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Methods for syntheses of *N*-methyl-*DL*-aspartic acid derivatives

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Summary. A novel practical method for the synthesis of *N*-methyl-*DL*-aspartic acid **1** (NMA) and new syntheses for *N*-methyl-aspartic acid derivatives are described. NMA **1**, the natural amino acid was synthesized by Michael addition of methylamine to dimethyl fumarate **5**. Fumaric or maleic acid mono-ester and -amide were regioselectively transformed into beta-substituted aspartic acid derivatives. In the cases of maleamic **11a** or fumaramic esters **11b**, the α-amide derivative **13** was formed, but hydrolysis of the product provided *N*-methyl-*DL*-asparagine **9** via base catalyzed ring closure to *DL*-α-methylamino-succinimide **4**, followed by selective ring opening. Efficient methods were developed for the preparation of NMA-α-amide **13** from unprotected NMA via sulphinamide anhydride **15** and aspartic anhydride **3** intermediate products. NMA diamide **16** was prepared from NMA dimethyl ester **6** and methylamino-succinimide **4** by ammonolysis. Temperature-dependent side reactions of methyl-amino-succinimide **4** led to diazocinone **18**, resulted from self-condensation of methylamino-succinimide via nucleophyl ring opening and the subsequent ring-transformation.

Keywords: *N*-methyl-aspartic acid – Regioselective ester and amide formation – Ring transformations – Methylamino succinimide – Diazocinone

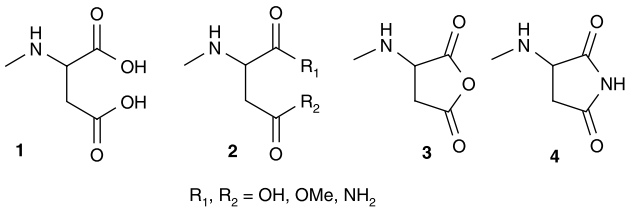
Introduction

N-methyl amino acids are natural compounds, exhibiting wide range of biological effects, partly as components of natural products (Ebata et al., 1966; Li and Joullié, 1992; Pettit et al., 1993; D'Aniello et al., 2003). *N*-alkyl amino acid derivatives are extensively used as peptidomimetic building blocks in combinatorial syntheses. In particular, *N*-methyl amino acids are useful tools to stabilize various peptide backbone conformations, and to obtain structure-activity information on synthetic analogues of endogenous peptides (Vitoux et al., 1986; Goodfellow et al., 1996).

N-methyl-*DL*-aspartic acid (NMA, **1**) and its optically active forms (*N*-methyl-*D*-aspartic acid, NMDA and *N*-methyl-*L*-aspartic acid, NMLA) have earlier been believed to be artificial compounds only (Lutz and Jirgensons, 1930). They have, however, been isolated from numerous plants and animal tissues (Sciuto et al., 1979; Sato et al., 1987; Watanabe et al., 1998; Todoroki et al., 1999; Seleni et al., 2000).

NMDA has attracted attention because of its agonist action on one of the glutamate receptor subtypes in the central nervous system (CNS) of higher animals (Hashimoto and Oka, 1997). L-glutamate is a major neurotransmitter in central excitatory pathways (Watkins and Evans, 1981; Gasic and Hollmann, 1992), and NMDA is a selective, potent agonist on an ionotropic receptor subtype. The *N*-methyl-D-aspartate receptor (NMDAR) is involved in the majority of neuroexcitatory events in the CNS (Mori and Mishina, 1995). Its specific features include calcium/sodium ion permeation, adaptive properties, learning. On the other hand, its misregulation (mostly overexcitation) is related to various neurodegenerative disorders, such as diabetes, Parkinson's and Alzheimer's diseases (Roher et al., 1993; Orpiszewski et al., 2000).

Only a few methods have been reported for the synthesis of **1**. It was prepared by Michael addition of methyl amine to maleic anhydride (Reppe and Ufer, 1937), methyl maleate (Zilkha and Bachi, 1959), and dimethyl maleate (Stadler and Hoffmann, 1962). Watkins synthesized **1** by nucleophyl substitution of 2-bromosuccinic acid with methylamine in aqueous solution (Watkins, 1962). NMDA was prepared from *N*-tosyl-*D*-aspartic acid by *N*-methylation and hydrolysis (Papaioannou et al., 1994). A general method has recently been reported for the synthesis of *N*-substituted aspartic acids using alkali maleates and



Scheme 1. Structures of NMA derivatives

amines (Piispanen and Pihko, 2005), but with the exception of *N*-methyl derivatives.

Since the early preparations of **1**, several methods aimed at the synthesis of NMA derivatives (**2**, Scheme 1). A shortcoming of these methods is, however, the propensity of asparagine, aspartic acid and their derivatives to form succinic-anhydride **3** or succinimide **4** cyclic intermediate products (Tam et al., 1988), leading to aspartyl and isoaspartyl residues, due to spontaneous ring opening. These types of reactions are also responsible for changing the structure and functions of endogenous peptides, and for causing problems in peptide syntheses (Clarke et al., 1992; Aswad, 1995; Mergler et al., 2003).

Recently, several efforts have been reported to convert L-amino acids to *N*-methyl amino acids without racemization, and for the preparation of scalemic *N*-methyl amino acids as well (Prashad et al., 2003). Unresolved weaknesses of these approaches are their inability to properly methylate trifunctional amino acids (i.e. histidine, tryptophan, lysine) (Aurelio et al., 2000; Burger et al., 2000), the harsh reaction conditions (Papaioannou et al., 1994; Wigniewski and Kotodziejczyk, 1997), the lack of generality due to racemization, low reactivity (Schedel and Burger, 2000; Burger et al., 1999) and the instability of the intermediate products.

N-methyl amino acids were synthesized by reductive amination of O'Donnell's Schiff bases with $NaBH_3CN$ (Chruma et al., 1997; Oppolzer et al., 1993), aldehydes and ketones with triacetoxyborohydride (AbdelMagid et al., 1996), but attempts to *N*-methylate aspartic acid under these conditions led to hardly separable mixtures of unmethylated, monomethylated, and dimethylated amino acids (Verardo et al., 2002). *N*-monoalkylation of amino esters was reported by reductive condensation of several carbonyl compounds in the presence of sodium borohydride (Chamoin et al., 2003; Verardo et al., 2002), but *N*-methyl analogues were not prepared.

The practical synthesis of *N*-methylamino acids by simultaneous protection-activation strategy has been reported using the highly toxic phosgene (Liwschitz and Zilkha, 1954), triphosgene (Thern et al., 2002), hexafluoroacetone (Burger and Spengler, 2000), and more recently, dichlorodialkyl silanes (van Leeuwen et al., 2002). The latter reagents were applied for regioselective preparation of L-aspartic acid amides probably via cyclic intermediates. Reductive cleavage of the intermediate 5-oxazolidinone derivative has also been developed to prepare L-amino acid *N*-methyl derivatives (Reddy and Iyengar, 1999; Aurelio et al., 2002, 2003, 2004). In the amino acid *N*-carboxy anhydride (NCA) approach, phosgene is used to protect the amino function and simultaneously to activate the carboxylic acid moiety (Deming, 1997).

In this paper we report the synthesis of NMA **1**, the biologically important standard material, by improved low cost method and the regioselective preparation of NMA ester and amide derivatives **2**, clarifying also the of mechanisms of selective isomer formation. Further synthetic modifications of NMA under mild and/or more practical conditions are also shown.

Materials and methods

All reagents and solvents were Aldrich or Sigma products, and were used as received. Melting points were taken on a Boetius apparatus and are uncorrected. Homogeneity of the products and follow up of the reactions were checked by ascending thin-layer chromatography (TLC, Camag Nanomat 4 instrument.). The following conditions were used for ester derivatives: Merck silica gel GF_{54} plates (layer thickness 0.1 mm), benzene-methanol (4:1) eluent. NMA and amide derivatives were cromatographed on cellulose plates: Macherey-Nagel Polygram Cel 300, layer thickness 0.1 mm, 20×20 cm (**A**); Merck HPTLC-Platten Cellulose 10×10 cm (**B**) and diethylamino-ethyl-cellulose plates: Macherey-Nagel Polygram Cel 300 DEAE, layer thickness 0.1 mm, 20×20 cm. The following eluent mixtures were used: n-butanol/acetic acid/water (60:15:25) (**a**), 2-propanol/2-butanon/1M HCl (60:15:25) (**b**). All ratios of the solvent systems are volume to volume (V/V), the eluents were HPLC grade Riedel-de Haën products (Chromasol V $\geq$ 99.9%); the eluent path was 15 cm. The spots were visualized with Ph.Eur.IV. 2% ninhydrine reagent, heated at 120–140 °C for about 30 min. Before ninhydrine treatment, densitograms of developed plates were taken with Shimadzu CS-9301PC densitometer. UV detection was used to control completion of methylamine addition to activated double bonds. Salts from the products were removed by flash column chromatography with ion exchange resin (Merck, Amberlyst 120, 200 mesh, strongly acidic) using pyridine-water (79:18) eluent mixture. Elemental analyses (C,H,N) were carried out on Carlo Erba NA-1500 analyzer, the results were within 0.4% of the theoretical values. Mass spectra were recorded with Thermo Finnigan LCQ Advantage LC/MS spectrometer, used in ESI positive or negative ion mode.

^{1}H NMR, HMQC spectra were recorded on 500 or 600 MHz Varian instruments, consol type: Inova Unity. DMSO-d_6 (TMS as internal standard) and H_2O/D_2O (DSS as internal standard) were used as solvents. Fast and convenient 1D ^{1}H-NMR spectroscopic distinction of *N*-methyl-aspartic-acid (NMA), NMA-α- **13** and β-amides **9** and their salts can be achieved with pD adjusted deuterium-oxide as a solvent. The protonation shift was an important identification tool in the process of distinction between monoamide regioisomers of NMA. At analogous protonation stages, each of the three compounds shows indistinguishably similar ABX proton patterns. Even in aprotic solvents, e.g. DMSO-d_6, the observed spectra of the free amino-acid and the sodium or chloride salt of

the same compound shows similar differences as in D_2O. The additional information of observing the amide $-CONH_2$ protons is not sufficient to distinguish between the amide isomers by routine 1D ^{1}H-NMR spectra, therefore HMQC measurements were used for identification.

N-methyl-DL-aspartic acid dimethyl ester ***6***

Method A: Dimethyl fumarate **5** (28.8 g, 0.2 mol) was suspended in 50 ml abs. ethanol, and 25 ml of 8 M ethanol solution of methylamine (0.2 mol) was added at 0 °C. The reaction mixture was stirred until complete dissolution of **5**, and for three additional hours. The solvent was evaporated in vacuo to dryness, yellowish oil of **6** was obtained in quantitative yield. For the analytical sample, the oil was distilled in vacuo to give colorless oil, bp 68–70 °C at 0.3 mmHg (lit. 60–61 °C/0.25 Torr; Stadler and Hofmann, 1962), $n_D = 1.4378$ at 20 °C. ^{1}H-NMR (DMSO-d_6, 600 MHz): $\delta = 3.64$ (3H, s, OCH_3), 3.58 (3H, s, OCH_3), 3.45 (1H, t, $^3J_1$(H,H) = 6.68 Hz, $^3J_2$(H,H) = 7.02 Hz, C–H), 2.65 (1H, d-d, $^3J_1$(H,H) = 6.68 Hz, $^2J_3$(H,H) = 15.80 Hz, H–CH), 2.55 (1H, d-d, $^3J_2$(H,H) = 7.02 Hz, $^2J_3$(H,H) = 15.80 Hz, HC–H), 2.23 (3H, s, NCH_3) MW = 175.19 ESI-MS m/z: 175.2 $[M+H]^+$ Anal. Calc. for $C_7H_{13}NO_4$ C = 43.95% H = 6.18% N = 9.29% Found C = 44.30% H = 6.08% N = 9.39%.

Method B: Methylamine hydrochloride (22.5 g, 0.3 mol) was added to the stirred solution of dimethyl fumarate **5** (28.8 g, 0.2 mol) in dry pyridine (150 ml), and the reaction mixture was warmed up to 100 °C. Triethylamine (27.3 g, 41 ml, 0.3 mol) was added dropwise to the suspension in 3 h. When the addition was completed, the reaction mixture was stirred under reflux for 2 additional hours. The solvent was evaporated in vacuo to dryness. The residue was suspended in ethyl acetate (150 ml) and the mixture was neutralized with saturated sodium carbonate solution. The organic phase was separated and the aqueous phase was extracted with ethyl-acetate (2 × 50 ml). The combined organic phases were washed with water (3 × 25 ml), dried over sodium sulfate and the solvent was evaporated in vacuo to give the product (31.4 g, 90%), as a yellowish oil, which was used for further transformations without additional purification.

N-methyl-DL-aspartic acid β-methyl ester ***8***

Method A: 2.5 ml of 8 M ethanol solution of methylamine (0.02 mol) was added to an ethanolic solution (8 ml) of methyl maleate **7a** or methyl fumarate **7b** (1.31 g, 0.01 mol) at 0 °C. After stirring at 0 °C for 1 h, ethanol and excess of methylamine were evaporated in vacuo to dryness. White crystals of **8** were obtained, which contained small amounts of methylamine. The crude product was dried overnight in vacuum desiccator over cc. sulfuric acid, then it was suspended in acetone, filtered off and washed with acetone to give amine free product (1.48 g, 91%), mp 207–8 °C (lit. 206 °C; Zilkha and Bachi, 1959). ^{1}H-NMR (DMSO-d_6, 600 MHz): $\delta = 3.58$ (3H, s, OCH_3), 3.42 (1H, d-d, $^3J_1$(H,H) = 5.17 Hz, $^3J_2$(H,H) = 6.84 Hz, C–H), 2.81 (1H, d-d, $^3J_1$(H,H) = 5.17 Hz, $^2J_3$(H,H) = 16.91 Hz, H–CH), 2.67 (1H, d-d, $^3J_2$(H,H) = 6.84 Hz, $^2J_3$(H,H) = 16.91 Hz, HC–H), 2.47 (3H, s, NCH_3) MW = 161.16 ESI-MS m/z: 161.2 $[M+H]^+$ Anal. Calc. for $C_6H_{11}NO_4$ C = 45.43% H = 6.06% N = 9.35% Found C = 44.93% H = 6.35% N = 9.28%.

Method B: To the stirred solution of methyl maleate **7a** (13 g, 0.1 mol) in dry pyridine (100 ml), methylamine hydrochloride (13.5 g, 0.2 mol) was added, and the reaction mixture was warmed up to 100 °C. Triethylamine (20.23 g, 27.3 ml, 0.2 mol) was added dropwise to the suspension in 2 h. When the addition was finished, the reaction mixture was stirred under reflux for 1 additional hour. The solvent was evaporated in vacuo to dryness. The residue was dissolved in minimal amount of pyridine (25 ml) and the solution was poured on ion exchange chromatographic column (80 g, strongly acidic resin) and the product was eluted with pyridine-water (79:18) mixture (150 ml). The eluent solution was evaporated in vacuo to dryness. The product **8** was crystallized from methanol to give white crystals (24.4 g, 76%), mp 206–207 °C.

N-methyl-DL-aspartic acid ***1***

6 (17.5 g, 0.1 mol) was dissolved in 5% sodium hydroxide solution (100 ml) and the reaction mixture was heated on water bath for 3 h at 60 °C. After cooling, the solution was neutralized with cc. hydrochloric acid to pH 4 and evaporated in vacuo to ~25 ml volume. The solution was poured on ion exchange chromatographic column (80 g, strongly acidic resin) and NMA was eluted with pyridine-water (79:18) mixture (250 ml). The eluent solution was evaporated in vacuo to dryness. The solid residue was suspended in isopropanol (50 ml) and the white crystals were filtered off, washed twice with cold isopropanol and ether, then dried at room temperature to give *N*-methyl-*DL*-aspartic acid monohydrate **1** (14.1 g, 86%), mp 141 °C (NMA × $1H_2O$) (lit. 138 °C; Watkins, 1962). After drying the product in vacuo, the melting point rose up to 178 °C (NMA) (lit. 175 °C; Watkins, 1962). R_f (**A**, **b**) = 0.61, R_f (**B**, **a**) = 0.36, R_f (**B**, **b**) = 0.54 ^{1}H-NMR (DMSO-d_6, 500 MHz): $\delta = 3.69$ (1H, d-d, $^3J_1$ = 8.56 Hz, $^3J_2$ = 4.51 Hz, C–H), 2.77 (1H, d-d, $^3J_1$ = 8.56 Hz, $^2J_3$ = 16.45 Hz, H–CH), 2.61 (1H, d-d, $^3J_2$ = 4.51 Hz, $^2J_3$ = 16.45 Hz, HC–H), 2.54 (3H, s, NCH_3) MW = 147.13 ESI-MS m/z: 147.13 $[M+H]^+$ Anal. Calc. for $C_5H_9NO_4$ C = 41.00% H = 6.65% N = 15.94% Found C = 40.60% H = 6.48% N = 16.02%.

N-methyl-DL-isoasparagine β-methyl ester ***12***

1.3 ml of 8 M ethanol solution of methylamine (0.01 mol) was added to an ethanol solution (8 ml) of methyl maleamate **11a** or methyl fumaramate **11b** (1.29 g, 0.01 mol) [prepared by acid catalyzed esterification of maleamic **10a**/fumaramic acid **10b**] at 0 °C. The reaction mixture was stirred for 12 h at 0 °C. The solvent was evaporated in vacuo to dryness. Transparent oil of **12** was obtained in quantitative yield. (1.60 g), R_f (**B**, **a**) = 0.84, R_f (**B**, **b**) = 0.85 ^{1}H-NMR (DMSO-d_6, 500 MHz): $\delta = 7.48$ (1H, s, NH), 7.11 (1H, s, NH), 3.59 (3H, s, OCH_3), 3.27 (1H, d-d, $^3J_1$(H,H) = 5.58 Hz, $^3J_2$(H,H) = 8.27 Hz, C–H), 2.57 (1H, d-d, $^3J_1$(H,H) = 5.58 Hz, $^2J_3$(H,H) = 15.38 Hz, H–CH), 2.39 (1H, d-d, $^3J_2$(H,H) = 8.27 Hz, $^2J_3$(H,H) = 15.38 Hz, HC–H), 2.21 (3H, s, NCH_3) MW = 160.17 ESI-MS m/z: 160.4 $[M+H]^+$ Anal. Calc. for $C_6H_{12}N_2O_3$ C = 44.99% H = 7.55% N = 17.49% Found C = 45.13% H = 7.62% N = 17.41%.

N-methyl-DL-asparagine ***9***

Method A: 25 ml 8 M ethanol solution of methylamine was added to a solution of maleamic acid **10a**/fumaramic acid **10b** (11.5 g, 0.1 mol) in dry pyridine (50 ml), and the reaction mixture was kept at 25 °C for 20 min. The solvent was evaporated in vacuo to dryness. The residue was dissolved in minimal amount of water (25 ml) and the solution was poured on ion exchange chromatographic column (50 g, weakly acidic resin) and the product was eluted with pyridine-water (79:18) mixture (150 ml). The eluent solution was evaporated in vacuo to dryness. The product was crystallized from methanol to give **9** as white crystals (12.4 g, 86%), mp 209 °C (lit. 208 °C; Liwschitz et al., 1956). R_f (**B**, **a**) = 0.25, R_f (**B**, **b**) = 0.40 ^{1}H-NMR (DMSO-d_6, 600 MHz): $\delta = 7.69$ (1H, s, NH), 7.05 (1H, s, NH), 3.39 (1H, d-d, $^3J_1$(H,H) = 4.04 Hz, $^3J_2$(H,H) = 7.96 Hz, C–H), 2.73 (1H, d-d, $^3J_1$(H,H) = 4.04 Hz, $^2J_3$(H,H) = 16.68 Hz, H–CH), 2.47 (1H, d-d, $^3J_2$(H,H) = 7.96 Hz, $^2J_3$(H,H) = 16.68 Hz, HC–H), 2.50 (3H, s, NCH_3) MW = 146.15 ESI-MS m/z: 147.07 $[M+H]^+$ Anal. Calc. for Found C = 40.89% H = 6.87% N = 15.79% Calc. for $C_5H_{10}N_2O_3$ C = 41.00% H = 6.65% N = 15.94%.

Method B: **12** (1.6 g, 0.01 mol), suspended in 10% sodium carbonate solution (20 ml), was heated at 60 °C for 2 h. After cooling, the solution was neutralized with cc. hydrochloric acid to pH 4 and evaporated in vacuo. The residue was purified on ion exchange chromatographic column (30 g, weakly acidic resin) and the product was eluted with pyridine-water (79:18) mixture (100 ml). The eluent solution was evaporated in vacuo to dryness. The solid residue was suspended in ethanol (10 ml), and the white crystals were filtered off, washed twice with cold ethanol to give **9** (1.14 g, 75%), mp 208–210 °C.

N-methyl-DL-isoasparagine ***13***

Method A: To a 15 ml suspension of **1** (1.47 g, 0.01 mol) in dry tetrahydrofuran, thionyl chloride (1.19 g, 0.73 ml, 0.01 mol) and triethylamine (1.01 g, 1.39 ml, 0.01 mol) were added dropwise at −20 °C. After dissolution of the suspended material, the reaction mixture was stirred for 30 additional minutes, and then 7 M methanol solution of ammonia (4.5 ml, 0.3 mol) was added at −10 °C. The reaction mixture was evaporated to dryness. The residue was suspended in acetone-water mixture (9:1), filtered and washed with cold acetone to give **13** (1.21 g, 82%), mp 194 °C (dec.) (lit. 191 °C; Zilkha and Bachi, 1959) 191 R_f (**A**, **b**) = 0.41, R_f (**B**, **a**) = 0.36, R_f (**B**, **b**) = 0.38 ^{1}H-NMR (DMSO-d_6, 500 MHz): δ = 7.71 (1H, s, NH), 7.29 (1H, s, NH), 3.43 (1H, d-d, $^3J_1$ = 4.77 Hz, $^3J_2$ = 9.43 Hz, C–H), 2.42 (1H, d-d, $^3J_1$ = 4.77 Hz, $^2J_3$ = 16.05 Hz, H–CH), 2.25 (1H, d-d, $^3J_2$ = 9.43 Hz, $^2J_3$ = 16.05 Hz, HC–H), 2.34 (3H, s, NCH_3) MW = 146.15 ESI-MS m/z: 147.07 $[M+H]^+$ Anal. Calc. for $C_5H_{10}N_2O_3$ C = 41.00% H = 6.65% N = 15.94%, Found C = 40.89% H = 6.87% N = 15.79%.

Method B: *DL*-α-methylamino-succinic anhydride · HCl **3**

1 (1.47 g, 0.01 mol) was dissolved in 8 ml glacial acetic acid. Usage of sonicator and heating (60 °C) may be necessary for complete dissolution, or the small amounts of undissolved substance must be filtered before performing the reaction. After cooling to 5 °C, NMA precipitated partly as amorphous material, but was dissolved again during the reaction with acetylchloride. 8 ml of acetylchloride was then added dropwise to the stirred solution. Carefully keeping the reaction mixture at 5 °C for 12 h, it was diluted with 20 ml of diethylether. The precipitated *DL*-α-methylamino-succinic anhydride hydrochloride **3** was filtered and washed with diethylether. The unstable, highly hygroscopic substance needs to be instantly transformed in the next reaction. (0.95 g, 74%) ^{1}H-NMR (DMSO-d_6, 600 MHz): δ = 3.97 (1H, d-d, $^3J_1$(H,H) = 4.13 Hz, $^3J_2$(H,H) = 7.65 Hz, C–H), 2.91 (1H, d-d, $^3J_1$(H,H) = 4.13 Hz, $^2J_3$(H,H) = 17.05 Hz, H–CH), 2.83 (1H, d-d, $^3J_2$(H,H) = 7.65 Hz, $^2J_3$(H,H) = 17.05 Hz, HC–H), 2.57 (3H, s, NCH_3) MW = 129.12 ESI-MS m/z: 127.87 $[M-H]^-$.

3 (1.29 g, 0.01 mol) was dissolved in 15 ml of 10 °C, 7 M methanol solution of ammonia (0.1 mol). After 1 h, the methanol and the excess of ammonia were evaporated in vacuo, keeping the temperature under 35 °C. To the obtained white crystals, acetone (5 ml) was added, filtered and the filtrate was washed with acetone. The product was dried over cc. sulfuric acid at 10 °C in vacuum desiccator for one night to give *N*-methyl-*DL*-isoasparagine **13** (1.16 g, 78%), mp 191–193 °C (dec.).

DL-α-methylamino-succinimide ***4***

Maleimide (1.94 g, 0.02 mol) was dissolved in ethanol (10 ml), and 2.5 ml, 8 M ethanol solution of methylamine (0.02 mol) was added at 0 °C. The reaction mixture was stirred for 20 min at 0 °C, then the solvent was evaporated in vacuo, keeping the temperature under 35 °C, to obtain amorphous mass of *DL*-α-methylamino-succinimide **4**. Substance was used for the following transformation without further purification. ^{1}H-NMR (DMSO-d_6, 600 MHz): δ = 3.61 (1H, d-d, $^3J_1$(H,H) = 4.82 Hz, $^3J_2$(H,H) = 8.51 Hz, C–H), 2.83 (1H, d-d, $^3J_1$(H,H) = 4.82 Hz, $^2J_3$(H,H) = 17.80 Hz, H–CH), 2.36 (1H, d-d, $^3J_2$(H,H) = 8.51 Hz, $^2J_3$(H,H) = 17.80 Hz, HC–H), 2.32 (3H, s, NCH_3) MW = 128.13 ESI-MS m/z: 129.2 $[M+H]^+$.

N-methyl-DL-aspartic acid diamide ***16***

Method A: **6** (2 g, 0.011 mol) was heated with excess of ammonia (0.07 mol, 10 ml 7 M methanol solution), in a sealed tube at 70 °C for 24 h. The mixture was evaporated in vacuo to dryness. The residue was dissolved in acetone (10 ml) at reflux temperature. After cooling down to room temperature, the precipitated white crystals were filtered and washed with acetone to give **16** (1.77 g, 61%), mp 145 °C. ^{1}H-NMR (DMSO-d_6, 600 MHz): δ = 7.39 (1H, s, NH), 7.34 (1H, s, NH), 7.02 (1H, s, NH), 6.81 (1H, s, NH), 3.16 (1H, d-d, $^3J_1$(H,H) = 4.52 Hz, $^3J_2$(H,H) = 8.99 Hz, T_1 = 1.27 s, C–H), 2.29 (1H, d-d, $^3J_1$(H,H) = 4.52 Hz, $^2J_3$(H,H) = 14.87 Hz, T_1 = 0.87 s, H–CH), 2.17 (1H, d-d, $^3J_2$(H,H) = 8.99 Hz, $^2J_3$(H,H) = 14.87 Hz, T_1 = 0.87 s, HC–H), 2.12 (3H, s, T_1 = 1.55 s, NCH_3) MW = 145.16 ESI-MS m/z: 146.2 $[M+H]^+$ Anal. Calc. for found C = 41.30% H = 7.72% N = 28.70% Calc. for $C_5H_{11}N_3O_2$ C = 41.37% H = 7.64% N = 28.95%.

Method B: **4** (2.63 g, 0.02 mol) was dissolved in 25% aqueous ammonium-hydroxide solution (20 ml) at 0 °C, and was allowed to react for five days at the same temperature. The precipitated crystals were filtered and washed with acetone to give **16** (1.2 g, 41%), mp 144–146 °C.

1,5-Dimethyl-4,8-dioxo-[1,5]diazocane-2,6-dicarboxylic-acid-diamide ***18***

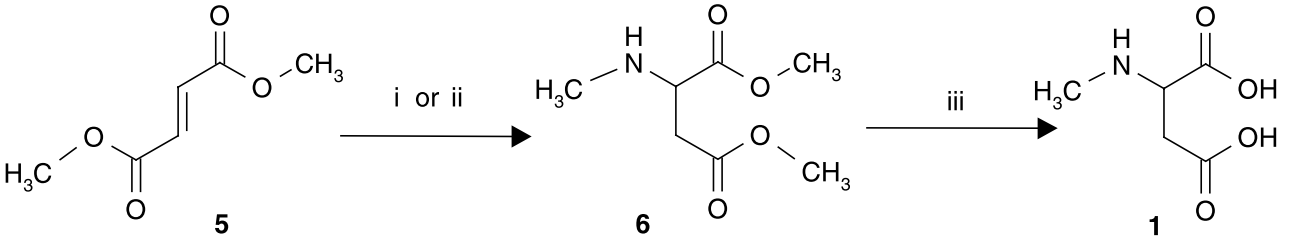
4 (2.63 g, 0.02 mol) was dissolved in 20 ml of 25 °C, 7 M methanol solution of ammonia, and was left for 12 h at room temperature. The precipitated crystals were filtered and washed with acetone (0.4 g, 15%), mp 173–175 °C. ^{1}H-NMR (DMSO-d_6, 600 MHz): δ = 7.41 (2H, s, NH), 6.85 (2H, s, NH), 4.126 (2H, d-d, $^3J_1$(H,H) = 4.50 Hz, $^3J_2$(H,H) = 4.04 Hz, T_1 = 1.36 s, C–H), 2.79 (2H, d-d, $^3J_1$(H,H) = 4.50 Hz, $^2J_3$(H,H) = 16.137 Hz, T_1 = 0.68 s, H–CH), 2.72 (2H, d-d, $^3J_2$(H,H) = 4.04 Hz, $^2J_3$(H,H) = 16.137 Hz, T_1 = 0.68 s, HC–H), 2.80 (6H, s, T_1 = 1.27 s, NCH_3) MW = 256.26 ESI-MS m/z: 257.2 $[M+H]^+$ Anal. Calc. for Found C = 46.69% H = 6.35% N = 21.80% Calc. for $C_{10}H_{16}N_4O_4$ C = 46.87% H = 6.29% N = 21.86%.

Results and discussion

Reported syntheses of NMA derivatives were accomplished upon using fairly common strategies for aspartic acid derivatives, including Michael addition of methylamine to substituted acrylates. We have utilized dimethyl fumarate **5** as a starting material, since reactions of primary amines with maleic anhydride or maleic acid esters yield monoamide derivatives that can be dehydrated to imides, polyimides (qv), or isoimides, depending on the reaction conditions (Orphanides, 1979). Pathways with favorable yields are difficult to achieve, because base catalyzed rapid isomerisation of maleic acid esters to fumarate esters, using catalytic amount of alkylamines is an exothermic process that leads to amide side product formation (Thomas, 1976).

We have found that reaction of equimolar dimethyl fumarate **5** and methylamine in ethanol solution at 0 °C in 1 h results in rapid formation of *N*-methyl-*DL*-aspartic acid dimethyl ester **6** (Method A) in quantitative yield. Under similar conditions, methyl maleate **7a** and methyl fumarate **7b** could be converted to *N*-methyl-*DL*-aspartic acid β-methyl ester **8** (Method A, Scheme 3).

In the reported syntheses of NMA **1** (Reppe and Ufer, 1937; Zilkha and Bachi, 1959; Stadler and Hoffmann, 1962), the insolubility of methylamine hydrochloride in organic solvents is the common technical problem. The use of aqueous solutions (Watkins, 1962) as reaction medium is unfavorable, in respect of too slow conversion and appearance of undesirable side reactions. In order to over-



i CH_3NH_2, EtOH, 0 °C, 1 h, 100%; **ii** CH_3NH_2·HCl, Pyridine, Et_3N, 100 °C, 3 h, 92%; **iii** 5% NaOH, 60 °C, 1 h, 87%

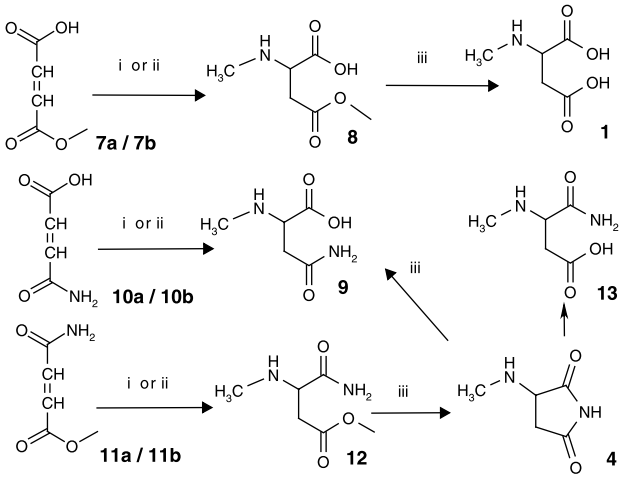
Scheme 2. Synthesis of NMA **1** from dimethyl fumarate **5**

come these difficulties, new reaction conditions were applied in the modified synthesis of **1**. Michael addition of methylamine to **5** was performed in pyridine solution at 100 °C. Dropwise addition of equimolar triethylamine continuously liberated methylamine from its hydrochloride salt in the reaction mixture. After optimization of reaction conditions, excess (2.5×) of methylamine hydrochloride was used for nearly quantitative conversion, without the occurrence of undesired amide side products (**6**, Method B).

The obtained ester derivative **6** was hydrolyzed with dilute sodium hydroxide solution. After neutralization, the separation of **1** from the salts and other compounds was carried out by flash column chromatography using strongly acidic ion exchange resin. The product was eluted with molar equivalent pyridine-water mixture. The synthetic route is illustrated in Scheme 2.

For preparation of *N*-methyl-*DL*-asparagine **9**, a general method was reported for the synthesis of any N^2-alkyl derivative of asparagine by heating maleamic acid and the appropriate alkylamine in pyridine at reflux (Liwschitz et al., 1956). For the *N*-methyl derivative, however, the volatility of methylamine restrains the reaction.

Practical modification of this procedure was carried out by using maleamic acid **10a** or fumaramic acid **10b**, methylamine hydrochloride and equimolar triethylamine in pyridine at 100 °C (**9**, Method A). The addition of methylamine to methyl maleamate **11a** or fumaramate **11b** was also performed in ethanol at 0 °C to give regioselectively *N*-methyl-*DL*-isoasparagine β-methyl ester **12** in quantitative yield. Attempts to hydrolyze the ester group of this compound under acidic or basic conditions led to a multicomponent mixture containing **1**, *N*-methyl-*DL*-isoasparagine **13**, and *N*-methyl-*DL*-asparagine **9** in various ratios, detected by TLC. NMR spectra showed the major component of the crude product to be **9**, whereas the two minor products were identified to be **1** and **13**, being formed over the cyclic succinimide **4** intermediate

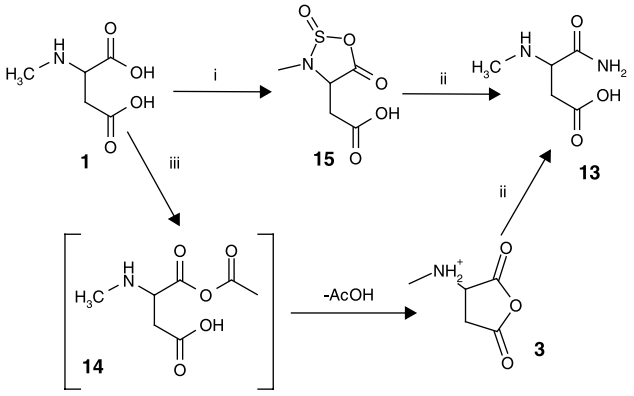


i CH_3NH_2, EtOH, 0 °C, 1 h; **ii** CH_3NH_2·HCl, Pyridine, Et_3N, 100 °C, 3 h; **iii** 10% Na_2CO_3, 60 °C, 1 h

Scheme 3. Selective addition of methylamine to substituted acrylates and subsequent transformations

product shown in Scheme 3. The formation of aspartimide from aspartic acid residues has been reported under both acidic and basic conditions (Oliyai and Borchardt, 1994; Nabuchi et al., 1997). Under aqueous conditions, the resulting cyclic intermediate can be converted to either the α- and the β-amide. Hydrolysis of the aminosuccinimide yields a mixture of α- and β-aspartyl residues consisting predominantly of the β-amide. In the case of *N*-methyl derivatives, *N*-methyl-*DL*-asparagine **9** could be isolated from the reaction mixture under weakly basic conditions (**9**, Method B, Scheme 3).

Investigation of regiochemistry was a challenging task in the presence of *two* free carboxylic acid moieties and one unprotected methylamino group. In NMA **1**, different protonation constants of functional groups can cause reactivity preferences (selectivity) under suitable conditions ($pKa_1 = 10.10$, $pKa_2 = 3.54$, $pKa_3 = 1.85$, Boros et al., 2006 in progress).



i $SOCl_2$, TEA, THF, -20 °C; **ii** NH_3, MeOH; **iii** Ac_2O / AcOH, 10 °C

Scheme 4. Synthesis of *N*-methyl-*DL*-isoasparagine **13**

In the course of investigation of the reaction between **1** and thionyl chloride under very mild conditions at −20 °C in the presence of equimolar triethylamine, and the subsequent reaction of the formed inner mixed anhydride **15** with amines, we have found the amidation to be regioselective. A possible explanation may be the five-membered sulphinamide anhydride **15** ring structure of the intermediate product, formed in the reaction of **1** and thionylchloride. In the next step, the thio-analogue of cyclic Leuchs-anhydride **15**, a well known sensitive agent against nucleophylic reagents, reacts with ammonia. Ring opening and elimination of sulphur dioxide take place after nucleophylic attack of ammonia, leading exclusively to the α-amide, **13** (Method A, Scheme 4). In small scale we could accomplish the reaction in good yield, but as a consequence of the unstable nature of the intermediate and the product, degradation occurred in larger scale before completion of the reaction.

Methods for preparation of *N*-alkyl-isoasparagines were reported by action of aqueous ammonia on *N*-alkyl-aspartic mixed anhydride hydrochloride, formed from *N*-alkyl-aspartic acids and a mixture of acetyl chloride-acetic acid (1:1) (Zilkha and Bachi, 1959). This procedure suffers from difficulties in technical conditions, reaction selectivity, isolation procedure and reproducibility. After reinvestigation of the reaction and variation of the reaction conditions, we investigated the products by chromatography. TLC analysis of the reaction mixture in different eluent systems indicated the presence of two main products among various amounts of recovered starting material. The proton NMR analysis of the crude mixture showed that the products seemed to be **13** and **9** in 5:1 ratio. Recording three ^{1}H-NMR spectra of **1**, **9**, **13** and their salts at three pD values (pD ∼ 1, pD ∼ 13 and pure D_2O) provide sufficient information not only on the chemical structures, but also on the protonation form of the substances (Table 1, see details in Materials and methods).

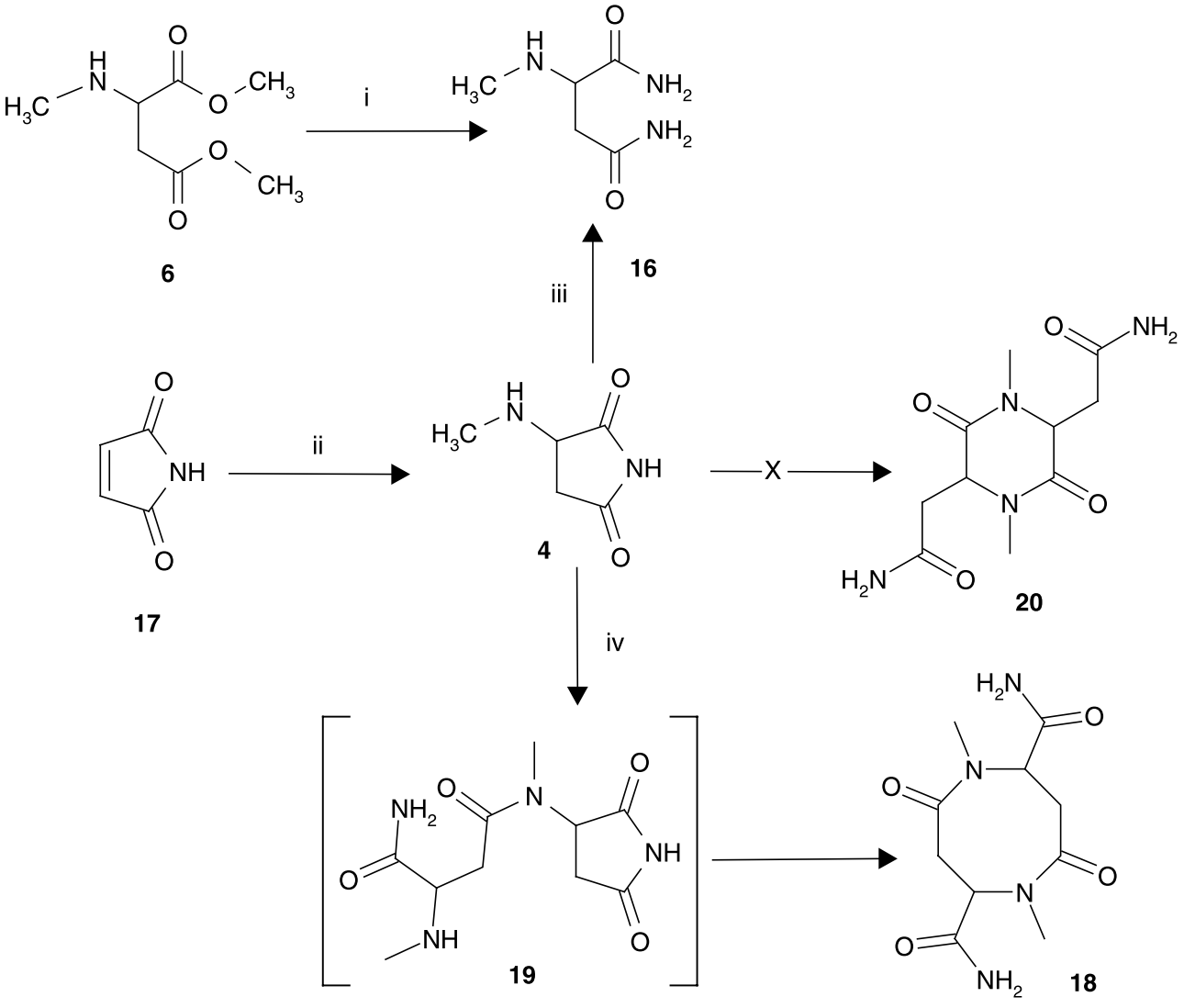
Table 1. pH (pD) dependent ^{1}H NMR chemical shifts of methine hydrogens of NMA (**1**), and its monoamide derivatives (**9**, **13**)

Compound	pD ∼ 1 (fully protonated form, hydrochloride salt)	pD ∼ 13 (fully deprotonated form, sodium or disodium salt)	Free amino acid in pure D_2O
NMA **1**	4.32	3.29	3.73
N-methyl-*DL*-isoasparagine **13**	4.26	3.43	4.11
N-methyl-*DL*-asparagine **9**	4.24	3.29	3.84

After modification of conditions, we can conclude that it is very important to keep some instructions for successful operation. Formation of anhydride **3** by acetyl chloride is an exothermic reaction. If temperature rises over 25 °C, undesired side reactions take place. We could detect the formation of *N*-acylated (ESI-MS m/z: 188 $[M-H]^-$) and dipeptide (ESI-MS m/z: 275 $[M-H]^-$) products by mass spectral analysis. At last, the reaction was performed by dropwise addition of acetyl chloride with intensive stirring, and temperature was kept below 10 °C. Under these conditions, acylation of the MeNH group was not observed, in contrast to the previously reported synthesis conditions. The precipitated anhydride hydrochloride **3** was isolated by filtration, and the cyclic anhydride structure was proved by NMR. The compound is stable for several hours in inert atmosphere. The reaction of **3** and excess of ammonia in methanol resulted in *N*-methyl-*DL*-isoasparagine **13** with a good yield and satisfactory purity (**13**, Method B, Scheme 4). The product was partly degraded during recrystallisation from any solvent; therefore purity could not be improved afterwards.

N-methyl-*DL*-aspartic acid diamide **16** was prepared by the classical reaction of **6** and large excess of ammonia (**16**, Method A, Scheme 5). Considering the easy formation and sensitive nucleophylic cleavage of *DL*-α-methyl-amino-succinimide **4**, we decided to study the addition of methylamine to maleimide and its ring opening reaction with ammonia, in order to open a new route to **16**. There are only few reports on the syntheses and ring opening reactions of 3-aminosuccinimides (Maddaluno et al., 1992; Briere et al., 1997; Katritzky et al., 1998).

The addition of methylamine to maleimide **17** was performed in methanol at 0 °C in quantitative yield. We observed a clean reaction, but the product was degraded after one day at room temperature or heating the solution over 40 °C for a few minutes. After the addition of equimolar



i NH_3, MeOH, 60 °C, 12 h, sealed tube; **ii** CH_3NH_2, EtOH, 0 °C; **iii** NH_3, MeOH, 0 °C, 3 days; **iv** NH_3, MeOH, 40 °C

Scheme 5. Formation and some reactions of *DL*-α-methylamino-succinimide **4**, synthesis of *N*-methyl-*DL*-aspartic acid diamide **16**

methylamine was complete, excess of ammonia was added to the reaction mixture in order to avoid side reactions of the product, and it was allowed to react at room temperature overnight. Diazocinone derivative **18** resulted from ring opening, followed by amine catalyzed self condensation of the formed *N*-methyl-*N*-(*N*-methylamino-aspartyl)-succinimide **19**. The structure was confirmed by routine ^{1}H NMR spectroscopy, and relaxation time [T_1] measurements, compared to T_1 values of **16**, and by comparison of the chemical shifts with ones of the known isomer, diketopiperazine derivative **20** (Howes et al., 1983; Falorni et al., 1997). Selective ring opening was observed if the reaction was carried out at 0 °C for 3 days. The formed **16** diamide crystallized out from the reaction mixture (Method B, Scheme 5).

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