Tetrahedron Vol. 39, No 24, pp. 4121 to 4126, 1983
Printed in Great Britain.

0040-4020/83 $3.00 + .00
Pergamon Press Ltd.

STUDIES ON AMINO ACIDS AND PEPTIDES - VII[+]
SYNTHESES OF ASPARTAME AND THIOASPARTAME

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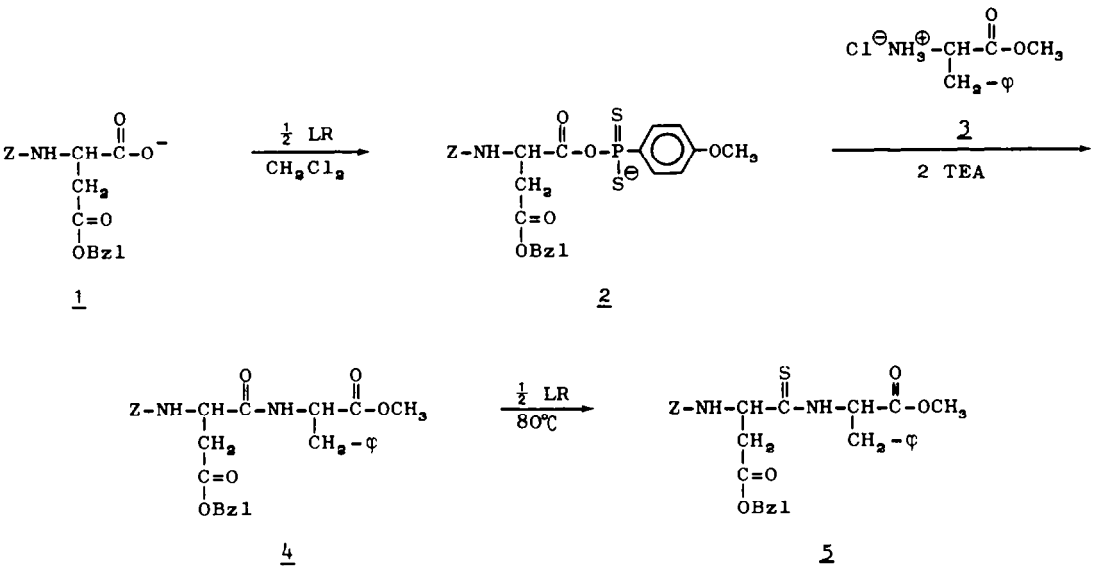
Abstract - The protected aspartame, 4, has been prepared from the benzyl ester of N-benzyloxycarbonyl-S-aspartic acid, 1, and the methyl S-phenylalanate, 3, using 2,4-bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane 2,4-disulfide, LR, as a coupling reagent. Another protected aspartame, 7, has been prepared from the tert-butyl ester of 1-[N-benzyloxycarbonyl-α-S-aspartyloxyl]-succinimide, 6, and methyl S-phenylalanate, 3. Thiations of 4/7 by LR produces a protected thioaspartame, 5/9. Deprotection of 7 and 9 gives aspartame, 8, and the slightly sweet thioaspartame, 10, in high yields.

The dipeptide sweetener, aspartame,[1] 8, was first synthesized in 1966,[2] and since the discovery of its sweet taste numerous studies have been carried out on methods of syntheses of 8 and other sweeteners.[3] Besides this, much synthetic work has been undertaken to understand the molecular basis for the sweet taste. For instance M. Goodman et al.[4-6] have made series of aspartame analogs, where the amide function has been modified. In connection with our investigations of peptide surrogates and our general studies of thiopeptides,[7-9] thioaspartame, 10, has now been prepared (the amide function has been converted to a thioamide function). Besides being a thiation reagent, LR has been used as a coupling reagent in peptide syntheses.[10-18] The protected aspartame and thioaspartame have now been synthesized, using LR as coupling- and thiation reagent. This present paper reports on a new synthetic way of preparing aspartame, and the synthesis of the until now unknown thioaspartame.

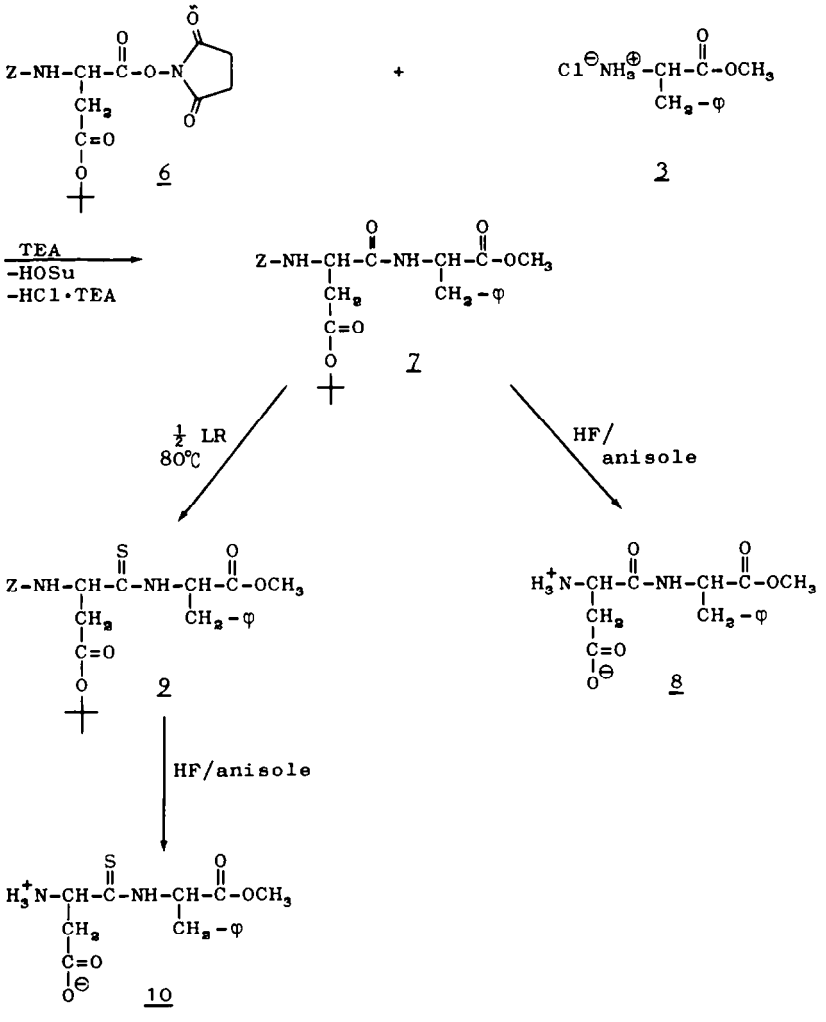
RESULTS

The protected aspartame was made in two different ways, namely a) by use of the novel racemization free coupling reagent LR[10,11] and b) by the active ester method. In method a) one mole of the N- and β-protected aspartic acid, 1, was reacted with triethylamine (TEA) in anhydrous CH_2Cl_2 at room temperature to give the salt of 1. Half a mole of LR was added at room temperature, and the intermediate, 2, was formed. The mixture was cooled to -60°C, and one mole of methyl S-phenylalanate hydrochloride, 3, and two moles of TEA were added. The temperature was allowed to rise to room temperature, and the mixture was stirred overnight, giving the protected aspartame, 4 in high yield (Scheme 1a and Table 1). The structural proof

[+]Part VI, see Ref. 24.



Scheme 1a



Scheme 1b

of the product was based on NMR, IR, UV and micro analysis. ^1H and ^{13}C NMR chemical shifts of the backbone, the β-hydrogens and the β-carbons are presented in Table 2 and the UV-data in Table 1. The protected aspartame, 4, was reacted with LR in anhydrous benzene at 80°C giving the corresponding protected endothiopeptide, 5. The structural proof of the product, 5, was based on NMR, UV (see Table 2 and 1), IR and micro analyses. It should be noted that LR selectively transformed the amide function to a thioamide function without affecting the carbonyls of the urethane functions and the ester functions. This was expected, since urethane[13] and ester[14] functions do not react with LR at 80°C but at 140°C.

In method b) the tert-butyl ester of 1-[N-benzylyloxycarbonyl-α-S-aspartyloxy]-succinimide, 6, was reacted with TEA and methyl S-phenylalanate hydrochloride, 3, in anhydrous dimethoxyethane at room temperature to give the protected aspartame, 7, in high yield. (Scheme 1b and Table 1). The structural proof of the product was based on NMR, UV (Table 2 and 1), IR and micro analyses.

The deprotections of the protected aspartame and the protected thioaspartame were made by use of the HF method. Earlier work[15-18] showed that this method indeed effected removal of the protecting groups without affecting the thioamide function.

EXPERIMENTAL

^1H NMR spectra were recorded at 60 MHz on a Varian EM-360 spectrometer. ^{13}C NMR were recorded at 20 MHz on a Varian CFT-20 spectrometer. $CDCl_3$ and D_2O were used as solvents, and chemical shifts are reported in ppm on the δ-scale, referenced to internal TMS or DDS as 0 ppm for ^1H NMR and to the $CDCl_3$ solvent as 77.0 ppm in ^{13}C NMR. IR spectra were recorded on a Beckman IR-18 spectrophotometer. UV spectra were recorded on a Perkin-Elmer 402 spectrophotometer. Mass spectra were recorded on a Micromass 7070 F spectrometer operating at 70 eV using direct inlet. Micro analyses were carried out by Løvens Kemiske Fabrik, DK-2750 Ballerup (Microanalytical Laboratory). Optical rotations were measured in a 1 dm cell in a Perkin-Elmer 241 polarimeter. Silica gel 60 (Merck) was used for chromatography. M.p.'s are uncorrected. LR (available from Fluka, Merck-Schuchardt, Aldrich Chemicals Co. and Riedel de Haen) was prepared as described earlier.[19] Samples for amino acid analysis were prepared by hydrolysis with 6 M HCl at 110°C for 16-20 hr (with 0.1% phenol added) and analysed on a Beckman 121 MB amino acid analyser.

Starting Materials

Methyl S-phenylalanate hydrochloride was prepared by the Fischer method[20] and the tert-butyl ester of 1-[N-benzyloxycarbonyl-α-S-aspartyloxy]-succinimide was purchased from Fluka. N-Benzyloxycarbonyl-β-benzyl-S-aspartate[3] was prepared by partial hydrolysis of the dibenzyl ester of benzyloxycarbonyl S-aspartic acid.[3]

Z-Asp(OBzl)-Phe-OMe, 4. A mixture of 3.57 g (0.01 mole) of the β-benzylester of N-benzyloxycarbonyl-S-aspartic acid and 1.01 g (0.01 mole) TEA in anhydrous CH_2Cl_2 was stirred for 5 min at room temperature. 2.02 g (0.005 mole) LR were added in small portions (exothermic reaction) and stirred until a clear solution was obtained. After cooling to about -60°C, 2.16 g (0.01 mole) of methyl S-phenylalanate hydrochloride were added to the mixture. Finally 2.02 g (0.02 mole) TEA were added dropwise to the mixture. The reaction mixture was stirred overnight, while the mixture was allowed to obtain room temperature. The reaction mixture was applicated directly to a silica gel column and the product was eluated in 10% ether/CH_2Cl_2 yielding 3.86 g (74%) of 4. Anal.: Found (Calc.): C, 67.03 (67.18); H, 5.79 (5.79); N, 5.36 (5.41).

Z-Aspt(OBzl)-Phe-OMe, 5. 3.00 g (5.6 mmole) of the protected aspartame, 4, and 1.17 g (0.028 mole) LR were heated in 15 ml anhydrous benzene at 80°C for 2 hr. After evaporation of the solvent the residue was chromatographed on a silica gel column in 50% CH_2Cl_2/ether, which after evaporation of the solvent yielded the product in 2.86 g (93%) yield, as a slightly yellow oil. Anal.: Found (Calc.): C, 65.15 (65.16); H, 5.75 (5.66); N, 5.22 (5.24).

Z-Asp(OtBu)-Phe-OMe, 7. The tert-butyl ester of 1-[N-benzyloxycarbonyl-α-S-aspartyloxy]-succinimide 2.73 g (6.5 mmole), 6, was mixed with a solution of methyl S-phenylalanate hydrochloride 1.40 g (6.5 mmole), 3. TEA 0.66 g (6.5 mmole) in 15 ml dry dimethoxyethane. The mixture was stirred at room temperature for 1 3/4 hr, filtered and evaporated under gentle heating. The residue was dissolved in CH_2Cl_2 and washed 4 times with water. The organic phase was dried ($MgSO_4$), and the solvent evaporated to give 3.14 g (100%) of crude solid, which was chromatographed on a silica gel column first in 100% ether, then

in 50% ether/PE yielding 2.94 g (93%) of the protected aspartame, 7. Anal.: Found (Calc.): C, 64.27 (64.45); H, 6.76 (6.66); N, 5.59 (5.78).

H-Asp-Phe-OMe, 8. The Z- and OtBu-protecting groups were cleaved simultaneously by using liquid HF with anisole added as a scavenger.

To a mixture of 0.44 g (0.9 mmole) of 7 and approximately 1.2 ml (12 mmole) anisole in a 100 ml polyethene-bottle were added 9 ml anhydrous HF at 0°C, and the mixture was kept at 0°C for 30 min. The HF was removed using a plastic water-suction pump. The oily residue was treated with 50 ml ether, whereupon the product solidified. The ether was decanted and the residue washed with 3 x 25 ml ether. The solid material was transferred to a 100 ml round bottomed flask by using MeOH, and the solvent was evaporated. Yield: 0.26 g (98%) of the sweetener aspartame, 8, which was recrystallized from water. Anal.: Found (Calc.): C, 64.27 (64.45); H, 6.76 (6.66); N, 5.59 (5.78).

Z-Aspt(OtBu)-Phe-OMe, 9. 1.26 g (2.6 mmole) 7 and 0.526 g (1.3 mmole) LR were heated in 14 ml anhydrous benzene at 80°C for 1 1/4 hr. After evaporation of the solvent the residue was chromatographed on a silica gel column in CH_2Cl_2. Eluation was continued with ether, and evaporation of the solvent yielded 1.20 g (92%) of a coloured oil. Anal.: Found (Calc.): C, 62.34 (62.38); H, 6.54 (6.45); N, 5.40 (5.60).

H-Aspt-Phe-OMe, 10. The protected thioaspartame, 9, was deprotected by liquid HF in anisole. The deprotection yielded 87% crude product, which was purified on HPLC. Precise mass measurement of $M-NH_3$: Found (Calc.): 293.07214 (293.07217).

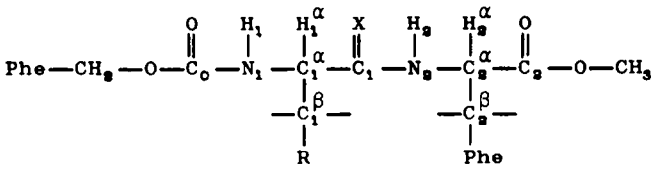
SPECTROSCOPIC SECTION

In ^1H NMR it was generally observed that going from carbonyl to thiocarbonyl compounds causes a downfield shift of the hydrogen attached to the corresponding amide nitrogen. All the other hydrogens were almost unaffected. In ^{13}C NMR the thiocarbonyl carbons appeared to resonate about 31 ppm downfield relative to the corresponding carbonyls. The corresponding change in shift values of the appropriate α-carbon was 5.76-7.27 ppm and for the appropriate $C_{2\beta}$-carbon the changes are 3.32-5.27 ppm. In IR absorptions were obsefved in the region 3300-3600 cm^{-1} (N-H stretching). Carbonyl absorptions were seen in the regions 1660-1670 cm^{-1} (amide I), 1720-1750 cm^{-1} (ester). The thiocarbonyl-containing compounds showed UV absorptions in the ranges 265-270 nm, and with log ε values from 3.98-4.24.[23] The mass spectra all showed the molecular ion $[M]^+$, with the base peak in all spectra being $[C_7H_7]^+$. The tert-butyl esters showed $[M-56]^+$ and $[M-73]^+$ corresponding to loss of 2-methylpropene, followed by loss of OH. The endothiopeptides showed the fragment ion $[M-SH]^+$.

Table 1. Experimental and Physical Data of Starting Materials and Products

Compound	Yield (%)	M.p.(°C) Found	M.p.(°C) Reported	$[\alpha]_D^{20}$ Found	$[\alpha]_D^{20}$ Reported	UV(EtOH) (nm)	UV(EtOH) log ε
4	74	116.7	116-17[8]	-13.00 c1,DMF	-15.3[8], -14.8[21] c1,DMF	210	-
5	93	oil	-	+23.6 c1,MeOH	-	210 267	- 4.07
7	93	71-73	-	-16.2 c1,MeOH	-	212	-
8	98	238-42	235-36[22]	+34.0 c1,AcOH	+32.0[22] c1,AcOH	210	
9	92	oil	-	+19.6 c1,MeOH	-	209 268	- 4.24
10	87	amorph	-	-	-	207 267	 3.98

Table 2. ^{1}H and ^{13}C NMR Data of Starting Materials and Products



X = O, S

^{1}H NMR ($CDCl_3$)						
Compound	H_1	$H_1\alpha$	$H_1\beta$	H_2	$H_2\alpha$	$H_2\beta$
4	5.70 b	4.20- 4.80 b	2.90 (d,6)	6.70 b	4.20- 4.80 b	2.70 b
5	6.00 (d,9)	5.20 (d,7)	3.10 (d,6)	8.60 b	4.70 m	2.90 (t,6,6)
7	5.70 (d,8)	5.00 s	2.85 (d,6)	6.85 s	4.40 m	2.50 (t,4,6)
8**	*	4.60 (t,6,6)	2.90 (t,6,6)	*	4.25 (t,6,6)	2.90 (t,6,6)
9	5.95 (d,8)	5.10 s	3.10 (d,7)	8.65 b	4.65 m	2.70 (t,4,6)

^{13}C NMR ($CDCl_3$)							
Compound	C_0	$C_1\alpha$	$C_1\beta$	C_1	$C_2\alpha$	$C_2\beta$	C_2
4	155.72	50.87	35.97	169.86	53.31	37.51	17.79
5	155.44	58.14	36.13	201.65	56.63	39.29	71.8
7	155.65	50.92	37.16	171.15	53.28	37.49	170.08
8**	-	58.80	40.88	176.94	56.86	38.26	178.19
9	155.39	56.68	40.32	201.98	58.55	36.13	170.32

* Not seen, because of proton exchanges.

** ^{1}H and ^{13}C NMR in D_2O, TFA.

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