Amino Acids (1995) 8: 109–112

Amino
Acids

Synthesis of sweet DOPA peptide and its analogues

Short Communication

Ł. Nakonieczna, W. Przychodzeń, and **A. Chimiak**

Faculty of Organic Chemistry, Technical University of Gdańsk, Gdańsk, Poland

Accepted April 26, 1994

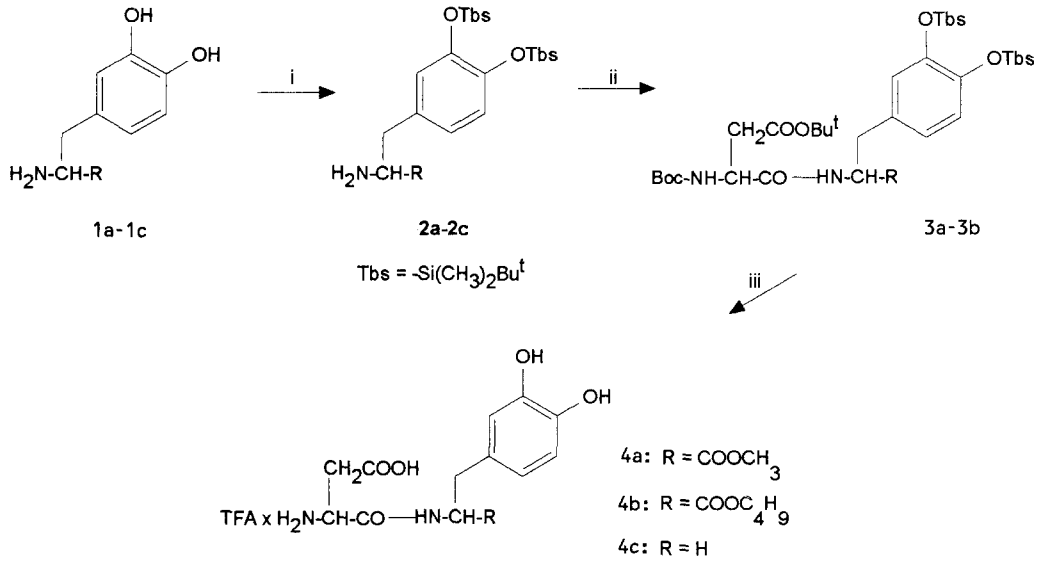
Summary. Asp-DOPA-OMe, Asp-DOPA-OnBu, and Asp-dopamine were prepared according to the new general procedure described for DOPA dipeptides.

Keywords: Amino acids – DOPA – Dopamine – Dipeptides – Sweetener

Because of the known effect of catecholamines on transduction of sense (including glucose-sensitive neurons) (Karadi et al., 1992) it is very promising to develop synthetic systems possessing both sweetener structure and catecholamine moiety. The well-known easiness of oxidation becomes difficult when working with DOPA and other catechols. Obviously, the phenolic groups must be protected during synthesis. L-Aspartyl-DOPA-OMe (**4a**) – the structural analogue of aspartame (Asp-Phe-OMe) – has previously been prepared (MacDonald et al., 1980) by coupling β-benzyl-*N*-Z-aspartate to DOPA-OMe × HCl in the presence of DCCI/HOBt. This procedure resulted in an impure, coloured material with erroneous elemental analyses (the carbon value was 3.18%) after the deprotection step.

In our recent paper, (Nakonieczna et al., 1992), we showed that the use of a *t*-butyldimethylsilyl protecting group provided a convenient preparative method for obtaining pure N- and C-terminal DOPA peptides. We involved *t*-butyldimethylsilyl protected DOPA esters and dopamine, coupling them with β-*t*-butyl-*N*-Boc-aspartate in the presence of a BOP reagent which gave fully protected dipeptides (**3a**), (**3b**) and dopamine amide (**3c**). The final and complete deprotection using 95% TFA (a common reagent in SPPS) provided pure dipeptide esters (**4a**), (**4b**) and N-aspartyldopamine (**4c**) as trifluoroacetate salts – aspartame analogues.

Sweetness tests showed that only substance **4a** had a moderately sweet taste, 2–3 times sweeter than sucrose. Substance **4b** was tasteless and substance **4c** had a sour taste.



[i] TbsCl/DBU/MeCN. [ii] BocAsp(OBu^t)OH/BOP/CH_2Cl_2. [iii] 95% TFA

Materials and methods

Optical rotations were measured on a Carl-Zeiss Polamat A polarimeter at 20°C. TLC was performed on silica gel plates (Merck, Darmstadt, Germany). Molecular weights were confirmed by mass spectrometry (LSIMS) using 3-nitrobenzyl alkohol matrix (AMD 604 apparatus, AMD Intectra GmbH, Germany). ^{1}H NMR spectra were recorded on a Varian EM 360 (60 MHz) spectrometer with TMS as external standard. L-DOPA and dopamine hydrobromide (**1c**) were purchased from Aldrich (Steinheim, Germany). *β-t*-butyl-*N*-(*t*-butoxycarbonyl)-L-aspartate was obtained from Sigma (St. Louis, USA).

L-3,4-dihydroxyphenylalanine methyl and n-butyl ester hydrochlorides (**1a** and **1b**) were prepared according to the procedure described in literature (Banerjee, 1976). 3,4-bis(*tert*-butyldimethylsilyloxy)-L-phenylalanine methyl ester (**2a**), n-butyl ester (**2b**) and 3,4-bis(*tert*-butyldimethylsilyloxy)phenethylamine (**2c**) were obtained by the action of *tert*-butyldimethylsilylchloride on (**1a**), (**1b**), and dopamine, respectively, in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (Nakonieczna, 1994). Equivalents for sweetness were establish by means of ranking tests with 5 judges. The taste intensities of aqueous solutions were related to a 8% sucrose solution.

N-Boc-β-t-butyl-L-aspartyl-L-3,4-bis(t-butyldimethylsilyloxy)-L-phenylalanine methyl ester (**3a**)

To a solution of *N*-Boc-*β-t*-butyl-L-aspartate (0.291 g, 1 mmol), the silyl protected DOPA methyl ester (**2a**) (0.442 g, 1 mmol *N*-methyl morpholine (0.22 mL, 2 mmol) in dichloromethane (8 mL), BOP reagent (0.442 g, 1 mmol) was added at room temperature. The mixture was stirred for 48 h. Removal of the solvent under reduced pressure gave an oil, which was taken up in ethyl acetate. The organic layer was washed with 0.1 M citric acid, water, aqueous 5% $NaHCO_3$ solution, water, and brine. After drying ($MgSO_4$), the solution was concentrated to give the crude product (**3a**) (0.57 g, 81%) as an oil. It was purified by silica gel column chromatography (chloroform). Yield 0.33 g (46%), colourless oil: CHN Found C, 59.25; H, 9.14; N, 4.31%). Calcd. for $C_{35}H_{62}N_2O_9Si_2$ C, 59.12; H, 8.79; N, 3.94%. $[\alpha]_D$ +30 ($CHCl_3$, c 1). ^{1}H NMR (CCl_4, δ): 0.10 (s, 12H, CH_3-Si), 0.90 (s, 18H, CH_3C-Si),

1.32 (s, 9H, Boc), 1.36 (s, 9H, OBu^t), 2.3–3.0 (2 × m, 4H, β-CH_2), 3.58 (s, 3H, OCH_3), 4.1–4.8 (2 × m, 2H, α-CH), 6.52 (m, 3H, Ar-H). m/z (LSIMS): 711(MH, 27%), 555([M-Boc-Bu^t]$^+$, 100%).

N-Boc-β-t-butyl-L-aspartyl-L-3,4-bis(t-butyldimethylsilyloxy)-L-phenylalanine n-butyl ester (**3b**)

A procedure similar to that used for (**3a**) provided 0.64 g (89%) of the crude product (**3b**) which was purified by silica gel column chromatography (chloroform). Yield 0.54 g (77%), colourless oil: CHN Found C, 60.26; H, 9.37; N, 4.17%). Calcd. for $C_{38}H_{68}N_2O_9Si_2$ C, 60.60; H, 9.10; N, 3.72%. $[\alpha]_D$ +22.4 ($CHCl_3$, c 1.4). ^{1}H NMR (CCl_4, δ): 0.13 (s, 12H, CH_3-Si), 0.90 (s, 21H, CH_3C-Si + CH_3-C), 1.35, 1.38 (2 × s, 22H, Boc + OBu^t + C-CH_2), 2.5–3.0 (2 × m, 4H, β-CH_2), 4.0 (t, 2H, OCH_2), 4.5 (2 × m, 2H, α-CH), 6.52 (m, 3H, Ar-H). m/z (LSIMS): 753(MH, 9%), 597([M-Boc-Bu^t]$^+$, 100%)

N-Boc-β-t-butyl-L-aspartyl-L-3,4-bis(t-butyldimethylsilyloxy)-phenethylamine (**3c**)

A procedure similar to that used for (**3a**) and (**3b**) provided 0.52 g (80%) of the crude product (**3c**) which was purified by silica gel column chromatography (chloroform). Yield 0.44 g (67%), colourless oil: CHN Found C, 60.53; H, 9.63; N, 4.26%). Calcd. for $C_{33}H_{60}N_2O_7Si_2$ C, 60.69; H, 9.26; N, 4.29%. $[\alpha]_D$ +9.8 ($CHCl_3$, c 4.2). ^{1}H NMR (CCl_4, δ): 0.13 (s, 12H, CH_3-Si), 0.93 (s, 18H, CH_3C-Si), 1.38 (s, 9H, Boc), 1.43 (s, 9H, OBu^t), 2.63 (2 × t, 4H, CH_2), 3.4 (q, 2H, CH_2N), 4.3 (m, 1H, α-CH), 5.7 (m, NH), 6.63 (m, 3H, Ar-H).

L-Aspartyl-L-DOPA-OMe (**4a**), *L-aspartyl-L-DOPA-OnBu* (**4b**), *and L-aspartyl-dopamine* (**4c**) *trifluoroacetates*

(**3a**), (**3b**) or (**3c**) (0.25 g) was treated with 95% TFA (2 mL). After 16 h NMR indicated completion, and the solution was taken to dryness. The residue (light foam) was dissolved in water (1.5 mL) and the aqueous layer was washed with ethyl acetate (0.5 mL) to give clear pale yellow solution of the product (**4a–c**). It moved as a single spot on TLC (BuOH/AcOH /H_2O/Py 18:3:12:10).

(**4a**): R_f 0.42; m/z(LSIMS): 330([M + 2D-TFA]$^+$, 25%); ^{1}H NMR (D_2O, δ): 2.85, 2.86 (2 × t, 4H, β-CH_2), 3.47 (s, 3H, OCH_3), 4.0–4.5 (m, 2H, α-CH), 6.33–6.67 (m, 3H, Ar-H).

(**4b**): R_f 0.60; m/z(LSIMS): 372([M + 2D-TFA]$^+$, 12%); ^{1}H NMR (D_2O, δ): 0.57–1.67 (m, 7H, $CH_3CH_2CH_2$), 2.85, 2.88 (2 × t, 4H, β-CH_2), 3.73–4.57 (m, 4H, α-CH + OCH_2), 6.36–6.73 (m, 3H, Ar-H).

(**4c**): R_f 0.39; m/z(LSIMS): 272([M + 2D-TFA]$^+$, 20%); ^{1}H NMR (TFA, δ): 2.1–2.8 (m, 4H, CH_2), 4.2 (m, 1H, α-CH), 6.4 (m, 3H, Ar-H), 7.0 (m, NH).

Acknowledgement

We thank Dr. J. Grabowska for carrying out the sweetness tests. This work was partly supported by a grant 936055-008 from the State Committee for Scientific Research (KBN), Poland.

References

Banerjee N, Ressler C (1976) Derivatives of 3,4-dihydroxyphenylalanine for peptide synthesis. J Org Chem V 41: 3056–3058

Karadi Z et al (1992) Responses of lateral hypothalmic glucose-sensitive and glucose-insensitive neurons to chemical stimuli in behaving rhesus monkeys. J Neurophysiol V 67: 389–400

MacDonald SA, Willson CG, Chorev M, Vernacchia FS, Goodman M (1980) Peptide sweeteners. 4. Hydroxy and metoxy substitution of the aromatic ring in L-aspartyl-L-phenylalanine methyl ester. Structure – taste relationships. J Org Chem V 23: 420–424

Nakonieczna Ł, Przychodzeń W, Chimiak A (1992) New stable t-butyldimethylsilyl ethers of 3,4-dihydroxyphenyl-L-alanine for DOPA-peptide synthesis. In: Schneider CH, Eberle AN (eds) Proceedings of the twenty-second european peptide symposium, Interlaken, Switzerland 1992. ESCOM, Leiden (Peptides 1992, pp 194–195)

Nakonieczna Ł, Przychodzeń W, Chimiak A (1994) Liebigs Ann Chem (in press)

Authors' address: Prof. A. Chimiak, Faculty of Organic Chemistry, Technical University of Gdańsk, Narutowicza 11/12, PL-80-952 Gdańsk, Poland.

Received February 15, 1994

Verleger: Springer-Verlag KG, Sachsenplatz 4–6, A-1201 Wien. – Herausgeber: Prof. Dr. Gert Lubec, Universitäts-Kinderklinik, Währinger Gürtel 18–20, A-1090 Wien. – Redaktion: Sachsenplatz 4–6, A-1201 Wien. – Satz und Umbruch: Asco Trade Typesetting Limited, Hong Kong; Offsetdruck: Novographic, Ing. W. Schmid, Maurer-Lange-Gasse 64, A-1238 Wien. – Verlagsort: Wien – Herstellungsort: Wien. Printed in Austria.

Offenlegung gem. §25 Abs. 1 bis 3 Mediengesetz: Unternehmensgegenstand: Verlag von wissenschaftlichen Büchern und Zeitschriften. An der Springer-Verlag KG ist beteiligt: Dr. Konrad F. Springer, Sachsenplatz 4-6, A-1201 Wien, als Kommanditist zu 52,38%. Geschäftsführer: Dr. Konrad F. Springer, Prof. Dr. Dietrich Götze, Ing. Wolfram F. Joos, Dipl.-Kfm. Claus Michaletz und Rudolf Siegle, alle Sachsenplatz 4-6, A-1201 Wien.