridine (0.70 mL, 9.10 mmol, 0.5 eq) and NaO$_4$ (15.6 g, 72.8 mmol, 4.0 eq) were added to a solution of 24 (62.0 g, 18.2 mmol, 1.0 eq) in t-BuOH (80 mL) and H$_2$O (120 mL) and the suspension was stirred at room temperature for 48 h. Then, the mixture was filtered and Na$_2$SO$_4$ (50 mL) and EtOAc (50 mL) were added to the solution. The aqueous layer was extracted with EtOAc (3 x 50 mL) and the combined organic layers were dried (Na$_2$SO$_4$), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (α = 8 cm, h = 16 cm, cHex/EtOAc 9:1 → 8:2, V = 80 mL). Colorless solid, mp 49–50 °C, yield 4.55 g (73%). C$_{31}$H$_{42}$N$_2$O$_7$ (343.4 g/mol). TLC: R$_f$ = 0.33 (cHex/EtOAc 8:2). 1H NMR (600 MHz, CDCl$_3$): δ (ppm) = 1.25 (t, J = 7.1 Hz, 3H, OCH$_2$CH$_3$), 1.27 (t, J = 7.1 Hz, 3H, OCH$_2$CH$_3$), 1.45 (s, 9H, C(CH$_3$)$_3$), 2.72 (dd, J = 18.0/1.4 Hz, 1H, 3/5-CH$_3$), 2.86 (dd, J = 18.0/1.4 Hz, 1H, 3/5-CH$_3$), 3.01–3.08 (m, 2H, 3-CH$_2$ax, 5-CH$_2$ax), 4.12–4.26 (m, 4H, 2 × OCH$_2$CH$_3$), 4.83 (d, J = 7.8 Hz, 1H, 2/6-CH$\beta$), 5.06 (d, J = 7.8 Hz, 1H, 2/6-CH). 13C NMR (151 MHz, CDCl$_3$): δ (ppm) = 14.2 (IC, OCH$_2$CH$_3$), 14.3 (IC, OCH$_2$CH$_3$), 28.3 (3C, C(3CH$_3$)$_3$), 40.6 (IC, C-3/5), 41.0 (IC, C-3/5), 53.0 (1C, C-2/6), 54.2 (1C, C-2/6), 61.9 (1C, OCH$_2$CH$_3$), 62.1 (1C, OCH$_2$CH$_3$), 81.9 (1C, C(3CH$_3$)$_3$), 154.5 (IC, N=O=O), 172.3 (IC, O=COEt), 172.4 (IC, CO=COEt), 203.8 (IC, C-4). IR (neat): $\bar{\nu}$ (cm$^{-1}$) = 2978 (CH$_{2}$-aliph.), 1736 (C=O-ester), 1701 (C=O-carbamate), 1188, 1115, 1026 (C-N, C-O). Exact mass (APCI): m/z = 344.1690 (calcd. 344.1704 for C$_{16}$H$_{26}$NO$_7$ [M+H$^+$]).

3.1.13. Synthesis of 1-Tert-Butyl 2,6-Diethyl Trans-(Ethoxycarbonylmethylene)-Piperidine-1,2,6-Tricarboxylate (31)

4-(Ethoxycarbonylmethylene)triphenylphosphorane (7.60 g, 21.7 mmol, 1.75 eq) was added to a solution of 30 (4.30 g, 12.4 mmol, 1.0 eq) in toluene (50 mL) and the mixture was heated at reflux for 48 h. After concentrating the mixture in vacuo, the residue was purified by flash column chromatography (α = 6 cm, h = 17 cm, cHex/EtOAc 9:1, V = 80 mL). Colorless oil, yield 3.9 g (75%). C$_{29}$H$_{35}$N$_2$O$_4$ (413.5 g/mol). TLC: R$_f$ = 0.35 (cHex/EtOAc 8:2). 1H NMR (600 MHz, DMSO-d$_6$): δ (ppm) = 1.11–1.22 (m, 9H, 3 × OCH$_2$CH$_3$), 1.35 (s, 9 × 0.54H, C(CH$_3$)$_3$), 1.36 (s, 9 × 0.46H, C(CH$_3$)$_3$)*, 2.71 (dd, J = 16.8/2.2 Hz, 0.54H, 3/5-CH$_2$), 2.78 (dd, J = 17.1/3.2 Hz, 0.46H, 3/5-CH$_2$*), 2.83–2.95 (m, 1.46H, 3/5-CH$_2$, 3-CH$_2$*, 5-CH$_2$*), 3.01 (ddm, J = 18.9/7.3 Hz, 0.54H, 3/5-CH$_2$), 3.61 (dm, J = 18.9 Hz, 0.54H, 3/5-CH$_2$), 3.68 (dm, J = 18.5 Hz, 0.46H, 3/5-CH$_2$*), 4.02–4.17 (m, 6H, 3 × OCH$_2$CH$_3$), 4.57 (dd, J = 6.7/2.3 Hz, 0.54H, 2/6-CH$\beta$), 4.62 (dd, J = 6.4/3.1 Hz, 0.46H, 2/6-CH), 4.67 (dd, J = 7.2/1.9 Hz, 0.46H, 2/6-CH$\beta$), 4.75 (dd, J = 7.3/2.3 Hz, 0.54H, 2/6-CH), 5.80 (s, 0.54H, R$_2$C=CH), 5.81 (s,
0.46H, R₂C=CH*). Ratio of rotamers is 54:46. Signals for the minor rotamer are marked with an asterisk (*). ¹³C NMR (151 MHz, DMSO-d₆): δ (ppm) = 13.9 (0.54C, OCH₂CH₃), 14.0 (0.46C, OCH₂CH₂*), 14.0 (0.46C, OCH₂CH₃*), 14.1 (0.54C, OCH₂CH₂), 14.11 (1C, OCH₂CH₂), 27.7 (3C, C(CH₃)₃), 29.8 (0.46C, C-3/5*), 30.4 (0.54C, C-3/5), 34.0 (0.46C, C-3/5*), 51.6 (0.54C, C-2/6), 53.0 (0.46C, C-2/6*), 53.1 (0.46C, C-2/6*), 54.1 (0.54C, C-2/6), 59.5 (1C, OCH₂CH₃), 60.8 (0.46C, OCH₂CH₂*), 60.9 (0.54C, OCH₂CH₃), 61.0 (0.46C, OCH₂CH₂*), 61.1 (0.54C, OCH₂CH₃), 80.3 (0.54C, C(CH₃)₃), 80.4 (0.46C, C(CH₃)₃*), 116.9 (0.54C, R₂C=CH₂), 117.0 (0.46C, R₂C=CH₂*), 151.4 (0.46C, R₂C=CH₂*), 151.6 (0.54C, R₂C=CH₂*), 154.0 (0.54C, N(C=O)O₂), 154.2 (0.46C, N(C=O)O₂*), 165.0 (1C, O=COEt*), 171.5 (0.46C, O=COEt*), 171.7 (0.54C, O=COEt*), 172.0 (0.54C, O=COEt*), 172.2 (0.46C, O=COEt*).

Ratio of rotamers is 54:46. Signals for the minor rotamer are marked with an asterisk (*). Purity (HPLC, method A): 98.9% (tₚ = 22.1 min). IR (neat): ν (cm⁻¹) = 2978 (C-H_aliph), 1744 (C=Oester), 1701 (C=O_carbamat), 1651 (C=C), 1184, 1142, 1026 (C-N, C-O). Exact mass (APCI): m/z = 414.2144 (calcd. 414.2122 for C₂₀H₃₂N₂O₈⁺ [M+H⁺]).

### 3.1.1. Synthesis of Tert-Butyl 4-Oxopiperidine-1-Carboxylate (18)

TFA (6.5 mL, 87.9 mmol, 30 eq) was added to a solution of 24 (1.0 g, 2.9 mmol, 1.0 eq) in dry CH₂Cl₂ (50 mL) and the mixture was stirred at room temperature for 16 h. Then, Na₂CO₃ was added, the layers were separated and the aqueous layer was extracted with CH₂Cl₂ (2 × 40 mL). The combined organic layers were dried (Na₂SO₄), filtered and concentrated in vacuo. The diastereomers were separated twice by flash column chromatography (1.50 g cartridge, cHex/EtOAc 8:2 → 6:4; 2.25 g cartridge, cHex/EtOAc 8:2). C₁₂H₁₅NO₃ (241.0 g/mol). cis-32: Yellow oil, yield 79 mg (11%). TLC: Rₗ = 0.31 (cHex/EtOAc 1:1). ¹H NMR (600 MHz CDCl₃): δ (ppm) = 1.29 (t, J = 7.1 Hz, 6H, 2 × OCH₂CH₃), 2.10−2.17 (m, 2H, 3-CH₂ax, 5-CH₂ax), 2.62 (dd, J = 13.5/2.7 Hz, 2H, 3-CH₂eq, 5-CH₂eq), 3.77 (s, J = 11.8/3.0 Hz, 2H, 2-CH₂ax, 6-CH₂ax), 4.22 (dq, J = 7.1/1.4 Hz, 4H, 2 × OCH₂CH₃), 4.87 (t, J = 1.7 Hz, 2H, R₂C=CH₂). NH signal is missing. ¹³C NMR (151 MHz, CDCl₃): δ = 14.3 (2C, 2 × OCH₂CH₃), 37.7 (2C, C-3, C-5), 58.9 (2C, C-2, C-6), 61.4 (2C, 2 × OCH₂CH₃), 111.4 (1C, R₂C=CH₂), 142.5 (1C, C-4), 171.9 (2C, 2 × O=COEt). IR (neat): ν (cm⁻¹) = 2982 (C-H_aliph), 1732 (C=Oester), 1651 (C=C), 1180, 1026 (C-N, C-O). Exact mass (APCI): m/z = 242.1360 (calcd. 242.1387 for C₁₂H₂₀N₂O₄⁺ [M+H⁺]). trans-32: Yellow oil, yield 623 mg (89%). TLC: Rₗ = 0.20 (cHex/EtOAc 1:1). ¹H NMR (600 MHz, CDCl₃): δ (ppm) = 1.29 (t, J = 7.1 Hz, 6H, 2 × OCH₂CH₃), 2.46 (dd, J = 13.2/7.0 Hz, 2H, 3-CH₂, 5-CH₂), 2.56 (dd, J = 13.2/5.0 Hz, 2H, 3-CH₂, 5-CH₂), 3.84 (dd, J = 7.0/5.0 Hz, 2H, 2-CH, 6-CH), 4.14−4.24 (m, 4H, 2 × OCH₂CH₃), 4.87 (s, 2H, R₂C=CH₂). NH signal is missing. ¹³C NMR (151 MHz, CDCl₃): δ (ppm) = 14.4 (2C, 2 × OCH₂CH₃), 36.3 (2C, C-3, C-5), 56.1 (2C, C-2, C-6), 61.2 (2C, 2 × OCH₂CH₃), 111.7 (1C, R₂C=CH₂), 141.3 (1C, C-4), 172.7 (2C, 2 × O=COEt). IR (neat): ν (cm⁻¹) = 3356 (NH-H), 2978 (C-H_aliph), 1728 (C=Oester), 1655 (C=C), 1200, 1165, 1026 (C-N, C-O). Exact mass (APCI): m/z = 242.1373 (calcd. 242.1387 for C₁₂H₂₀N₂O₄⁺ [M+H⁺]).

### 3.1.1.5. Synthesis of Diethyl Trans-4-Oxopiperidine-2,6-Dicarboxylate (33)

TFA (3.60 mL, 47.0 mmol, 30 eq) was added to a solution of 30 (0.54 g, 1.57 mmol, 1 eq) in CH₂Cl₂ (15 mL) and the mixture was stirred at room temperature for 16 h. Then, the mixture was washed with NaHCO₃ (30 mL). The aqueous phase was extracted with CH₂Cl₂ (3 × 20 mL) and the combined organic layers were dried (Na₂SO₄), filtered and concentrated in vacuo. Yellow oil, yield 0.27 g (71%). C₁₁H₁₇NO₃ (243.3 g/mol). TLC: Rₗ = 0.22 (cHex/EtOAc 1:1). ¹H NMR (600 MHz, CDCl₃): δ (ppm) = 1.28 (t, J = 7.1 Hz, 6H, 2 × OCH₂CH₃), 2.61 (ddd, J = 15.1/7.0/1.4 Hz, 2H, 3-CH₂, 5-CH₂eq), 2.71 (ddd, J = 15.0/5.5/1.3 Hz, 2H, 3-CH₂, 5-CH₂eq), 4.05 (dd, J = 7.0/5.5 Hz, 2H, 2-CH, 6-CH), 4.21 (q, J = 7.1 Hz, 4H, 2 × OCH₂CH₃). The signal for NH is not observed in the spectrum. ¹³C NMR (151 MHz, CDCl₃): δ (ppm) = 14.3 (2C, 2 × OCH₂CH₃), 42.7 (2C, C-3, C-5), 54.8 (2C, C-2, C-6), 61.8 (2C, 2 × OCH₂CH₃), 171.7 (2C, 2 × O=COEt), 204.7 (1C, C-4).
3.1.16. Synthesis of Diethyl Trans-4-(2-Ethoxy-2-Oxoethylidene)Piperidine-2,6-Dicarboxylate (34)

TFA (2.10 mL, 28.4 mmol, 30 eq) was added to a solution of 31 (0.39 g, 0.95 mmol, 1 eq) in CH₂Cl₂ (10 mL) and the mixture was stirred at room temperature for 16 h. Then, the mixture was washed with NaHCO₃ (20 mL). The aqueous phase was extracted with CH₂Cl₂ (3 × 15 mL) and the combined organic layers were dried (Na₂SO₄), filtered and concentrated in vacuo. Yellow oil, yield 0.27 g (90%). C₁₄H₂₃NO₆ (313.4 g/mol). TLC: Rf = 0.35 (cHex/EtOAc 1:1). ¹H NMR (600 MHz, CDCl₃): δ (ppm) = 1.25 (t, J = 7.1 Hz, 3H, OCH₃), 1.28 (t, J = 7.1 Hz, 3H, OCH₂CH₃), 1.28 (dd, J = 13.3/7.1 Hz, 1H, 3-/5-C), 1.52 (s, 1H, R₂=C≡CH), 1.66.9 (1C, C-6), 162.9 (1C, O=CH), 166.0 (1C, O=CH₂). ¹³C NMR (151 MHz, CDCl₃): δ (ppm) = 14.3 (1C, OCH₂CH₃), 14.4 (2C, 2 × OCH₂CH₃), 31.2 (1C, C-3/5), 38.2 (1C, C-3/5), 55.6 (1C, C-2/6), 56.0 (1C, C-2/6), 60.0 (1C, OCH₂CH₃), 61.3 (1C, OCH₂CH₃), 61.4 (1C, OCH₂CH₃), 117.5 (1C, R₂=C≡CH), 153.4 (1C, R₂=C=CH), 166.0 (1C, O=COEt), 172.2 (1C, O=COEt), 172.6 (1C, O=COEt).

3.1.17. Synthesis of Diethyl 4-Methylpyridine-2,6-Dicarboxylate (35)

At 0 °C, NaOCl (0.75 m in H₂O, 3.90 mL, 2.90 mmol, 2 eq) was added to a solution of 32 (350 mg, 1.45 mmol, 1 eq) and AcOH (0.17 mL, 2.90 mmol, 2 eq) in t-BuOH (0.20 mL, 1.74 mmol, 1.2 eq) and methyl t-butyl ether (8 mL) and the mixture was stirred for 1 h. Then, DIPEA (2.05 mL, 11.6 mmol, 8 eq) was added and the mixture was stirred at room temperature for 16 h. After the addition of H₂O (10 mL), the mixture was extracted with EtOAc (3 × 15 mL). The combined organic layers were dried (Na₂SO₄), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (25 g cartridge, cHex/EtOAc 95:5 → 9:1). Yellow oil, yield 0.19 g (6%). C₁₄H₂₇NO₄ (237.3 g/mol). TLC: Rf = 0.59 (cHex/EtOAc 9:1). ¹H NMR (600 MHz, CDCl₃): δ (ppm) = 1.45 (t, J = 7.2 Hz, 6H, 2 × OCH₂CH₃), 2.51 (s, 3H, CH₃), 4.47 (q, J = Hz, 4H, 2 × OCH₂CH₃), 8.10 (s, 2H, 3-CH₃, 5-CH₃). ¹³C NMR (151 MHz, CDCl₃): δ (ppm) = 14.4 (2C, 2 × OCH₂CH₃), 21.3 (1C, CH₃), 62.4 (2C, 2 × OCH₂CH₃), 128.8 (2C, C-3, 5), 148.6 (2C, C-2, C-6). 150.2 (1C, C-4, C-4), 165.1 (2C, 2 × O=COEt).

3.1.18. Synthesis of Diethyl-4-Oxo-1,2,3,4-Tetrahydroxypyrrole-2,6-Dicarboxylate (36)

At 0 °C, NaOCl (0.75 m in H₂O, 1.70 mL, 1.28 mmol, 1.2 eq) was added to a solution of 33 (260 mg, 1.07 mmol, 1 eq) and AcOH (0.07 mL, 1.28 mmol, 1.2 eq) in t-BuOH (0.12 mL, 1.28 mmol, 1.2 eq) and methyl t-butyl ether (10 mL) and the mixture was stirred for 1 h. Then, DIPEA (0.90 mL, 5.35 mmol, 5 eq) was added and the mixture was stirred at room temperature for 16 h. H₂O (15 mL) was added and the mixture was extracted with EtOAc (3 × 15 mL) and dried (Na₂SO₄), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (25 g cartridge, cHex/EtOAc 7:3 → 1:1). Colorless oil, yield 101 mg (39%). C₁₁H₁₅NO₅ (241.2 g/mol). TLC: Rf = 0.40 (cHex/EtOAc 1:1). ¹H NMR (600 MHz, CDCl₃): δ (ppm) = 1.30 (t, J = 7.1 Hz, 3H, OCH₂CH₃), 1.35 (t, J = 7.1 Hz, 3H, OCH₂CH₃), 2.7 (dd, J = 16.5/12.3 Hz, 1H, 3-CH₂), 2.8 (dd, J = 16.5/5.8 Hz, 1H, 3-CH₂), 4.23–4.29 (m, 3H, 2H, 2-CH₂, OCH₂CH₃), 5.77 (s, 1H, 5-CH), 6.07 (s, 1H, NH). ¹³C NMR (151 MHz, CDCl₃): δ (ppm) = 14.1 (1C, OCH₂CH₃), 14.2 (1C, OCH₂CH₃), 38.2 (1C, C-3), 54.8 (1C, C-2), 62.4 (1C, OCH₂CH₃), 63.0 (1C, OCH₂CH₃), 102.2 (1C, C-5), 147.7 (1C, C-6), 162.9 (1C, O=COEt), 169.9 (1C, O=COEt), 172.9 (1C, C-4).

3.1.19. Synthesis of Diethyl (E)- and (Z)-(RS)-4-(2-Ethoxy-2-Oxoethylidene)-1,2,3,4-Tetrahydroxypyrrole-2,6-Dicarboxylate (37)

At 0 °C, NaOCl (0.75 m in H₂O, 2.20 mL, 1.65 mmol, 2 eq) was added to a solution of 34 (260 mg, 0.82 mmol, 1 eq) and AcOH (0.10 mL, 1.65 mmol, 2 eq) in t-BuOH (0.10 mL, 1.00 mmol, 1.2 eq) and methyl t-butyl ether (8 mL) and the mixture was stirred for 1 h. Then, DIPEA (1.15 mL, 6.61 mmol, 8 eq) was added and the mixture was stirred at room
temperature for 16 h. H2O (10 mL) was added and the mixture was extracted with EtOAc (3 × 15 mL) and dried (Na2SO4), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (25 g cartridge, cHex/EtOAc 8:2 → 6:4). Yellow oil, yield 77 mg (30%). C15H23NO3 (311.3 g/mol). TLC: Rf = 0.49 (cHex/EtOAc 7:3). 1H NMR (600 MHz, CDCl3): δ (ppm) = 1.24–1.32 (m, 6H, 2 × OCH2CH3, 2 × OCH2CH3*), 1.34 (t, J = 7.1 Hz, 3 × 0.55C, OCH2CH3), 1.35 (t, J = 7.1 Hz, 3 × 0.45C, OCH2CH3*), 2.76 (ddd, J = 15.5/9.6/1.5 Hz, 0.45H, 3-CH2*), 2.83 (ddd, J = 15.5/5.0/1.2 Hz, 0.45H, 3-CH2*), 3.10 (ddd, J = 17.0/10.2/2.0 Hz, 0.55H, 3-CH2*), 3.70 (ddd, J = 17.0/4.8/1.6 Hz, 0.55H, 3-CH2*), 4.00 (dd, J = 10.2/4.8 Hz, 0.55H, 2-CH), 4.06 (dd, J = 9.6/5.0 Hz, 0.45H, 2-CH*), 4.12–4.27 (m, 4H, 2 × OCH2CH3, 2 × OCH2CH3*), 4.30 (q, J = 7.1 Hz, 1.10H, OCH2CH3), 4.31 (q, J = 7.1 Hz, 0.9H, OCH2CH3*), 5.43 (s, 0.45H, 5-CH*), 5.66 (s, 0.55H, 5-CH), 6.08 (s, 0.55H, CHCOOEt), 7.37 (s, 0.45H, CHCOOEt*). Ratio of diastereomers is 55:45. The signals for the minor diastereomer are marked with an asterisk (*). 13C NMR (151 MHz, CDCl3): δ (ppm) = 14.25, 14.28, 14.20, 14.31 (2C, 2 × OCH2CH3, 2 × OCH2CH3*), 14.50, 14.51 (1C, OCH2CH3, OCH2CH3*), 28.4 (0.55C, 3-C), 33.7 (0.45C, C-3*), 53.5 (0.55C, C-2), 55.8 (0.45C, C-2*), 59.82 (0.55, OCH2CH3), 59.84 (0.45C, OCH2CH3*), 61.8 (0.45C, OCH2CH3*), 61.9 (0.55C, OCH2CH3*), 62.06 (0.55C, OCH2CH3), 62.13 (0.45C, OCH2CH3*), 102.4 (0.45C, CHCOOEt*), 107.1 (0.55C, CHCOOEt), 112.3 (0.45C, C-5*), 113.2 (0.55C, C-5), 137.9 (0.55C, C-6), 138.1 (0.45C, C-6*), 146.1 (0.45C, C-4*), 148.0 (0.55C, C-4), 163.2 (0.55C, O=COEt), 163.9 (0.45C, O=COEt*), 166.7 (0.45C, O=COEt*), 167.0 (0.55C, O=COEt), 170.8 (0.45C, O=COEt*), 171.2 (0.55C, O=COEt). Ratio of diastereomers is 55:45. The signals for the minor diastereomer are marked with an asterisk (*).

4. Conclusions

In order to learn more about the relevance of the carboxy moieties of betalamic acid (9), the seven-step synthesis of the betalamic acid analog 13 without carboxy groups in positions C-2 and C-6 was designed and carried out. Due to low stability, in particular against O2, the free amine could be characterized only by NMR spectroscopy. However, the Boc-protected precursor 21 could be isolated. In the Folin–Ciocalteu assay, 21 did not show any antioxidative properties, indicating that a free amine within the piperidine ring is essential for its antioxidative activity. Analogous 1,2,3,4-tetrahydropyridines 15–17 with two ester moieties in positions C-2 and C-6 and different substituents in position C-4 showed different levels of stability, i.e., different antioxidative properties in NMR studies.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/molecules27217423/s1, Page S2: Folin–Ciocalteu Assay; Page S3: NMR spectra.

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