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(5-Oxooxazolidin-4-yl)acetic Acid Derivatives as a Protection for the α-Carboxyl Group of Aspartic Acid: A Word of Caution

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Abstract—Objective: (*S*)-2-{3-[(Benzyloxy)carbonyl]-5-oxooxazolidin-4-yl}acetic acid is an useful intermediate in the preparation of β-branched peptide derivatives of aspartic acid. However, we and others observed the formation of side products in the reaction of activated esters of this compound with amino acid derivatives. This suitably protected aspartic acid derivative exists as a mixture of *E* and *Z* rotamers that complicates the interpretation of NMR spectra of the products obtaind from it. Therefore, some of the failures in the preparation of β-branched aspartic acid derivatives could be due to misinterpretation of spectral data. **Methods:** The selectivity of the reaction of pentafluorophenyl (*S*)-2-{3-[(benzyloxy)carbonyl]-5-oxooxazolidin-4-yl}acetate with amino acid esters was explored with particular emphasis on the rigorous structure assignment of the products using high-temperature NMR experiments. **Results and Discussion:** The isomeric products of the reaction were isolated and identified. One of the isomers is an expected dipeptide, and its isomer is an *N*-(hydroxymethyl-amino)succinimide formed by methylene bridge cleavage. The steric bulkiness of amino acid esters favors dipeptide formation, but the reaction selectivity is quite unpredictable. **Conclusions:** The protection of the α-carboxyl group of aspartic acid in the form of (5-oxooxazolidin-4-yl)acetic acid derivatives has a number of limitations that must be taken into account, when this derivative is considered for use in the preparation of β-branched peptides.

Keywords: aminosuccinimide, β-branched aspartic acid dipeptide, *N*-(hydroxymethylamino)succinimide

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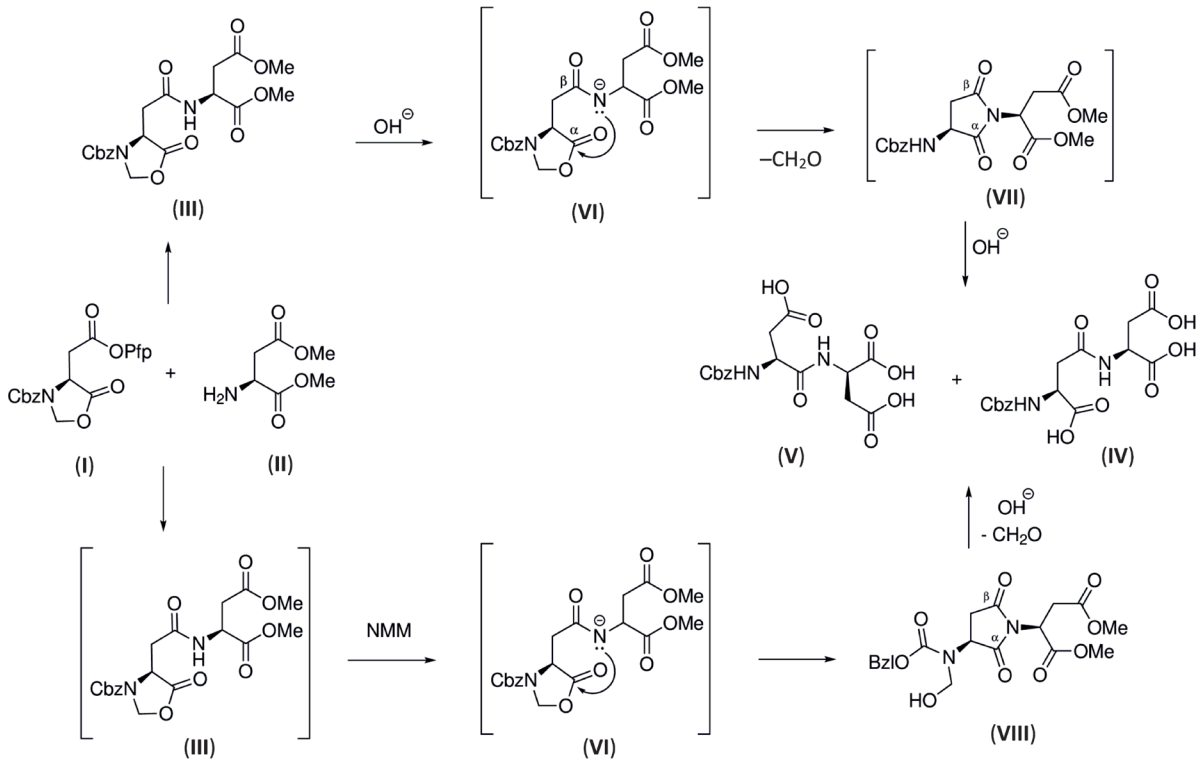
INTRODUCTION

The reaction of Nα-Cbz-aspartic acid with formaldehyde forms (*S*)-2-{3-[(benzyloxy)carbonyl]-5-oxooxazolidin-4-yl}acetic acid in a high yield [1, 2]. The α-COOH group in this compound is protected from reaction with electrophilic reagents, and the second remains free and can be converted into activated esters or mixed anhydrides for the synthesis of β-branched aspartic acid derivatives [1, 3, 4].

We earlier found that the reaction of pentafluorophenyl (*S*)-2-{3-[(benzyloxy)carbonyl]-5-oxooxazolidin-4-yl}-acetate (**I**) with dimethyl aspartate (**II**) gives a compound which we identified as dipeptide (**III**) on the basis of its spectral data. Dipeptide (**III**) was a mixture of the *E* and *Z* rotamers, which complicated the interpretation of the spectra. By alkaline hydrolysis, this intermediate was converted into the target triacid (**IV**) and, probably, its isomer (**V**) (Scheme 1) [5]. We assumed that isomerization occurs under alkaline hydrolysis conditions through the intermediate formation of an intramolecular cyclization product aminosuccinimide (**VII**), which, under the same conditions, undergoes hydroxide-induced ring opening

Abbreviations: APCI, atmospheric pressure ionization; Cbz, benzyloxycarbonyl group; Hep, heptane.



Scheme 1. The formation of two isomeric products in a two-step preparation of (**IV**).

at either of the two carbonyl groups. Such cyclization followed by ring opening is a fundamental chemical property of asparagine derivatives [6]. Numerous examples of this side reaction are available in the literature, and its contribution depends on the specific peptide sequence.

At the same time, a precedent of the isolation of an *N*-[(hydroxymethyl)amino]succinimide (also as a mixture of the *E* and *Z* rotamers) as a product of the reaction of hydroxybenzotriazolyl (*S*)-2-{3-[(benzyloxy)carbonyl]-5-oxoazolidin-4-yl}acetate with ethyl phenylalaninate has been reported [4]. Thus, it could not be excluded that we isolated a similar *N*-(hydroxymethylamino)succinimide (**VIII**) as a result of the reaction between compounds (**I**) and (**II**)**,** while compound **(III)** formed as an intermediate of this reaction. The hydrolysis of compound (**VIII**), also gives a mixture of isomers (**IV**) and (**V**). With this in mind, we set ourselves the task to explore the selectivity of the reaction of compound (**I**) with dimethyl aspartate (**II**) and other amino acid esters and the structure of the products of these reactions.

RESULTS AND DISCUSSION

An unusual feature of the ^{1}H NMR spectrum of the reaction product of compounds (**I**) and (**II**) is that the amide (or hydroxyl) proton signal is observed in the region of 6 ppm (Fig. 1a), which amide protons are often appear more downfield (at ~8 ppm). However, such a chemical shift could be caused by the magnetic anisotropy of the benzene ring of the Cbz group. The multiplicity of this signal could to be determined in thoroughly dried DMSO-d_6 solutions (Fig. 1b); at the same time, at 298 K we were unable to unambiguously identify this signal: it could be either an overlapping doublet of a mixture of the *E* and *Z* isomers of compound (**III**) or as a triplet of the hydroxyl group of compound (**VIII**).

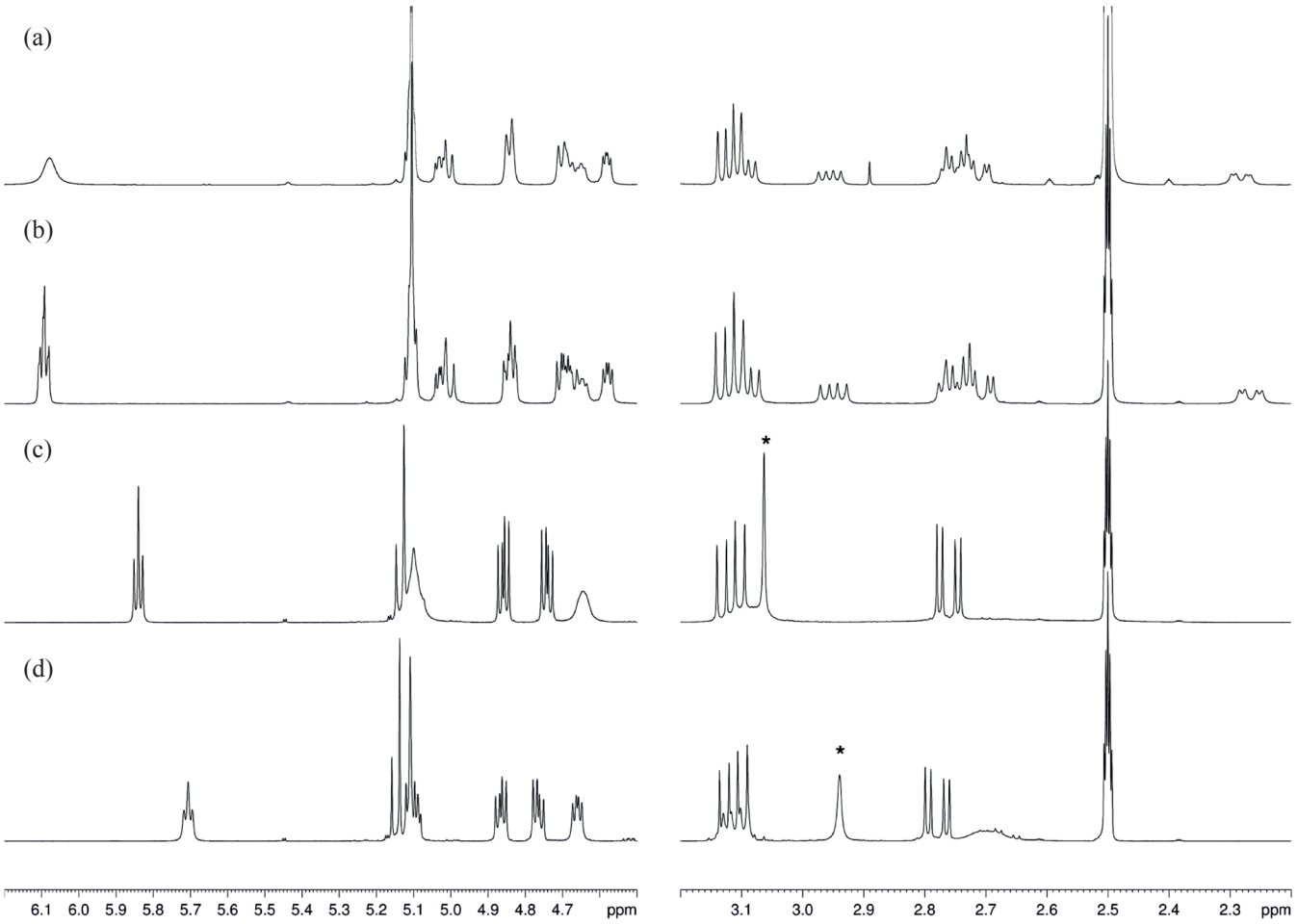


Fig. 1. Comparison of two spectral regions of the reaction product of compounds (**I**) and (**II**): (a) at 298 K in commercial DMSO-d_6; (b) at 298 K in anhydrous DMSO-d_6; (c) at 353 K; and (d) at 383 K. The asterisk indicates the signal of residual water.

Like many other *N*-acylated heterocyclic compounds [7], (*S*)-2-{3-[(benzyloxy)carbonyl]-5-oxooxazolidin-4-yl}acetic acid derivatives are present in solution as *E*/*Z* isomer mixtures, and this complicates the interpretation of NMR spectra. To unambiguously interpret such spectral data it is required to acquire the spectra at elevated temperatures, when the interconversion of the *E* and *Z* isomers is accelerated. As far as we know, such NMR experiments with compounds similar to those studied in the present work have been described only once previously [4].

Upon heating to 353 K, the signal at ~6 ppm has already clearly begun to appear as a triplet (Fig. 1c), and in the COSY spectrum we observed correlations with the AM system of proton signals at 4.76 and 4.87 ppm. It is interesting to note that at this temperature the α-proton and some β-proton signals of aspartic acid residues are still strongly broadened, and at 383 K (Fig. 1d), only some of them acquired a well-defined multiplicity. It should also be emphasized that the spectra of the sample before and after heating were identical (data not shown). Thus, from the reaction products of compounds (**I**) and (**II**) in the presence of a weak amine (*N*-methylmorpholine was used to neutralize salt (**II**)), we isolated *N*-(hydroxymethylamino)succinimide derivative (**VIII**) rather than β-branched peptide (**III**).

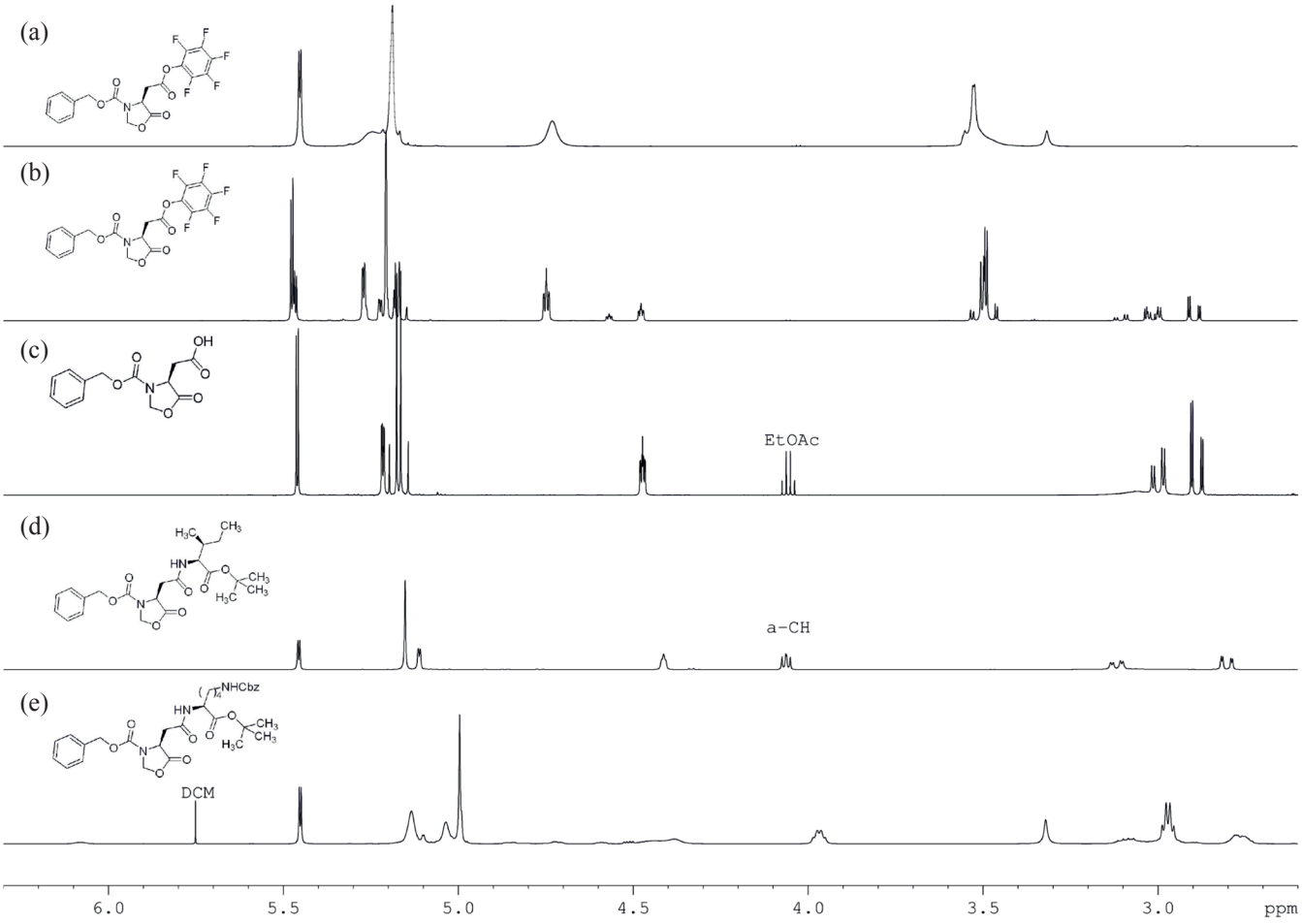


Fig. 2. Comparison of the 6.2–2.6 ppm ranges of the ^{1}H NMR spectra of products containing the oxazolidine rings: (a) compound (**I**) at 298 K; (b) compound (**I**) at 353 K; (c) starting acid of the synthesis of compound (**I**) at 353 K; (d) product (**IX**) at 353 K; (e) product (**X**) at 298 K.

Separately, we established that the methylene bridge is not cleaved at the stage of formation of activated ester (**I**) from the corresponding acid. At 298 K, compound (**I**) also exists as a mixture of the *E* and *Z* isomers (Fig. 2a). The ^{1}H NMR spectrum of ester (**I**) at 353 K contains no signals from the CH_2OH group, and the diastereotopic methylene protons are coupled to each other with a constant of 3.68 Hz (Fig. 2b). It should be noted that during heating compound (**I**) undergoes partial hydrolysis under the action of residual water in the deuterated solvent: the spectrum (Fig. 2b) displays multiplets characteristic of the starting acid at 4.4, 2.95, and 2.85 ppm (Fig. 2c) and, in addition, after the reaction with compound (**I**), the signal of water itself disappears. Further on, upon heating, another by-product is formed (multiplets at 4.55, 3.1, and ~3.0 ppm), presumably by the reaction of compound **I** with DMSO-d6: sulfoxides are known to react with various electrophiles [8].

The selectivity of the formation of compound (**VIII**) was not affected by the nature of the base (NMM, TEA, or pyridine) or the nature of the solvent (DCM, MeCN, or THF). Taking into account the availability in the literature of successful precedents of the synthesis of β-branched peptides from activated (*S*)-2-{3-[(benzyloxy)-carbonyl]-5-oxooxazolidin-4-yl}acetic acid derivatives,

we investigated the compound (**I**) reaction selectivity with a number of amino acid esters. In most cases, mixtures of products formed, and a highly selective formation of compounds with an intact methylene bridge was observed only with the sterically hindered *tert*-butyl esters. As an example, Fig. 2d demonstrates a region of the spectrum of dipeptide (**IX**) formed by the reaction of compound (**I**) with *tert*-butyl isoleucinate. The spectrum contains no signals of the AMX system of the CH_2OH group, and the diastereotopic methylene protons appear as a doublet of doublets at 5.05 (*J* 0.76 and 3.34 Hz) and a doublet at 5.45 ppm. (*J* 3.34 Hz). In addition, the amide proton signal was observed in the usual region of the spectrum (~8 ppm) (not shown in Fig. 2). Abell et al. [4] described the ^{1}H NMR spectrum of a dipeptide with a methylene bridge (also recorded on heating in DMSO-d_6), in which similar signals were observed.

Further study of the selectivity of the reaction of compound (**I**) with a series of *tert*-butyl esters of amino acids showed that oxooxazolidine is not the only reaction product. For example, the reaction of compound (**I**) with the lysine derivative H-Lys(Cbz)-OtBu gave a mixture containing, as judged from the ^{1}H NMR data, both isomers in a ratio of 9 : 1 in favor of oxooxazolidine (**X**) . Figure 2e shows the spectral region of a sample containing oxooxazolidine (**X**). It can be identified by a doublet at 5.45 ppm, and the admixture of *N*-(hydroxymethylamino) succinimide by a multiplet of hydroxyl protons in the region of 6.1 ppm. Attempted separating of this mixture by flash chromatography or preparative TLC was unsuccessful because of the instability of the products on silica gel. This is probably why Abell et al. [4] resorted to an exotic but fast version of radial chromatography for these purposes.

NMR experiments at elevated temperatures are time-consuming due to rather lengthy sample thermal equilibration in the NMR probe. Therefore, it is important to note that the above-described NMR study led us to the preliminary conclusion that the ^{1}H NMR spectra measured even at the standard recording temperature of 298 K show signals that allow us to assign the structure in hand to one or another isomer. First of all, this is a multiplet of the hydroxyl proton in the region of 6.1 ppm for *N*-(hydroxymethylamino)succinimide or a doublet in the region of 5.5 ppm for its isomer with the methylene bridge. Amino acids and their derivatives very rarely give proton signals in the range 5.2–7 ppm, unlike what was observed in the present work. These features were used to identify the products of the reaction of compound (**I**) with the lysine derivative H-Lys(Cbz)-OtBu (Fig. 2e). The position of the amide proton signal can also be considered, provided it does not overlap with other signals. These observations could not be compared with published data because of the differences in the conditions of recording NMR spectra (first of all, solvent and temperature).

With regard to the mechanism of the side ring opening reaction, it should be noted that, probably, in addition to the close spatial proximity of the nucleophilic (amide) and electrophilic (ester) centers, which leads to the kinetic preference for the formation of a five-membered ring [6], it is the experimentally demonstarted [1] fundamental property of the oxooxazolidine ring to function both as a protecting group and as an activated ester (possibly, due to the inductive effect of the *N*α-carbamoyl group).

EXPERIMENTAL

All solvents, sodium bicarbonate, potassium carbonate, anhydrous magnesium sulfate, and hydrochloric acid were obtained from commercial sources (Reakhim and Khimmed, Russia). If necessary, they were purified by known methods [9]. All standard amino acid derivatives were purchased from Reanal (Hungary) and IRIS Biotech (Germany). The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Biospin Avance III spectrometer at 600 and 125 MHz, respectively, for DMSO-d_6 solutions and calibrated against the residual proton and carbon signals of the solvent. The high-resolution mass spectra were recorded on a Thermo Fisher Scientific Orbitrap Elite

Hybrid Ion Trap-Orbitrap instrument. Analytical TLC was performed on Merck F254 silica gel G plates (part no. 1.05554.0001). Preparative TLC was performed on Merck glass plates TLC silica gel 60 F_{254} (part no. 1057150001). Spot visualization on TLC plates was carried out using: 1) a ninhydrin solution (0.5 g ninhydrin, 250 mL of butan-1-ol, 50 mL of acetic acid, 10 mL of *sym*-collidine) followed by heating; 2) iodine vapor; 3) UV irradiation; and 4) a saturated solution of phosphomolybdic acid in ethanol (12 wt %) followed by heating.

Pentafluorophenyl [(4*S*)-3-(benzyloxycarbonyl)-5-oxooxazolidin-4-yl]acetate (I). Pentafluorophenyl trifluoroacetate (2 mL, 10 mmol) and pyridine (1 mL, 12 mmol) were successively added to a solution of [(4*S*)-3-(benzyloxycarbonyl)-5-oxooxazolidine-4-yl]acetic acid (2.5 g, 9 mmol) in 15 mL of DCM. The reaction mixture was stirred for 4 h and then diluted with DCM to a volume of 40 mL. The resulting solution was washed with 0.1 M aqueous HCl (20 mL), 5% $NaHCO_3$ (20 mL), and water (20 mL), dried over $MgSO_4$, filtered, and the drying agent was rinsed with DCM (20 mL). The combined organic phases were evaporated to a small volume, after which heptane was added in portions until cloudy. The resulting mixture was kept overnight at 4°C, and the precipitate that formed was filtered off, washed with heptane, and dried in a vacuum over KOH and paraffin to obtain compound (**I**). Yield 3.13 g (79%); R_f 0.52 (EtOAc–Hep, 1 : 1 v/v); mp 120–122; ^{1}H NMR (353 K) (*J*, Hz): 3.48 dd (*J* 3.80 and 17.41 Hz, 1H, HβAsp), 3.52 dd (*J* 5.17 and 17.41 Hz, 1H, HβAsp), 4.75 ddd (*J* 1.09, 3.80, and 5.23 Hz, 1H, HαAsp), 5.21 br s (2H, $PhCH_2$), 5.11 dd (*J* 1.12 and 3.68 Hz, 1H, NCH_2O), 5.48 d (*J* 3.66 Hz, 1H, NCH_2O), 7.31–7.41 m (5H, $PhCH_2$); ^{13}C NMR (333 K): 170.83, 166.09, 152.26, 140.22 dm (*J* 248.00 Hz), 138.96 dm (*J* 252.00 Hz), 137.26 dm (*J* 251.00 Hz), 135.67, 128.07, 127.74, 127.33, 123.83 m, 77.86, 66.73, 51.17, 33.76; MS (+ APCI-HRMS): *m/z* 446.0666 $[M + H]^+$; $C_{19}H_{13}F_5NO_6^+$; Calculated: 446.0658.

Reaction of compound I with salts of amino acid esters (*general procedure*). A solution of compound (**I**) (89.1 mg, 0.2 mmol), amino acid ester hydrochloride (0.22 mmol), and NMM (22 μL, 0.2 mmol) in THF (1–1.5 mL) was stirred for 16 h. The reaction mixture was diluted with EtOAc (10–15 mL), the organic phase was washed with 5% $NaHCO_3$ (20 mL), 0.1 M aqueous HCl (2 × 10 mL), and water, dried over Na_2SO_4, filtered, and the drying agent was washed with EtOAc (10 mL). The combined organic phases were evaporated on a rotary evaporator, dried in vacuum, and the resulting mixture was separated by preparative TLC or silica gel column chromatography.

(S)-Dimethyl 2-{(*S*)-3-[(benzyloxycarbonyl)(hydroxymethyl)amino]succinimido} succinate (VIII) was prepared using the general procedure from dimethyl L-aspartate hydrochloride (43.5 mg). Yield 50 mg, 60%; R_f 0.46 (PhMe–AcOH, 2 : 1 v/v); ^{1}H NMR (383 K) (*J*, Hz): 2.7 br s (1H, Hβ), 2.78 dd (*J* 5.61 and 17.92 Hz, 1H, Hβ), 3.07–3.15 m (2H, 2Hβ), 3.64 s (3H, OCH_3), 3.65 s (3H, OCH_3), 4.66 dd (*J* 5.53 and 9.12 Hz, 1H, Hα), 5.49 dd (*J* 6.66 and 10.35 Hz, 1H, CH_2OH), 5.59 dd (*J* 6.56 and 10.35 Hz, 1H, CH_2OH), 5.08–5.13 m (2H, Hα, $PhCH_2$), 5.15 d (*J* 12.62 Hz, 1H, $PhCH_2$), 5.71 t (*J* 6.74 Hz, 1H, OH), 7.29–7.39 m (5H, $PhCH_2$); ^{13}C NMR (383 K): 173.57, 172.64, 169.21, 167.47, 153.77, 135.81, 127.72, 127.28, 126.98, 71.17, 66.40, 54.17, 51.98, 50.97, 47.75, 34.18, 32.38; MS (+ ESI-HRMS): *m/z* 445.1201 $[M + Na]^+$; $C_{19}H_{22}N_2NaO_9^+$; Calculated: 445.1218.

***tert*-Butyl {*Nα*-[(4*S*)-3-(benzyloxycarbonyl)-5-oxooxazolidin-4-yl]acetyl}isoleucinate (IX)** was prepared using the general procedure from L-isoleucine *tert*-Butyl Ester Hydrochloride (49.2 mg). An aliquot (10.3 mg) of the crude reaction mixture (115 mg) was separated by preparative TLC (PhMe–AcOH, 2 : 1 v/v) to obtain

4.68 mg of the dipeptide (extrapolated yield 58%). R_f 0.57 (EtOAc–heptane, 1 : 1 v/v); ^{1}H NMR (353 K) (*J*, Hz): 0.84–0.89 m (6H, CH_3γIle, CH_3δIle), 1.16–1.24 m (1H, Hγ Ile), 1.36–1.47 m (10H, OBu^t, Hγ Ile), 1.70–1.78 m (1H, Hβ Ile), 2.80 dd (*J* 2.90, 16.65, 1H, Hβ Asp), 3.12 dd (*J* 4.49, 16.80, 1H, Hβ Asp), 4.06 dd (*J* 6.27, 7.84, 1H, Hα Ile), 4.66 (t, *J* 3.33, 1H, Hα Asp), 5.11 dd (*J* 0.76, 3.34, 1H, NCH_2O), 5.15 br s (2H, $PhCH_2$), 5.46 d (*J* 3.43, 1H, NCH_2O), 7.31–7.41 m (5H, $PhCH_2$), 8.03 d (*J* 7.84, 1H, NH); ^{13}C NMR (383 K): 171.83, 169.77, 168.46, 151.95, 135.76, 127.94, 127.47, 127.04, 80.07, 77.40, 66.30, 56.93, 51.27, 35.96, 34.70, 27.27, 24.64, 14.90, 10.63; MS (+ ESI-HRMS): *m/z* 471.2121 $[M + Na]^+$; $C_{23}H_{32}N_2NaO_7^+$; Calculated: 471.2102.

***tert*-Butyl *N*α-{[(4*S*)-3-(benzyloxycarbonyl)-5-oxooxazolidin-4-yl]acetyl}-*N*ε-[(benzyloxy)carbonyl]-lysinate (X)** was prepared by the general procedure from $N_ε$-Cbz-L-lysine *tert*-butyl ester hydrochloride (82 mg). The yield of compound (**X**) with an admixture of ~10% of the isomer was 80 mg (67%). R_f 0.21 (1 : 1 v/v EtOAc/Hep); ^{1}H NMR spectrum of the main isomer (298 K, the *E* and *Z* rotamers are observed at 298 K, as characteristic of acylated heterocyclic compounds [7]): (*J*, Hz), 1.23–1.31 m (2H, Hγ Lys), 1.32–1.41 m (11H, OBu^t, Hδ Lys), 1.49–1.57 m (1H, Hβ Lys), 1.58–1.65 m (1H, Hβ Lys), 2.72–2.83 m (1H, Hβ Asp), 2.98 dd (*J* 6.45, 13.05, 1H, Hε Lys), 3.05–3.15 m (1H, Hβ Asp), 3.97 dd (*J* 7.94, 13.70, 1H, Hα Lys), 4.33–4.50 m (1H, Hα Asp), 5.00 s (2H, $PhCH_2$), 5.04 br s (1H, NCH_2O), 5.14 br s (2H, $PhCH_2$), 5.46 d (*J* 3.27, 1H, NCH_2O), 7.27 (t, *J* 5.76, 1H, NHε Lys), 7.28–7.42 m (10H, $PhCH_2$), 8.39 d (*J* 7.11, 1H, NHα); MS (+ ESI-HRMS): *m/z* 620.2598 $[M + Na]^+$; $C_{31}H_{39}N_3NaO_9^+$; Calculated: 620.2579.

CONCLUSIONS

The protection of the α-carboxyl group of aspartic acid in the form of (5-oxooxazolidin-4-yl)acetic acid derivatives, while being quite attractive in terms of the high selectivity of the formation of such derivatives, has a number of limitations that must be taken into account, when they are planned for use in the synthesis of β-branched peptides. First of all, careful monitoring of the reaction products is required by measuring high-temperature NMR spectra in anhydrous solvents. In addition, in each specific case it is impossible to predict the quantity of the formed isomer [*N*-(hydroxymethylamino)-succinimide]. However, significant selectivity can be obtained in the reactions of *tert*-butyl esters, which is consistent with published data.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This article does not contain any studies involving patients or animals as test objects.

Informed consent was not required for this article.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

AUTHOR CONTRIBUTION

All authors made equal contributions to the writing of the article.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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