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The peptides of α-aminosuberic acid I
New intermediates for synthetic studies

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Summary. The paper describes the synthesis of α-aminosuberic acid derivatives suitable for the synthesis of peptides. These include Z-, Boc- and Fmoc-protection on the α-amino group, benzyl ester, Boc-hydrazide and Z-hydrazide as well as the free carboxylic function in the side chain, and methyl ester, benzyl ester or free α-carboxylic group. Their use is demonstrated on the synthesis of the respective derivatives of Asu-Val-Leu. The enzyme catalyzed reaction was succesfully used both as a route to L-Asu from the D,L-compound as well as for the direct synthesis of the optically active tripeptide derivative from the Z-D,L-Asu-OH.

Keywords: α-Aminosuberic acid – Enzymatic resolution – Peptide synthesis – Synthetic intermediates

Abbreviations: Asu: L-α-aminosuberic acid, ASU: DL-α-aminosuberic acid, Z: benzyloxycarbonyl-, Boc: *tert*-butyloxycarbonyl-, Fmoc: 9-fluorenylmethyloxycarbonyl-, Me: methyl-, Bu^t: *tert*-butyl-, Ph: phenyl, Bzl: benzyl, Nb: 4-nitrobenzyl-, Su: succinimidyl-, DCHA: dicyclohexylammonium, TLC: thin layer chromatography

Introduction

Based on a well known synthetic strategy, Treibs and Reinheckel (1956) described the first synthesis of racemic α-aminosuberic acid directly from the suberic acid. Nearly ten years later Rudinger's group in Prague (1965) prepared the L-α-aminosuberic acid from tosyl-L-glutamic acid via multiple Arndt-Eistert (1935, 1936) reaction, see also Walker (1940), while Sakakibara and coworkers (1968) synthesized the racemic form from ethyl ε-bromocapronate and ethyl acetamidomalonate with the subsequent enzymatic splitting either with takadiastase or better via a fractional salt pair

crystallization according to Vogler (1967). Both laboratories synthesized these derivatives with the intention to prepare the deamino-dicarba-analogs of cyclic cystine containing peptide factors, in those times oxytocin, vasopressin and calcitonin. As starting materials Rudinger and Jošt (1967) employed benzyloxycarbonyl-L-α-aminosuberic acid ω-methylester for the preparation of deamino-dicarba-oxytocin, while Sakakibara and coworkers (1968) used benzyloxycarbonyl-L-α-aminosuberic acid ω-*tert*-butylester prepared from either Z-Asu-OBzl, Kobayashi et al. (1969) or Z-Asu-OMe, Hase et al. (1969) and also H-Asu-OMe, Morikawa et al. (1976) in their syntheses of deamino-dicarba-8-lysin-vasopressin, deamino-dicarba-8-arginin-vasopressin, deamino-dicarba-oxytocin and the last one for the preparation of deamino-dicarba-eel-calcitonin ("ELCATONIN").

With the goal to develop a new strategy for the synthesis of several calcitonin analogs we investigated new ways of preparation of α-aminosuberic acid peptides starting with the not so expensive racemic material. In this paper we detail the first necessary step – the procedures and preparations of the derivatives of α-aminosuberic acid itself.

Results and discussion

Using the method described by Kobayashi and coworkers (1969) for the synthesis of Z-Asu-OBzl we prepared Z-ASU-OBzl and further utilized it in a preparation of Z-ASU(NHNHBoc)-OH and analogously also Boc-ASU(NHNHZ)-OH via Boc-ASU-OH, Boc-ASU-ONb and Boc-ASU(NHNHZ)-ONb. Both ω-protected hydrazide derivatives can be coupled with the dipeptide esters H-Val-Leu-OMe or H-Val-Leu-OBzl in good yields affording the tripeptides Z-ASU(NHNHBoc)-Val-Leu-OMe or Boc-ASU(NHNHZ)-Val-Leu-OBzl. The N-deprotection by the respective hydrogenolysis or acidolysis results in the amino free tripeptide esters.

The ω-esterification of the aminodicarboxylic acids according to Guttmann and Boisonnas (1958) is successful for both the D,L and L- α-aminosuberic acid when applied with a modified work-up procedure. It provided H-ASU(OBzl)-OH and H-Asu(OBz)-OH in acceptable yields and in good crystalline form when crystallized from hot acetic acid. Both these ζ-benzyl esters display rather poor solubility in solvents used in peptide synthesis, even in mixtures with water and in the presence of one equivalent of a base (NaOH, tertiary amines, quarternary bases). Under these circumstances the Schotten-Baumann acylation with various acyl donors is not successful. However, in the mixtures of tetrahydrofuran with water (especially in a ratio of 1:1) and in the presence of at least 2 equivalents of sodium carbonate, the N-acylation proceeds with excelent yield with *tert*-butyl-, benzyl- and fluorenymethyl-N-hydroxysuccinimidylcarbonate. Condensations of the N-protected ASU/Asu derivatives prepared in this manner (e.g. Boc-ASU(OBzl)-OH, Boc-Asu(OBzl)-OH, Z-Asu(OBzl)-OH, Fmoc-ASU(OBzl)-OH and Fmoc-Asu(OBzl)-OH) with the respective dipeptides H-Val-Leu-OMe, H-Val-Leu-OBu^t and H-Val-Leu-NHNH(Boc)

proceed smoothly by the Wünsch-Weygand procedure. The appropriate means of N-deprotection afford then the following tripeptide esters: a) H-ASU(OBzl)-Val-Leu-OMe and H-Asu(OBzl)-Val-Leu-OMe isolated as hydrochlorides after the acidolysis (HCl in dioxane) of the Boc derivatives; b) H-Asu-Val-Leu-OBu^t by hydrogenolysis of the Z-, ζ-benzyl derivative; c) H-ASU(OBzl)-Val-Leu-NHNH(Boc), H-Asu(OBzl)-Val-Leu-NHNH(Boc) and H-Asu(OBzl)-Val-Leu-OBu^t by morpholine splitting off the Fmoc-group. The products mentioned last were isolated with advantage together with fluorenylmethylmorpholine in quantitative yield. The following coupling step then requires addition of 1 equivalent of N-hydroxysuccinimide to neutralise the tertiary base (this will be detailed in the Part II of this series).

Our efforts towards an affordable way of obtaining L-α-aminosuberic acid from the racemate lead to a papain catalyzed synthesis of Z-Asu-NH(Ph) from Z-ASU-OH and aniline. Hydrolysis of the anilide provides the required L-α-aminosuberic acid in good yield. Next, we attempted to utilize N-acyl-ASU-OH directly in an enzyme catalyzed enantioselective synthesis of the tripeptides N-acyl-Asu-Val-Leu-OR. The positive result which we obtained demonstrates a possibility to prepare the required Asu peptides of correct configuration without a separate racemate splitting step and also without the material-costly preparation of the starting Asu intermediates. In this way we successfully prepared Z-Asu-Val-Leu-OMe, -OBzl and -ONb from Z-ASU-OH and the corresponding dipeptide esters by papain or better thermolysin catalysed condensation. (For DL-ASU compounds see Tables 1 and 2).

Materials and methods

Melting points were determined on a Totolli's capillary melting point apparatus and are uncorrected. Optical rotations were measured in a jacketed 1 dm cell on Perkin Elmer polarimeter (model 241). Prior to elemental analyses the compounds were dried under vacuum. Thin layer chromatography was carried out on Merck silica-gel 60 plates using the following eluents: A: butanol-acetic acid-water (3:1:1); B: heptane-*tert*-butylalcohol-pyridine (3:1:1); C: heptane-butanol-acetic acid-water-pyridine (7.5:15:1.8:7.2:1); D: cyclohexane-chloroform-acetic acid (45:45:10). The peptidic compounds were visualized with chlorine/tolidine and ninhydrine reagents.

Table 1. D,L-ASU derivatives

	Yield	m.p.	TLC system
Z-ASU(NHNHBoc)-OH.DCHA	76%	131–132°	B/D
Boc-ASU-OH	87%	120–121°	B/D
Boc-ASU-ONb	70%	95–96°	C/D
Boc-ASU-(NHNHZ)-ONb	90%	97–99°	D
Boc-ASU-(NHNHZ)-OH	80%	123–124°	B/D
H-ASU(OBzl)-OH	64–71%	223°	A
Boc-ASU(OBzl)-OH.DCHA	91%	103–104°	B/D
Fmoc-ASU(OBzl)-OH	96%	95–96°	B/D

Table 2. D,L-ASU-peptide derivatives

	Yield	m.p.	TLC system
Z-ASU(NHNHBoc)-Val-Leu-OMe	quant.	102–104°	D
H-ASU(NHNHBoc)-Val-Leu-OMe.HCl	96%	130° (dec)	A
Boc-ASU(NHNHZ)-Val-Leu-OBzl	79%	106–108°	D
H-ASU(NHNHZ)-Val-Leu-OBzl.HCl	97%	125° (dec)	A
Boc-ASU(OBzl)-Val-Leu-OMe	90%	oil	A
H-ASU(OBzl)-Val-Leu-OMe.HCl	85%	150°	A
Fmoc-ASU(OBzl)-Val-Leu-NHNHBoc	85%	133–135°	A/D
Fmoc-ASU(OBzl)-Val-Leu-OBu^t	88%	104–105°	A/D

Table 3. Synthesis of H-Val-Leu-X

X	N-Protection	Condensation Solvent	Yield	m.p.	$[\alpha]^{20}_D$; $[\alpha]^{20}_{546}$ (c = 1, MeOH)
OMe	Boc	CH_3CN	87%	129–130°	−53.02; −62.95
OBzl	Boc	DMF	94%	90–91°	not measured
OBu^t	Z	DMF	92%	65–67°	−47.80; −56.83
NHNHBoc	Z	Dioxane	88%	172–173°	−53.18; −63.59[a]

X	Method	Deprotection Reaction conditions	Yield	m.p.	$[\alpha]^{20}_D$; $[\alpha]^{20}_{546}$ (c = 1, MeOH)
OMe.HCl	2 M HCl/dioxane	3 h, r.t.	97%	precipitate	−6.39; −6.99
OBzl.HCl	4 M HCl/AcOH	2 h, r.t.	100%	190–192°	−18.12; −21.25
OBu^t.HCl	H_2/Pd, MeOH	titrate HCl/MeOH	97%	184–185°	−16.89; −19.72
NHNHBoc	H_2/Pd, MeOH-H_2O		90%	147–149°	−57.96; −69.27[a]

[a] optical rotation measured in ethanol.

The C-protected dipeptides H-Val-Leu-X (X = OMe, OBzl, OBu^t, NHNHBoc) were prepared by condensing the appropriate N-protected Val-OSu derivatives with the corresponding H-Leu-X components. The subsequent N-deprotection afforded the above dipeptide derivatives which were further used in the enzymatic enantiomer recognition/tripeptide synthesis. The details are summarizd in Table 3.

1. H-Asu(Bzl)-OH

Concentrated sulphuric acid (98%) was added to 100 ml of diethyl ether cooled to −50°C and then, under shaking, 100 ml of freshly distilled benzyl alcohol (aldehyde free) was added. Most of the ether was evaporated under reduced pressure and 19 g of H-Asu-OH was added. The reaction mixture was shaked until all dissolved (5 hours); the temperature rose to 20°C. The oil which separated after dilution with 1.5 l of diisopropyl ether was decanted and washed three times with diisopropyl ether. The residue was quickly dissolved in 200 ml of hot 2-propanol and the solution treated immediately with pyridine (50 ml). After cooling, the fine precipitate was filtered, dried and recrystallized from acetic acid with small amount of water. Yield 18.9–20 g (68–71%, 80–83% with respect to the starting acid recovered below), m.p. >220°C, $[\alpha]^{20}_D = +18.6°$ and $[\alpha]^{20}_{546} = 22.58°$, c = 1

(Acetic acid), (pure by TLC in system A). For $C_{15}H_{21}NO_4$ (279.34) calc. 64.49%C, 7.58%H, 5.01%N, found 64.39%C, 7.59%H, 5.05%N. The mother liquors from this experiment were evaporated to dryness, the residue dissolved in 80% acetic acid and the solution shaked with DOWEX 44 (Acetate form) 1 hour. The filtrate was immediately hydrogenolyzed on palladium catalyst. (Monitoring of the reaction by TLC in system A.) The filtrate was evaporated under reduced pressure and the residue crystallized from a small volume of 80% acetic acid yielding 3–3.3 g (15.8–17.4%) of the starting H-L-Asu-OH, $[\alpha]^{20}_D = 20.5 \pm 1°$, c = 1 (6 M HCl).

2. Boc-Asu(OBzl)-OH.DCHA

H-Asu(OBzl)-OH (11.2 g) and sodium carbonate (8.4 g) were added to 200 ml of tetrahydrofuran/water 1:1 mixture. After addition of 8.6 g Boc-OSu the reaction mixture was stirred 2 hours and diluted with water (2:3). Another portion of Boc-OSu (8.6 g) and sodium carbonate (4.2 g) was added and stirring was continued for 10 hours. After neutralization with diluted sulphuric acid tetrahydrofuran was removed under reduced pressure and extracted with ethyl acetate. The organic layer was washed as usually, dried and evaporated under reduced pressure with addition of small amount of toluene. The oily residue was dissolved in small volume of diisopropyl ether and treated with 7.3 g of dicyclohexyl amine in diisopropyl ether. After dilution with light petroleum the crystallization started. Yield 21 g (93.5%), m.p. 102–103°C, $[\alpha]^{20}_D = +9.80°$, $[\alpha]^{20}_{546} = +11.56°$, c = 1 (methanol). For $C_{32}H_{52}N_2O_6$ (560.76) calc. 68.54%C, 9.35%H, 4.99%N, found 68.37%C, 9.37%H, 5.04%N.

3. Fmoc-Asu(OBzl)-OH

H-Asu(OBzl)-OH (5.6 g) and sodium carbonate (4.25 g) were under stirring mostly dissolved in tetrahydrofuran/water 1:1 (150 ml) and treated with Fmoc-OSu (7.2 g). Stirring was continued for 12 hours and the reaction mixture was diluted with water to 1:3 THF/water ratio, evaporated to remove most THF, and acidified with diluted sulphuric acid. The oil which separated was taken in ethyl acetate, the solution washed as usually and after drying evaporated under reduced pressure. The residue crystallized from diethyl ether/light petroleum and it was recrystallized from ethyl acetate/light petroleum. Yield 9.9 g (98%), m.p. 99–100°C, $[\alpha]^{20}_D = +14.38°$, $[\alpha]^{20}_{546} = +17.04°$ c = 1 ($CHCl_3$), TLC pure in systems B and D. For $C_{30}H_{31}NO_6$ (501.58) calc. 71.84%C, 6.23%H, 2.79%N, found 71.87%C, 6.40%H, 2.66%N.

4. Z-Asu(OBzl)-OH.DCHA

H-Asu(OBzl)-OH (5.6 g) in tetrahydrofuran/water 1:1 (150 ml), sodium carbonate (4.2 g) and Z-OSu (5.5 g, 10% excess) were let to react in usual way for 12 hours. Most of the tetrahydrofuran was removed under diminished pressure and the reaction mixture acidified with sulphuric acid and exhaustively extracted with ethyl acetate. The organic layer was washed, dried and treated with ethereal solution of dicyclohexyl amine (3.6 g). The precipitate was separated by filtration and dried in vacuo. Yield 11.5 g (96%), m.p. 118–119°C, $[\alpha]^{20}_D = 10.92°$, $[\alpha]^{20}_{546} = 12.82°$ c = 1 ($CHCl_3$), TLC pure in systems B and D. For $C_{35}H_{50}N_2O_6$ (594.80) calc. 70.68%C, 8.47%H, 4.71%N, found 70.72%C, 8.65%H, 4.74%N.

5. Boc-Asu(OBzl)-Val-Leu-OMe

The oily Boc-Asu(OBzl)-OH released from 17 g of the respective DCHA salt, 8.5 g of H-Val-Leu-OMe.HCl, 4.2 ml of triethyl amine and 3.5 g of N-hydroxysuccinimide were

dissolved in 150 ml of DMFA and the solution treated at −5°C with 6.2 g of DCCI. After 12 hours and reaching 20°C the mixture was evaporated under reduced pressure and the residue worked up in the usual way. The solution of the resulting oil in diethyl ether was thoroughly extracted with sodium carbonate solution, washed with water and evaporated under reduced pressure. The oil obtained crystallized from ethyl acetate/diisopropyl ether/light petroleum. Yield 12 g (80%, calculated with respect to 3 g of the starting DCHA salt recovered from mother liquors), m.p. 92–93°C, TLC (system A), $[\alpha]^{20}_{D}$ = −47.03°, $[\alpha]^{20}_{546}$ = −56.03° c = 1 (MeOH). For $C_{32}H_{51}N_3O_8$ (605.78) calc. 63.43%C, 8.48%H, 6.93%N, found 63.42%C, 8.59%H, 6.78%N.

6. H-Asu(OBzl)-Val-Leu-OMe.HCl

11 g of the Boc-derivative was suspended in 100 ml of 3 M HCl in dioxane at 0°C and set aside 3 hours at 20°C. The mixture was evaporated under reduced pressure and the residue triturated with dry diethyl ether. The solid obtained was separated by filtration and dried thoroughly over KOH. Yield 9.6 g (98%), m.p. 190°C, TLC (system A), $[\alpha]^{20}_{D}$ = −25.5°, $[\alpha]^{20}_{546}$ = −30.2° (c = 1, methanol). For $C_{27}H_{44}N_3O_7Cl$ (542.12) calc. 59.82%C, 8.18%H, 7.75%N, found 59.58%C, 8.06%H, 7.94%N.

7. Fmoc-Asu(OBzl)-Val-Leu-NHNHBoc

5.25 g of DCCI was added at −10°C to the solution of 4.15 g H-Val-Leu-NHNHBoc, 6.03 g Fmoc-Asu(OBzl)-OH and 1.4 g N-hydroxysuccinimide in 200 ml dichloromethane and the reaction mixture was stirred at 20°C for 12 hours. The filtrate from DCHU was evaporated under reduced pressure. The residue was dissolved in 50 ml DMFA and poured under stirring to 1 L water. The precipitate was filtered, dried, stirred in 250 ml MeOH, filtered again and dried under reduced pressure. Yield 9.2 g (93%), m.p. 165–166°C, TLC (system A,D), $[\alpha]^{20}_{D}$ = −54.08°, $[\alpha]^{20}_{546}$ = −64.61° c = 1 (MeOH). For $C_{46}H_{61}N_5O_9$ (828.03) calc. 66.73%C, 7.43%H, 8.46%N, found 6.62%C, 7.51%H, 8.42%N.

8. H-Asu(OBzl)-Val-Leu-NHNHBoc

7.5 g of Fmoc-Asu(OBzl)-Val-Leu-NHNHBoc was dissolved in 100 ml morpholine. After 40 minutes at 20°C the reaction mixture was poured into 1.5 l water cooled to 0°C. The precipitate after filtration was washed with water, dried under reduced pressure and disolved in ethyl acetate. The solution was washed with water, dried with sodium sulphate, the solvent removed under reduced pressure and the residue after addition of toluen dried under reduced pressure. Yield 8 g (crude product with 1 equivalent of fluorenylmethylmorpholine), TLC (system A, D).

9. Fmoc-Asu(OBzl)-Val-Leu-OBu^t

To 5.02 g Fmoc-Asu(Bzl)-OH and 3.25 g H-Val-Leu-OBu^t.HCl in 150 ml dichloromethane triethylamine (1.4 ml) was added and then at −10°C 2.1 g DCCI. The reaction mixture was stirred 24 hours at 20°C. The filtrate after removal of DCHA was evaporated under reduced pressure and processed in the standard way. The residue was dissolved in small amount of ethyl acetate and diluted with diethyl ether/light petroleum. The voluminous precipitate was after filtration dried under very low pressure. Yield 8.15 g (98%), m.p. 143–144°C, TLC (system A, D), $[\alpha]^{20}_{D}$ = −44.29°, $[\alpha]^{20}_{546}$ = −52.95° (c = 1 MeOH). For $C_{45}H_{59}N_3O_8$ (769.99) calc. 70.20%C, 7.72%H, 5.46%N, found 70.32%C, 7.78%H, 5.52%N.

10. H-Asu(OBzl)-Val-Leu-OBu^t

5.93 g Fmoc-Asu(OBzl)-Val-Leu-OBu^t was dissolved in morpholine (50 ml), set aside for 40 minutes at 20°C and poured into 1 l water cooled to 0°C. The precipitate was washed after filtration with water and dissolved in ethyl acetate/methyl tert.butyl ether. The solution was washed with water, dried over sodium sulphate and evaporated to dryness under reduced pressure, finally with the addition of toluene. Yield 6.4 g (a mixture of the product and fluorenylmethyl-morpholine), TLC (system A).

11. Z-Asu(OBzl)-Val-Leu-OBu^t

The oily Z-Asu(OBzl)-OH released with sulphuric acid/potassium hydrogen sulphate from the DCHA salt (11.9 g) was dissolved in 80 ml DMFA and condensed in the standard way with H-Val-Leu-OBu^t.HCl (6.5 g) upon addition of triethylamine (2.8 ml), hydroxybenzotriazole (3.25 g) and water soluble carbodiimide (ECD.HCl) (4.7 g). The residue after removal of the solvent under reduced pressure was dissolved in diethyl ether/methyl *tert*.butyl ether, the solution washed with water, diluted sulphuric acid and sodium carbonate solution, dried with sodium sulphate and evaporated under reduced pressure. A sticky crystalline mass was obtained from diethyl ether/light petroleum. Yield 13 g, TLC (system A, D).

12. H-Asu-Val-Leu-OBu^t

The crude Z-Asu(OBzl)-Val-Leu-OBu^t (13 g) was hydrogenolyzed in the usual way in 300 ml of 90% aqueous MeOH with several drops of AcOH. The filtrate after removal of catalyst was diluted with large volume of diethyl ether and the voluminous and slightly hydroscopic product was after filtration dried under reduced pressure. Yield 8.6 g (90.5%, calc. for monohydrate and both steps), m.p. 195°C, TLC (system A), $[\alpha]^{20}_{D} = -28.66°$, $[\alpha]^{20}_{546} = -34.32°$ (c = 1 AcOH). For $C_{23}H_{43}N_3O_6$ (475.63) calc. 58.08%C, 9.54%H, 8.84%N, found 58.20%C, 9.26%H, 8.76%N.

13. Z-Asu-Val-Leu-OMe

H-Val-Leu-OMe.HCl (0.85 g, 3 mMol) was added to the solution of Z-ASU-OH (1.62 g, 5 mMol) in the mixture of 8.5 ml 1 M NaOH and 1.5 ml water. After addition of 10 mg $CaCl_2.2H_2O$ and 20 mg thermolysin the reaction mixture was stirred at pH 6.5 24 hours; during this time the product precipitated. The precipitation was supported by adding 1 M HCl and the product was washed with 1 M HCl and water. Its solution in ethyl acetate was washed with 0.5 M sodium acetate buffer pH 6.0, water, dried with sodium sulphate and evaporated to a small volume. The product crystallized from ethyl acetate/light petroleum. Yield 1.14 g (83%), m.p. 153–154°C, $[\alpha]^{20}_{D} = -50.3°$ (c = 0.48 MeOH). For $C_{28}H_{43}N_3O_8$ (549.7) calc. 61.18%C, 7.89%H, 7.64%N, found 61.30%C, 8.04%H, 7.71%N. $(M + H)^+ = 550.6$.

14. Z-Asu-Val-Leu-OBzl

Solution of Z-ASU-OH (0.26 g, 0.8 mmol) in a mixture of 2 M NaOH (0.6 ml) and 0.2 M sodium acetate buffer pH 6 containing 0.05 M calcium chloride (0.4 ml) was mixed with the solution of HCl.Val-Leu-OBzl (0.285 g, 0.8 mmol) in a mixture of dimethylformamide (0.6 ml) and 0.2 M sodium acetate buffer pH 6 containing 0.05 M calcium chloride (0.4 ml). After the addition of thermolysin (5 mg) the mixture was stirred at

room temperature 24 h, during which time the product precipitated. 1 M HCl was added to complete precipitation of the product and the precipitate was washed with 1 M HCl and water. Product was dissolved in ethyl acetate and washed extensively with the solution of 0.5 M sodium acetate buffer pH 6 and water. After drying with sodium sulfate and evaporation, the residue was crystallized from ethyl acetate-light petroleum. Yield 0.15 g (60%), m.p. 130°–132°C, $[\alpha]^{20}_D = -53.4°$ (c = 0.25 MeOH). For $C_{34}H_{47}N_3O_8$ (625.8) calc. 65.26%C, 7.57%H, 6.71%N, found 65.25%C, 7.66%H, 6.72%N. $(M + H)^+ =$ 626.6.

15. Z-Asu-Val-Leu-ONb

Solution of Z-ASU-OH (0.324 g, 1 mmol) in a mixture of 2 M NaOH (0.75 ml) and 0.2 M sodium acetate buffer pH 6 containing 0.05 M calcium chloride (0.5 ml) was mixed with the solution of HCl.Val-Leu-ONb (0.402 g, 1 mmol) in a mixture of dimethylformamide (0.75 ml) and 0.2 M sodium acetate buffer pH 6 containing 0.05 M calcium chloride (0.5 ml). After the addition of thermolysin (6 mg) the mixture was stirred at room temperature for 24 h, during which time the product precipitated. 1 M HCl was added to complete precipitation of the product and the precipitate was washed with 1 M HCl and water. Product was dissolved in ethyl acetate and washed sequentially with 1 M HCl, water, 0.5 M sodium acetate buffer pH 6 and water. After drying with sodium sulfate and evaporation, the residue was crystallized from ethyl acetate/light petroleum. Yield 0.186 g (55.5%), m.p. 122°–124°C, $[\alpha]^{20}_D = -47.32°$ (c = 0.42 MeOH). For $C_{34}H_{46}N_4O_{10}$ (670.8) calculated; 60.88%C, 6.91%H, 8.35%N, found 60.74%C, 6.90%H, 8.33%N. $(M + H)^+ =$ 671.3.

16. Z-Asu-NH(Ph)

44.7 g of Z-ASU-OH was added to 1 M NaOH (140 ml) and then water (111 ml), borate buffer pH 5 (111 ml), L-cysteine (1.28 g), papaine (7.77 g) and aniline (25.5 ml) were also added. The reaction mixture was kept for 40 hours at 37°. The product which separated was after filtration dissolved in ethyl acetate (600 ml) at 70°, cooled, washed with 1 M HCl (100 ml), filtered, and washed successively with 1 M HCl (2 × 100 ml) and water (400 ml). The organic layer was dried with magnesium sulphate and evaporated to dryness. Yield 19.12 g.

17. H-Asu-OH

19.12 g of Z-Asu-NH(Ph) was refluxed with 230 ml 6 M HCl. It was let sit overnight and refluxed another 2 hours until all dissoved. 1.3 g of charcoal was added, the solution was refluxed 30 more minutes, filtered and evaporated under reduced pressure. The yellow residue was dissolved in water and washed with ethyl acetate. The solution was adjusted to pH 3 by addition of concentrated ammonia and cooled. The product which separated was after filtration dried under low pressure. Yield 8.6 g (90%), $[\alpha]^{20}_D = +20.6 \pm 1°$, $[\alpha]^{20}_{546} = +24.8°$ c = 1 (6 M HCl).

References

Arndt F, Eistert B (1935) Ein Verfahren zur Überführung von Carbonsäuren in ihre höheren Homologen bzw. deren Derivate. Ber 68: 200–208

Arndt F, Eistert B (1936) Bemerkungen zur unserem Verfahren zur Überprüfung von Carbonsäuren in ihre Homologen und zu Arbeiten von Preobrashenski und Mitarbeitern. Ber 69: 1805–1806

Farkašová H, Rudinger J (1965) Amino acids and peptides LVII. A synthesis of L-α-aminopimelic acid and L-α-aminosuberic acid. Coll Czech Chem Commun 30: 3117–3122

Guttmann S, Boissonnas RA (1958) Synthèse du N-acétyl-L-séryl-L-tyrosyl-L-séryl-L-méthionyl-γ-L-glutamate de benzyle et de peptides apparentés. Helv Chim Acta 41: 1852–1867

Hase S, Kiyoi R, Sakakibara S (1968) Synthesis of DL-α-aminosuberic acid and its optical resolution. Bull Chem Soc Japan 41: 1266–1267

Hase S, Morikawa T, Sakakibara S (1969) Synthesis of a biologically active analog of deamino-8-arginine-vasopressin which does not contain a disulphide bond. Experientia 25: 1239–1240

Jošt K, Rudinger J (1967) Amino acids and peptides LXIX. Synthesis of two biologically active analogues of deamino-oxytocin not containing a disulphide bond. Coll Czech Chem Commun 32: 1229–1241

Kobayashi A, Hase S, Kiyoi R, Sakakibara S (1969) Oxytocin analogues containing no disulfide bond I. Synthesis of the lactam of L-tyrosyl-L-isolecucyl-L-glutaminyl-L-asparaginyl-L-α-aminosuberyl-L-prolyl-L-leucylglycine amide. Bull Chem Soc Japan 42: 3491–3495

Lane JF, Wallis ES (1941) Molecular rearrangements involving optically active radicals. X. The retention of configuration in the Wolf rearrangement. J Am Chem Soc 63: 1674–1676

Morikawa T, Munekata E, Sakakibara S, Noda T, Otani M (1976) Synthesis of Eel-calcitonin and [Asu^{1,7}]-Eel-calcitonin: contribution of the disulfide bond to the hormone activity. Experientia 32: 1104–1106

Sakakibara S, Hase S (1968) Synthesis of an analogue of desamino-lysine-vasopressin containing no disulfide bond. Bull Chem Soc Japan 41: 2816

Treibs W, Reinheckel H (1956) Synthesen mit Dicarbonsäuren, XVII. Mitteil. Reduktion von Oximino-Dicarbonsäureestern mittels Lithiumaluminiumhydrid zu Aminoalkoholen. Ber 89: 51–57

Vogler K, Lanz P (1966) L-Tyrosinhydrazid als optisch aktive Base zur Spaltung von DL-Prolin, DL-Alanin und DL-Isoleucin in die optischen Antipoden. Helv Chim Acta 49: 1348–1354

Walker J (1940) The synthesis of ωω′-Bis-2′-amino-4′-thiazolylalkanes and N^4-2′-thiazolylsulphanilamides. J Chem Soc: 1304–1307

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