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SYNTHESIS OF CHIRAL TRIAZINE COUPLING REAGENTS BASED ON ESTERS OF *N*-ALKYLPROLINE AND THEIR APPLICATION IN THE ENANTIOSELECTIVE INCORPORATION OF D OR L AMINO ACID RESIDUE DIRECTLY FROM RACEMIC SUBSTRATE

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Abstract: Esters of *N*-methylproline and *N*-allylproline were prepared and used as component for synthesis of chiral triazine based coupling reagents. *N*-Triazinylammonium tetrafluoroborate obtained from methylester of L-*N*-methylproline, 2-chloro-4,6-dimethozxy-1,3,5-triazine and tetrafluoroboric acid in the coupling of *rac*-Z-Ala-OH with glycine methylester preferred formation of D-Z-AlaGly-OMe with L/D ratio 21/79. Coupling reagent prepared from D enantiomer of *N*-methylproline gave L-Z-AlaGly-OMe with L/D ratio 75/25.

Keywords: enantioselective condensation, 2-chloro-4,6-dimethoxy-1,3,5-triazine, 2,4-dichloro-6-methoxy-1,3,5-triazine, peptide synthesis

The majority of the biomolecules which are crucial for living systems are chiral e.g., amino acids, proteins, nucleic acids, sugars, isoprenoids etc. The interesting feature is that in the Nature in most cases there is existing only one of the two possible enantiomeric forms. Therefore, in the enantiospecific environment of biomolecules, for many of therapeutics, their enantiomers can provide diversified selectivity for their biological targets, different therapeutic indices, and/or pharmacokinetics. There are no doubts that the stereoselective pharmacokinetics and pharmacodynamics of chiral drugs has wide ranging implications in practical therapeutics, health care and pharmacy. According to the guidelines on the development of chiral drugs, presented by European Union (EU) in 1994 in a document entitled Investigation of Chiral Active Substances (1), it is recommended to recognize the occurrence of chirality in new drugs, attempts to separate the stereoisomers, assessed the contribution of the various stereoisomers to the activity of interest and finally made a rational selection of the stereoisomeric form that is proposed for marketing (2).

Even more complex relations are observed between living system and diastereomeric pharmaceutically active compounds (3). For the peptides, peptoids or other complex molecules prepared by condensation of a number of the chiral building blocks, the accurate composition of stereochemistry of all components is crucial factor determining activity, resistance to enzymatic degradation and all other pharmacological features and attributes. Therefore, ready access to a whole range of chiral building blocks is essential to acquire full profits from the systematic exploration of a structural diversity of such molecules.

In the search based on application methods of combinatorial chemistry and high throughput screening, the classical approach based on asymmetric synthesis or resolution of a racemic precursor seems to be non-optimal because most substrates are used in small amounts, in single experiments only and usually they are discarded after a preliminary selection procedure as not promising. More advantageous and less time-consuming would be evaluated an alternative approach based on the enantiodifferentiating transformation of racemic substrates, which are usually more easily available than enantiomerically pure equivalents. Such an approach would be considered particularly attractive for incorporation of non coded chiral amino acids into the peptide chain.

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Although several chiral coupling reagents and chiral acylation catalysts have been proposed for the enantioselective acylation and synthesis of enantiomerically enriched peptides directly from racemic substrates, including optically active 4-dimethylaminopyridine (DMAP) analogues (4), benzotetramisol (5), *N*-methylimidazole (6), *N*-hydroxysuccinimide (7), diacylamines (8), tertiary amines (9), phosphines (10) and others (11), until recently, none of the proposed enantioselective coupling methods has been accepted for peptide synthesis because of the unpredictable results of coupling experiments including configuration, enantiomeric purity, optimal coupling conditions and the yield of the final product. The unpredictability of the enantioselective synthesis of peptide bonds results from the complex mechanism of this process, consisting of two subsequent stages, e.g., activation of carboxylic group, followed by aminolysis proceeding *via* two independent tetrahedral intermediates and four independent transition states with additional stereogenic centers temporary formed on the carbon atom of the activated carboxylic group.

EXPERIMENTAL

Thin layer chromatography experiments (TLC) were carried out on silica gel (Merck; 60 Å F254), and spots were located with: UV light (254 and 366 nm), 1% ethanolic 4-(4-nitrobenzyl)pyridine (NBP) or, in the case of secondary amines, with a mixture of 2% solution of acetaldehyde in DMF and 2% solution of chloranil in DMF. Derivatives of *N*-methylproline were visualized by spraying plates with 1% $KMnO_4$ in 2% aqueous K_2CO_3 solution.

Analytical RP-HPLC was performed on a Waters 600S HPLC system (Waters 2489 UV/VIS detector, Waters 616 pump, Waters 717 plus autosampler, HPLC manager software from Chromax) using a Vydac C18 column (25 cm × 4.6 mm, 5 µm; Sigma). HPLC was performed with a gradient of 0.1% TFA in H_2O (A) and 0.08% trifluoroacetic acid in acetonitrile (B), at a flow rate of 1 mL/min with UV detection at 220 nm, t_R in min. Preparative column chromatography were performed on silica gel 60, 240–600 mesh (Merck).

LC/MS spectra were recorded on a Dionex UltiMate 3000. IR spectra were recorded in KBr pellets or film on a Bruker ALPHA spectrometer or a PerkinElmer Spectrum 100 apparatus. ^{1}H-NMR and ^{13}C-NMR, spectra were recorded on a Bruker Avance DPX 250 (250 MHz) and Varian (300 MHz) spectrometers. Chemical shifts (ppm) are relative to TMS used as an internal standard. Multiplicities are marked as s = singlet, d = doublet, t = triplet, q = quartet, qu = quintet, m = multiplet. Melting points were determined using a Büchi apparatus, model 510.

Synthesis of L-*N*-methylproline (1). Typical procedure

The vigorously stirred suspension of zinc dust (6.50 g; 100 mmol), L-proline (5.57 g; 50 mmol) and NaH_2PO_4 (11.90 g; 100 mmol) in water (22 mL) was treated with 35% aq. formaldehyde (2.10 mL). Stirring was continued for 48 h at 30°C. The suspension was discarded, the filtrate was neutralized with 2 M aq. ammonia to pH 8, concentrated under vacuum, the solid residue was dissolved in small amount of water and lyophilized. Dry residue was extracted with hot mixture of benzene-ethanol (1 : 1, v/v). Collected extracts were evaporated to dryness and then recrystallized from the mixture methanol/ether affording L-*N*-methylproline (**1**) (5.68 g; yield 88%) as white crystals m.p. 115–120°C, lit. (12) m.p. 115–116°C. IR (film, cm^{-1}): 3008–2978 (CH), 1738 (C=O), 1467 (CH), 1353 (CH), 1243 (C-N). ^{1}H-NMR (250 MHz, D_2O, δ, ppm): 1.9–2.1 (m, 4H, CH_2-CH_2-CH-), 2.5–2.7 (m, 1H, CH_2-N-), 2.8 (s, 3H, CH_3-N-), 3.0–3.2 (m, 1H, CH_2-N-), 3.6–3.7 (m, 1H, -CH-CO-).

L-*N*-Methylproline *n*-propyl ester hydrochloride (3b). General procedure

Stirred suspension of L-*N*-methylproline (6.45 g; 50 mmol) in *n*-propanol (100 mL) was cooled to –50°C and thionyl chloride (6 mL; 78 mmol) was added dropwise in such a rate to maintain the temperature below –45°C. Then, the solution was allowed to warm up and stirring was continued for 20 h at room temperature followed by boiling under reflux for additional 5 h. Clear solution was concentrated under vacuum and the solid residue was twice dissolved in dichloromethane (50 mL), and concentrated to oily residue. The residue was dissolved in dioxane and lyophilized yielding L-*N*-methylproline *n*-propyl ester hydrochloride (**3b**) as white plates (8.3 g; yield 86%). IR (film, cm^{-1}): 2957 (CH), 2849 (CH), 1737 (C=O), 1311 (C-O), 1231 (C-N). ^{1}H-NMR (250 MHz, CD_3OH, δ, ppm): 0.9–1.0 (m, 3H, CH_3-CH_2-), 1.6–1.8 (m, 2H, -CH_2-CH_3), 1.9–2.3 (m, 4H, -CH_2-CH_2-CH-), 2.5–2.7 (m, 1H, CH_2-N-), 2.9 (s, 3H, CH_3-N-), 3.2–3.3 (m, 1H, CH_2-N-), 3.6–3.8 (m, 1H, -CH-CO-), 4.2–4.4 (m, 2H, -O-CH_2-).

L-*N*-Methylproline methyl ester hydrochloride (3a)

L-*N*-Methylproline methyl ester hydrochloride was prepared according to the general procedure described above from L-*N*-methylproline (6.45 g;

50 mmol), methanol (100 mL) and thionyl chloride (6 mL; 78 mmol). The isolated product was dissolved in dioxane and lyophilized yielding L-*N*-methylproline methyl ester hydrochloride (**3a**) as white plates (8.12 g; yield 90%). IR (film, cm^{-1}): 2957 (CH), 2849 (CH), 1737 (C=O), 1311 (C-O), 1231 (C-N). ^{1}H-NMR (250 MHz, D_2O, δ, ppm): 1.90–2.20 (m, 4H, -CH_2-CH_2-CH-), 2.45–2.55 (m, 1H, CH_2-N-), 2.9 (s, 3H, CH_3-N-), 3.10–3.20 (m, 1H, CH_2-N-), 3.70 (s, 3H, O-CH_3), 4.20–4.30 (m, 1H, -CH-CO-).

Synthesis of L-proline *n*-propyl ester hydrochloride (H-Pro-OC_3H_7 × HCl) (2d). Typical procedure

A vigorously stirred suspension of L-proline (5.76 g; 50 mmol) in *n*-propanol (20 mL) was cooled to 0°C and thionyl chloride (6 mL; 78 mmol) was added dropwise in such a rate to maintain temperature below 5°C. The stirring was continued at room temp. for 21 h, then concentrated under reduced pressure. The oily residue was dissolved again with *n*-propanol (10 mL) and evaporated under reduced pressure. The treatment with *n*-propanol and evaporation was repeated twice to solidify the residue. After drying to the constant weight in vacuum desiccator under P_2O_5, proline *n*-propyl ester hydrochloride (**2d**) (9.29 g, 96% yield) was obtained as a white solid. IR (film, cm^{-1}): 3382 (NH), 2971–2881 (CH), 1732 (C=O), 1232 (C-O), 1102 (C-N). ^{1}H-NMR (250 MHz, $CDCl_3$, δ, ppm): 0.9–1.0 (m, 3H, CH_3-), 1.6–1.65 (m, 2H, -CH_2-CH_2-CH_3), 1.7–1.8 (m, 2H, -CH_2-CH_2-), 2.1–2.2 (m, 1H, -CH_2-CH_2-), 2.4–2.5 (m, 1H, -CH_2-CH_2-), 3.5–3.7 (m, 2H, -CH_2-CH_2-), 4.1–4.3 (m, 2H, -CH_2-CH_2-CH_3), 4.4–4.5 (m, 1H, -CH_2-CH-C-O).

Synthesis of L-*N*-allylproline methyl ester (3c). Typical procedure

To the stirred solution of proline methyl ester hydrochloride (6.45 g; 50 mmol) in DMF (20 mL) finely grounded anhydrous potassium carbonate (27.64 g; 200 mmol) was added and then allyl bromide was added dropwise (4.25 mL; 50 mmol). Stirring was continued at room temperature for 4 days. The slurry was filtered. The filtrate was diluted with water (100 mL) and extracted with ethyl acetate (4 × 50 mL). The filtrate cake was hashed with ethyl acetate (30 mL). Washing was combined with ethyl acetate extract, dried with anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The residue (7.5 g) was poured on silica gel column and eluted with hexane – ethyl acetate (8 : 1, v/v) to isolate first fraction (1.21 g; R_f = 0.46 in hexane : ethyl acetate), then eluted with hexane – ethyl acetate (4 : 1, v/v) to isolate *N*-allylproline methyl ester (**3c**) (3.14 g; 37% yield). R_f = 0.41 hexane : ethyl acetate (6 : 1, v/v). IR (film, cm^{-1}): 3078, 2951, 2876, 2796, 1731, 1681, 1643, 1435, 1420, 1356, 1275, 1195, 1167, 1086, 1038, 995, 917, 760, 706, 670, 606, 557, 442, 422. ^{1}H-NMR (250 MHz, $CDCl_3$, δ, ppm): 1.68 (m, 3H, -CH_2-CH_2-CH_2-), 2.10–2.15 (m, 1H, -CH_2-CH_2-CH-), 2.28–2.38 (m, 1H, -CH_2-CH_2-CH-), 3.02–3.14 (m, 3H, =CH-CH_2-N- + -CH_2-CH_2-CH_2-), 3.22–3.29 (m, 1H,-N-CH-CH-), 3.65 (s, 3H, -$COCH_3$), 5.00–5.16 (m, 2H, CH_2=CH-), 5.78–5.94 (m, 1H, CH_2=CH-). LC/MS: 170.0878 ([M + H]$^+$, $C_9H_{16}NO_2^+$, 170.23).

Synthesis of L-*N*-allylproline *n*-propyl ester (3d)

In the synthesis there were used proline *n*-propyl ester hydrochloride (10.08 g; 52 mmol) DMF (20 mL), anhydrous potassium carbonate (29.17 g; 211 mmol) and allyl bromide (4.50 mL; 52 mmol). Product was isolated by column chromatography on silica gel. The first fraction (0.623, R_f = 0.58) was eluted with hexane : ethyl acetate (10 : 1, v/v). *N*-Allylproline *n*-propyl ester (**3d**) (6.13 g, yield 78%). R_f = 0.28 (hexane : ethyl acetate 6 : 1, v/v) was eluted with hexane : ethyl acetate (10 : 1, v/v) as the second fraction. ^{1}H-NMR (250 MHz, $CDCl_3$, δ, ppm): 0.93 (t, 3H, J_1 = 7.48 Hz, CH_3-CH_2-), 1.59–1.73 (m, 2H, -CH_2-CH_2-CH_3), 1.8–2.02 (m, 3H, -CH_2-CH_2-CH-), 2.12–2.23 (m, 1H, -CH_2-CH_2-CH-), 2.44–2.53 (m, 1H, -CH_2-CH_2-CH-), 3.13–3.28 (m, 3H, -CH_2-CH_2-N- + -CH_2-CH_2-), 3.34–3.42 (m, 1H, -CH_2-CH_2-CH-), 4.10 (t, 2H, J_1 = 6.79 Hz, -O-CH_2-CH_2-), 5.08–5.23 (m, 2H, CH_2-CH=), 5.87–6.03 (m 1H, -CH_2=CH). IR (film, cm^{-1}): 3079, 2967, 2878, 2797, 1728, 1686, 1643, 1460, 1419, 1392, 1378, 1355, 1269, 1170, 1086, 1059, 1039, 992, 916, 766, 707, 659, 558, 424. LC-MS: 198.2 ([M + H]$^+$, $C_{11}H_{20}NO_2^+$, 198.28).

Synthesis of L-*N*-allylproline allyl ester (3e)

To the stirred solution of L-proline (11.50 g; 100 mmol) in DMF (40 mL) finely grounded anhydrous potassium carbonate (41.46 g; 300 mmol) was added and then allyl bromide was added dropwise (24 mL; 300 mmol). Stirring was continued at room temperature until all proline was consumed. The slurry was filtered. The filtrate was diluted with water (150 mL) and extracted with ethyl acetate (5 × 50 mL). The filtrate cake was hashed with ethyl acetate (50 mL). Washing was combined with ethyl acetate extract, dried with anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The

oily residue (18.31 g) was poured on silica gel column and eluted with hexane : ethyl acetate (8 : 1, v/v) to isolate first fraction (13.35 g) R_f = 0.27 in hexane : ethyl acetate (8 : 1, v/v), then eluted with hexane : ethyl acetate (4 : 1, v/v) to isolate *N*-allylproline allyl ester (**3e**) (3.87g, yield 21%) R_f = 0.35 (hexane : ethyl acetate, 4 : 1, v/v) as colorless oil. ^{1}H-NMR (250 MHz, $CDCl_3$, δ, ppm): 1.74–2.03 (m, 3H, -CH_2-CH_2-CH_2-), 2.10–2.19 (m, 1H, -CH_2-CH_2-CH-), 2.35–2.45 (m, 1H, =CH-CH_2-N-), 3.08–3.23 (m, 3H, CH_2=CH- + -CH_2-CH-), 3.3–3.38 (m, 1H, -CH_2-CH-), 4.6–4.63 (m, 2H, -O-CH_2-CH), 5.06–5.35 (m, 4H, CH_2=CH-CH_2-N), 5.84 (m, 2H, CH_2=CH-CH_2-N + CH_2=CH-CH_2-O). IR (film, cm^{-1}): 3080, 2950, 2876, 2797, 2026, 1730, 1680, 1646, 1443, 1419, 1384, 1356, 1269, 1166, 1087, 1035, 989, 918, 768, 717, 658, 554, 488. LC-MS: 196.1 ([M + H]$^+$, $C_{11}H_{18}NO_2^+$, 196.26).

Condensations of 4-methoxybenzoic acid (6) with 4-toluidine (8) mediated by CDMT (4) and *n*-propyl ester of L-*N*-allylproline 3d. Typical procedure

CDMT (0.044 g; 0.25 mmol) was added to the stirred and cooled to 0°C solution of L-*N*-allylproline *n*-propyl ester (0.049 g; 0.25 mmol) in acetonitrile (2 mL). Progress of activation was monitored by TLC (mobile phase: dichloromethane; visualization by spraying plate with 0.5% solution of 4,4'-nitrobenzylpyridine in ethanol). After formation of red spot R_f = 0, *p*-methoxybenzoic acid (0.038 g; 0.25 mmol) and DIPEA (45 µL; 0.25 mmol) were added. Stirring was continued until TLC confirmed disappearing of red spot R_f = 0.6 characteristic for CDMT. Then *p*-toluidine (0.026 g; 0.25 mmol) was added and stirring was continued for additional 24 h at room temperature and solvent was removed under reduced pressure. Dry solid residue was dissolved in dichloromethane (15 mL) and washed successively with water (15 mL), 1 M aq. HCl (15 mL), water (15 mL), 1 M aq. sodium bicarbonate (15 mL) and again with water (15 mL). Organic layer was dried with magnesium sulfate, filtered and the filtrate was concentrated to dryness. White solid residue was dried to constant mass in vacuum desiccator yielding **9** (0.055 g; yield 91.2%). M.p. = 140°C, lit. (13) m.p. = 150°C. ^{1}H-NMR (250 MHz, $CDCl_3$, δ, ppm): 2.34 (s, 3H, CH_3-), 3.87 (s, 3H, -OCH_3), 7.1 (dd, 4H, -C_6H_4, J_1 = 8.23 Hz, J_2 = 48.96 Hz), 7.65 (dd, 4H, -C_6H_4, J_1 = 8.93 Hz, J_2 = 48.96 Hz). IR (film, cm^{-1}): 3339, 3080, 3006, 2962, 2917, 2840, 1901, 1746, 1707, 1650, 1595, 1578, 1514, 1499, 1464, 1444, 1402, 1378, 1321, 1307, 1295, 1237, 1178, 1122, 1101, 1030, 967, 936, 898, 837, 811, 792, 760, 653, 639, 626, 610, 541, 504, 430, 399.

Synthesis of L-5b (DMT/L-*N*-MePro-OMe/BF_4^-) from L-*N*-methylproline methyl ester tetrafluoroborate and CDMT

To the solution of L-*N*-methylproline methyl ester (5.50 g; 39 mmol) in dichloromethane (50 mL) equimolar amount of HBF_4 in ether (4.02 mL; 39 mmol) was added. Solvent was removed under reduced pressure yielding quantitatively L-*N*-methylproline methyl ester tetrafluoroborate as white solid. Solid residue was dissolved in acetonitrile (100 mL), anhydrous $NaHCO_3$ (6.10 g; 72 mmol) and CDMT (6.30 g; 39 mmol) were added and suspension was vigorously stirred at room temperature until all CDMT was consumed as monitored by TLC. The suspension was filtered. The filter cake was washed several times with acetonitrile and combined filtrates were evaporated under reduced pressure affording **5b** (DMT/L-*N*-MePro-OMe/BF_4^-) (12.0 g, yield 83%) as pale oil. ^{1}H-NMR (250 MHz, CD_3CN, δ, ppm): = 1.77–1.81 (m, 4H, -CH_2-CH_2-), 3.01–3.33 (m, 2H, -N-CH_2-), 3.46–3.66 (m, 1H, -O-CH-), 3.70 (s, 3H, CH_3-N), 3.81 (s, 3H, CH_3O-), 3.96 (s, 6H, 2 × CH_3-O).

Synthesis of D-5b (DMT/D-*N*-MePro-OMe/BF_4^-) from D-*N*-methylproline methyl ester tetrafluoroborate and CDMT

The synthesis was performed according to general procedure (see above) from D-*N*-methylproline methyl ester (5.50 g; 39 mmol), dichloromethane (50 mL), HBF_4 in ether (4.02 mL; 39 mmol), acetonitrile (100 mL), anhydrous $NaHCO_3$ (6.10 g; 72 mmol) and CDMT (6.30 g; 39 mmol). Product **D-5b** (DMT/D-*N*-MePro-OMe/BF_4^-) (3.92 g, yield 88%) was obtained as yellow oil. Spectroscopic data identical as presented above for **L-5b.**

Synthesis of L-5d (DMT/L-*N*-MePro-OMe/ClO_4^-) from L-*N*-methylproline methyl ester, CDMT and silver perchlorate

To the vigorously stirred and cooled to 0°C solution of CDMT (0.840 g; 4.8 mmol) in acetonitrile (8 mL), the solution of L-*N*-allylproline allyl ester (0.936 g; 4.8 mmol) in acetonitrile (5 mL) and solution of silver perchlorate (0.995 g; 4.8 mmol) in acetonitrile (5 mL) were added dropwise. The reaction progress was monitored by TLC. After all CDMT was consumed (disappeared spot R_f = 0.6, mobile phase dichloromethane) a deposit of silver chloride was filtered off and the filtrate was concentrated to dryness under reduced pressure. Product **L-5d** (DMT/L-*N*-MePro-OMe/ClO_4^-) (0.437g, yield 21%) was obtained as solid, m.p. = 103°C. IR (film, cm^{-1}): 2965, 2877, 1573, 1551, 1516, 1474, 1455,

1400, 1364, 1312, 1236, 1214, 1185, 1086, 1048, 804, 682, 621, 556, 530, 492, 396. LC-MS: 179 ([M + Na]/2, $C_{16}H_{23}N_4O_4^+$, 335.37).

Enantioselective synthesis of Z-AlaGly-OMe from *rac*-Z-Ala-OH using L-5b (DMT/L-*N*-MePro-OMe/BF_4^-). General procedure

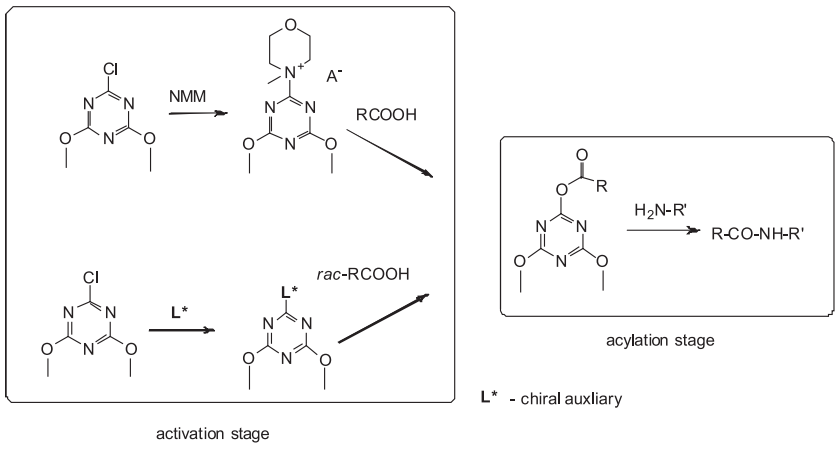
The vigorously stirred solution of **L-5b** (DMT/L-*N*-MePro-OMe/BF_4^-) (0.142 g; 0.5 mmol) in acetonitrile (5 mL) was cooled to 0°C and then *rac*-Z-Ala-OH (0.223 g; 1 mmol) and DIPEA(17.6 µL; 0.1 mmol) were added. Stirring was continued for 1 h and HCl × H-Gly-OMe (0.069 g; 0.5 mmol) and NMM (55 µL; 0.5 mmol) were added and the mixture was left at 0°C for 2 h. Then, it was allowed to warm-up to room temperature overnight. Solvent was removed under reduced pressure and residue was dissolved in dichloromethane (10 mL) and washed successively with water (3 mL), 1 M aq. HCl (3 mL), water (3 mL), 1 M aq. $NaHCO_3$ (3 mL) and again water (3 mL). Organic phase was dried with $MgSO_4$, filetered and evaporated to dryness yielding Z-D-Ala-Gly-OMe (99 µg, yield 67%), m.p. = 87–89°C; lit. m.p. = 94 – 96°C (14), $[\alpha]_D^{25}$ = (+) 18.5 (c = 1.0, MeOH); lit. $[\alpha]_D^{25}$ = (+) 26 (c = 1.0, MeOH) (14). IR (film/NaCl, cm^{-1}): 3321, 3017, 2966, 2937, 2903, 2274, 2143, 1984, 1815, 1759, 1695, 1666, 1586, 1536, 1480, 1448, 1410, 1374, 1362, 1320, 1247, 1191, 1168, 1127, 1072, 1054, 1017. ^{1}H-NMR (250 MHz, $CDCl_3$, δ, ppm): 1.41 (d, 3H, *J* = 6.5 Hz, CH_3-CH-), 3.78 (s, 3H, CH_3O-), 4.11 (q, 1H, *J* = 7 Hz, CH_3-CH-), 4.39 (m, 2H, -CH_2-CO-), 5.10 (s, 2H, -CH_2O-), 6.61 (broad s, 1H, NH), 7.40 (s, 5H, arom.).

Enantioselective synthesis of Z-AlaGly-OMe from *rac*-Z-Ala-OH using D-5b (DMT/D-*N*-MePro-OMe/BF_4^-).

Synthesis was carried out according to general procedure described above using **D-5b** (DMT/D-*N*-MePro-OMe/BF_4^-) (0.142 g; 0.5 mmol), rac-Z-Ala-OH (0.223 g; 1 mmol), DIPEA (17.6 µL; 0.1 mmol), HCl × H-Gly-OMe (0.069 g; 0.5 mmol) and NMM (55 µL; 0.5 mmol). Z-L-Ala-Gly-OMe (96 µg) was obtained (yield 65%), m.p. = 90–91°C; lit. m.p. = 94–96°C (15), $[\alpha]_D^{25}$ = (–) 17.2; lit. (15) $[\alpha]_D^{25}$ = (–) 23.2 (c = 1.0, MeOH). IR (film/NaCl, cm^{-1}): 3321, 3017, 2966, 2937, 2903, 2274, 2143, 1984, 1815, 1759, 1695, 1666, 1586, 1536, 1480, 1448, 1410, 1374, 1362, 1320, 1247, 1191, 1168, 1127, 1072, 1054, 1017. ^{1}H-NMR (250 MHz, $CDCl_3$, δ, ppm): 1.41 (d, 3H, *J* = 6.5 Hz, CH_3-CH-), 3.78 (s, 3H, CH_3O-), 4.11 (q, 1H, *J* = 7 Hz, CH_3-CH-), 4.39 (m, 1H, -CH_2-CO-), 5.10 (s, 2H, -CH_2O-), 6.61 (broad s, 1H, NH), 7.40 (s, 5H, aromatic).

RESULTS AND DISCUSSION

The problem of unpredictable results of enantioselective peptide synthesis from racemic substrates was resolved by designing the traceless enantioselective coupling reagents (16). According to this concept, traceless reagents are prepared by temporary attachment of the chiral fragment to a classic



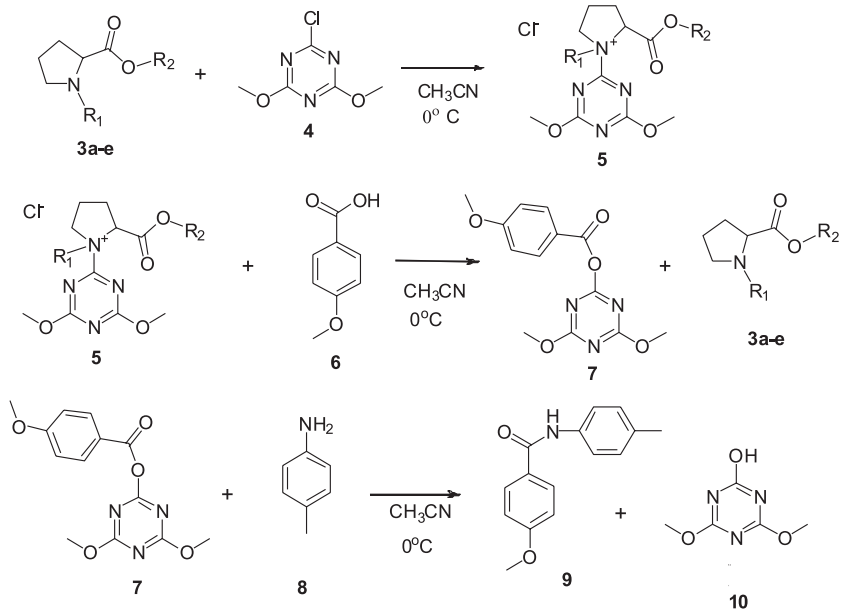
Scheme 1. Treatment of racemic carboxylic component with chiral traceless coupling reagent yields appropriate enantiomerically enriched activated derivative, the same for all chiral components in the whole family of enantioselective reagents as well as the entity identical with obtained using appropriate achiral counterpart

peptide-bond-forming agent. Thus, after selection of the enantiomer at the activation stage and departure of the chiral component, the activated intermediate is converted to a well-known form of a conventional achiral acylating species.

Moreover, it has been shown that configuration and enantiomeric enrichment once established at the activation stage remained intact and independent on the structure of acylated counterpart. This advantage is extremely important because all reaction parameters including configuration, optical purity of the product, optimal reaction conditions and the efficiency of coupling are predictable on the basis of a single experiment of the model reaction.

The first traceless enantioslective coupling reagents were designed based on chiral *N*-triazinylammonium salts derived from alkaloids (brucine, strychnine, quinine) (17). The versatility of the approach was fully confirmed by the high yield of peptide synthesis within up to 99% enantiomeric purity from racemic substrate, with expected configuration. However, until now, the main limitation in the broad application of chiral *N*-triazinylammonium salts are toxicity of the alkaloids and limited access to both enantiomeric forms of the chiral component of coupling reagent. To overcome this drawback, the search were undertaken for more versatile chiral components useful in enantioselective synthesis of peptide bond. In the introductory experiments it has been noticed that *N*-methylpyrrolidine is very versatile amino component in triazine mediated coupling reactions (18). Therefore, in order to open access to the representative collection of chiral nontoxic coupling reagents, the systematic studies were undertaken to develop procedures for synthesis of chiral *N*-methylpyrrolidines from proline which is readily available in both enantiomeric forms. In the first attempts an efforts were made to evaluate *N*-methyl and *N*-allylproline esters as chiral component in enantioselective activation of *N*-protected amino acids.

N-Methylproline (**1**) was prepared by treatment of proline with aqueous formaldehyde in the presence of zinc dust according to the modified procedure (12) with 92% yield. Methyl ester of *N*-methylproline (**3a**) and propyl esters of *N*-methylproline (**3b**) were obtained under classic conditions in reaction with appropriate alcohols and thionyl chloride in 90 and 86% yield, respectively. *N*-Allylproline methyl ester (**3c**) and *n*-propyl ester **3d** were



3a R_1= CH_3; R_2= CH_3
3b R_1= CH_3; R_2= $CH_2CH_2CH_3$
3c R_1= $CH_2CH{=}CH_2$; R_2= CH_3
3d R_1= $CH_2CH{=}CH_2$; R_2= $CH_2CH_2CH_3$
3e R_1= $CH_2CH{=}CH_2$; R_2= $CH_2CH{=}CH_2$

Scheme 2. Condensation of 4-methoxybenzoic acid (**6**) with 4-toluidine (**8**) mediated by 2-chloro-4,6-dimethoxy-1,3,5-triazine (**4**) in the presence of esters of *N*-alkylproline **3a-e**

obtained in 90% yield according to procedure of Hassner (18) in reaction of appropriate proline ester **2c** or **2d** with allyl bromide in the presence of potassium carbonate. The structure of **3c** was confirmed by MS analysis based on intensive (M + 1) signal m/z = 170.08 expected for *N*-allylproline methyl ester (M = 169.23). *N*-Allylproline allyl ester (**3e**) was prepared by direct *N*- *O*- dialkylation of proline with two equivalents of allyl bromide in the presence of potassium carbonate. Its structure was confirmed by the presence of double triplet at δ = 3.20 ppm and triplet at δ = 4.50 ppm in ^{1}H-NMR spectrum corresponding respectively to the allyl methylene fragment attached to nitrogen and oxygen and by the presence of $[M + 1]^+$ ion m/z = 196.1.

Model coupling reactions of 4-methoxybenzoic acid (**6**) with 4-toluidine (**8**) by 2-chloro-4,6-dimethoxy-1,3,5-triazine (**4**) in the presence of esters of *N*-alkylproline **3a-e** gave expected amide (**9**) with good yield (Scheme 2).

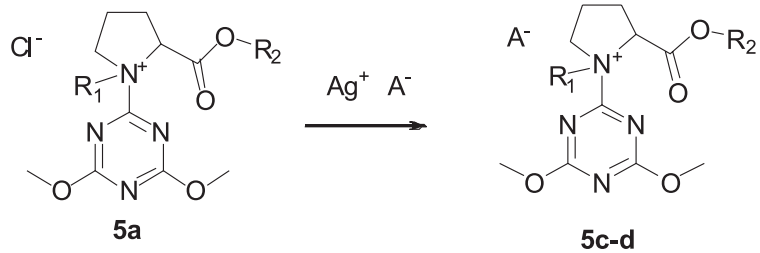
This confirmed that simple derivatives of *N*-alkylproline **3a-e** can be potentially used as chiral components in enantioselective condensations, although in all cases studied, progress of condensation was relatively slow and 24 h coupling time was necessary for reaction to be completed.

Careful examination of reacting mixture evidenced, that the rate limiting step is formation of *N*-triazinylammonium chloride **5a** in reaction of CDMT (**4**) with *N*-alkylproline ester **3a-e**. This circumstance is adverse for enantiodifferentiating procedures because prolonged contact of reactants forecast dealkylation of **5a**, racemization of chiral component **3a-e** and favors formation of *mezo*-anhydrides by acylation discriminated enantiomer of carboxylic acid with carboxylic acid enantiomer preferred in the activation step.

The remedy preventing this shortcoming is to separate the rate limiting quaternization of *N*-alkylproline ester **3a-e** in reaction with CDMT (**4**) and

Table 1. Condensations of 4-methoxybenzoic acid (**6**) with 4-toluidine (**8**) mediated by CDMT (**4**) and esters of *N*-alkylproline **3a-e**.

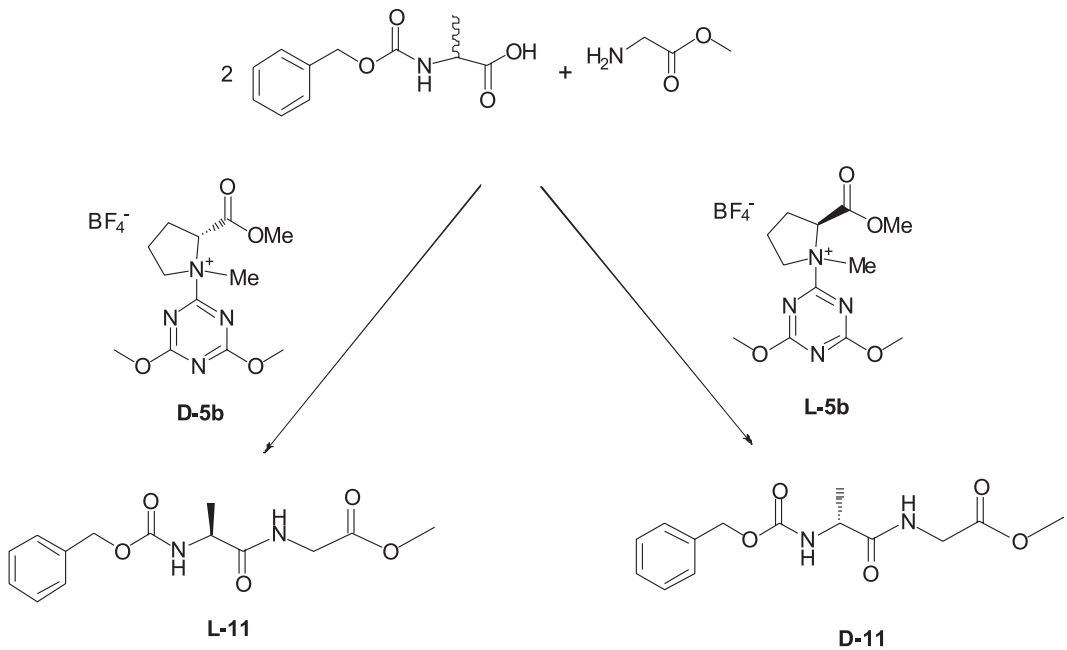
Entry	*N*-alkylproline ester **3a-e**	Solvent	Activation time [h]	Yield [%]
1	**3a**	acetonitrile	24	78.7
2	**3b**	acetonitrile	24	60
3	**3a**	THF	24	72.9
4	**3b**	THF	24	65.5
5	**3c**	acetonitrile	24	93
6	**3d**	acetonitrile	24	98
7	**3e**	acetonitrile	24	98



5c A$^-$ = 4-$CH_3C_6H_4$-SO_3^-

5d A$^-$ = ClO_4^-

Scheme 3. Stable salts of triazine based coupling reagents **5c-d**



Scheme 4. Enantioselective coupling of racemic Z-Ala-OH with H-Gly-OMe to **11**, mediated by enantiomeric coupling reagents **L-5b** and **D-5b**

Table 2. Synthesis of Z-Ala-Gly-OMe from racemic Z-Ala-OH in the presence of chiral coupling reagents **L-5b** and **D-5b**.

Coupling reagent	Yield [%]	$[\alpha]_D^{25}$ (c = 1.0. MeOH)	Lit. $[\alpha]_D^{25}$ (c = 1.0, MeOH)	Preferred configuration	D/L [%]
L-5b	67	(+) 18.5	(+) 26 (14)	D	85/15
D-5b	65	(–) 17.2	(–) 23.2 (15)	L	13/87

substitute unstable *N*-triazinylammonium chlorides **5a** with stable salts of triazine based coupling reagents prepared in the form of appropriate salt with non-nucleophilic tetrafluoroborate, tosylate or perchlorate counterion **5b-d**. The treatment of L *N*-methylproline methyl ester (**3a**) with tetrafluoroboric acid solution and then reaction with CDMT gave appropriate tetrafluoroboric salt **L-5d** (DMT/L-*N*-MePro-OMe/BF_4^-) in 83% yield and **D-5b** (DMT/D-*N*-MePro-OMe/BF_4^-) in 88% yield, respectively. Appropriate perchlorate **L-5d** (DMT/L-*N*-MePro-OMe/ClO_4^-) was prepared by treatment of L-*N*-methylproline methyl ester (**3a**) with CDMT in the presence of silver perchlorate. Unfortunately, the poor solubility of **L-5d** in solvents used in peptide synthesis severely limited its synthetic applications. Furthermore, both procedures failed in the attempts to prepare 4-toluenesulfonate **5c** (DMT/L-*N*-MePro-OMe/TsO_3^-).

The versatility and selectivity of **5a-d** were studied both in model condensation of **6** with 4-toluidine (**8**) and in enantioselective coupling of racemic Z-Ala-OH with H-Gly-OMe.

It has been found that **L-5b** obtained from L enantiomer of methyl esters **3a** prefers formation of D dipeptide with L/D ratio 79/21, but enantiomeric form of **D-5b** prepared from D enantiomer of methyl esters **3a** prefers formation of L dipeptide with reverse L/D ratio 25/75. This demonstrated that relatively simple, non-toxic, non-expensive and readily accessible in both enantiomeric forms components for synthesis of traceless enantioselective coupling reagents can be obtained from proline.

CONCLUSION

The deteriorating effect of electron withdrawing ester group of proline severely reducing nucleophilicity of nitrogen in *N*-alkylproline esters in reaction with CDMT, as compared to not substituted *N*-alkylpyrrolidines, has been overcome by transformation of unstable quaternary chlorides into stable *N*-triazinyl-*N*-methylpyrrolidinium tetrafluoroborates **L-5b** and/or **D-5b.**

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REFERENCES

1. Committee for Proprietary Medical Products (1993) Working parties on quality, safety and efficacy of medical products. Note for guidance: investigation of chiral active substances. III/3501/91.
2. Agranat I., Caner H., Caldwell J.: Nat. Rev. Drug Discov. 1, 753 (2002).
3. Hava C., Efrat G., Liron L., Israel A.: Drug Discov. Today 9, 105 (2004).
4. a) Garret C.E., Fu G.C.: J. Am. Chem. Soc. 120, 7479 (1998); b). Arai S., Bellemin-Laponnaz S., Fu G.C.: Angew. Chem. 113, 240 (2001); c). Arai S., Bellemin-Laponnaz S., Fu G.C.: Angew. Chem. Int. Ed. 40, 234 (2001); d) Shaw P., Aleman S.A., Christy J., Kampf J. ., Vedejs E.: J. Am. Chem. Soc. 128, 925 (2006); e) Mermerian. A.H., Fu G.C.: Angew. Chem. 117, 971 (2005); f) Mermerian A.H., Fu G.C.: Angew. Chem. Int. Ed. 44, 949 (2005); g) Spivey A.C., Zhu F., Mitchell M.B., Davey S.G., Jarvest R.L.: J. Org. Chem. 68, 7379 (2003); h) Kawabata T., Stragies R., Fukaya T., Nagaoka Y., Schedel H., Fuji K.: Tetrahedron Lett. 44, 1545 (2003); i) Priem G., Pelotier B., Madonald S.J.F., Anson M.S., Campbell I.B.: J. Org. Chem. 68, 3844 (2003); j) Dalaigh C.O., Hynes S.J., O'Brien J.E., McCabe T., Maher D.J., Watson G.W., Connon S.J.: Org. Biomol. Chem. 4, 2785 (2006); k) Anstiss M., Nelson A.: Org. Biomol. Chem. 4, 4135 (2006).
5. Birman V.B., Li X.: Org. Lett. 8, 1351 (2006).
6. a) Jarvo E.R., Miller S.J.: Tetrahedron 58, 2481 (2002); b) Sibi M.P., Maneyem S.: Tetrahedron 56, 8033 (2000); c) Copeland G.T., Jarvo E.R., Miller S.J.: J. Org. Chem. 63, 6784 (1998); d) Copeland G.T., Miller S.J.: J. Am. Chem. Soc. 121, 4306 (1999); e) Harris R.F., Nation A.J., Copeland G.T., Miller S.J.: J. Am. Chem. Soc. 122, 11270 (2000); f) Vasbinder M.M., Jarvo E.R., Miller S.J.: Angew. Chem. 113, 2906 (2001); g) Vasbinder M.M., Jarvo E.R., Miller S.J.: Angew. Chem. Int. Ed. 40, 2824 (2001); h) Birman V.B., Uffman E.W., Jiang H., Li X., Kilbane C.J.: J. Am. Chem. Soc. 126, 12226 (2004).
7. a) Takeda, K. Tsuboyama K., Suzuki A., Ogura H.T.: Chem. Pharm. Bull. 33, 2545 (1985); b) Teramoto T., Deguchi M., Kurosaki T.: Tetrahedron Lett. 22, 1109 (1981); c) Teramoto T., Kurosaki T.: Tetrahedron Lett. 18, 1523 (1977); d) Markowicz S.W., Karolak-Wojciechowska J.: Pol. J. Chem. 68, 1973 (1994); e) Kricheldorf H.R., Au M., Mang T.: Int. J. Pept. Protein Res. 26, 149 (1985); f) Kricheldorf H.R., Mang T.: Makromol. Chem. 183, 2093 (1982).
8. a) Murakami Y., Kondo K., Miki K., Akiyama Y., Watanabe T., Yokoyama Y.: Tetrahedron Lett. 38, 3751 (1997); b) Kondo K., Murakami Y.: Chem. Pharm. Bull. 46, 1217 (1998); c) Kondo K., Kurosaki T., Murakami Y.: Synlett 725 (1998); d) Al-Sehemi A.G., Atkinson R.S., Fawcett J., Russel D.R.: J. Chem. Soc., Perkin Trans. 1, 4413 (2000); e) Günster E.J., Scholtz R.C.: Makromol. Chem. 181, 643 (1980); f) Evans D.A., Andersen J.C., Taylor M.K.: Tetrahedron Lett., 34, 5563 (1993); g) Jencks P.W., Gilchrist M.: J. Am. Chem. Soc. 90, 2622 (1968); h) Maezaki N., Furusawa A., Uchida S., Tanaka T.: Tetrahedron 57, 9309 (2001).
9. a) Wegler R.: Liebigs Ann. Chem. 498, 62 (1932); b) Oriyama T., Hori Y., Imai K., Sasaki R.: Tetrahedron Lett. 37, 8543 (1996); c) Sano T., Imai K., Ohashi K., Oriyama T.: Chem. Lett. 265 (1999); d) Oriyama T., Imai K., Sano T., Hosoya T.: Tetrahedron Lett. 39, 3529 (1998); e) Terakado D., Koutaka H., Oriyama T.: Tetrahedron Asymmetry 16, 1157 (2005).
10. a) Vedejs E., Diner S.T.: J. Am. Chem. Soc. 115, 3358 (1993); b) Vedejs E., Bennett N.S., Conn L.M., Diver S.T., Gingras M., Lin S., Oliver P.A., Peterson M.J.: J. Org. Chem. 58, 7286 (1993); c) Bogatkov S.V., Golovina Z.P., Fild V.R. , Cherkasova E.M.: Zh. Org. Khim. 13, 2271 (1977); d) MacNeil P.A., Roberts N.K., Bosnisch B.: J. Am. Chem. Soc. 103, 2273 (1981); e) Gladiali S., Dore A., Fabbri D., De Lucchi O., Manassero M.: Tetrahedron:

Asymmetry 5, 511 (1994); f) Burk M.J., Feaster J.E., Harlow R.L.: Tetrahedron: Asymmetry 2, 569 (1991); g) Vedejs E., Daugulis O., Harper L.A., MacKay J.A., Powell D. R.: J. Org. Chem. 68, 5020 (2003); h) MacKay J.A., Vedejs E.: J. Org. Chem., 69, 6934 (2004); i) Methot J.L., Roush W.R.: Adv. Synth. Catal. 346, 1035 (2004).

11. a) Srseniyadis S., Valleix A., Wagner A., Mioskowski C.: Angew. Chem. 116, 3376 (2004); b) Srseniyadis S., Valleix A., Wagner A., Mioskowski C.: Angew. Chem. Int. Ed.: 43, 3314 (2004); c) Pelotier B., Priem G., Macdonald S.J.F., Anson M.S., Upton R.J., Campbell I.B.: Tetrahedron Lett. 46, 9005 (2005); d) Lewis C.A., Sculimbrene B.R., Xu Y., Miller S.J.: Org. Lett. 7, 3021 (2005); e) Birman V.B., Jiang H., Li X.: Org. Lett. 9, 3237 (2007); f) Kosugi Y., Akakura M., Ishihara K.: Tetrahedron 63, 6191 (2007); g) Stamatov S.D., Stawinski J.: Org. Biomol. Chem. 5, 3787 (2007); h) Karnik A.V., Kamath S.S.: J. Org. Chem. 72, 7435 (2007); i) Notte G.T., Sammakia T., Steel P.J.: J. Am. Chem. Soc. 127, 13502 (2005); j) Terakado D., Oriyama T.: Org. Synth. 83, 70 (2006); k) Moretto A., Peggion C., Formaggio F., Crisma M., Kaptein B., Broxterman Q.B., Toniolo C.: Chirality, 17, 481 (2005).
12. Moehrle R., Siekeor Z.: Arch. Pharm. (Weinheim) 309, 380 (1974).
13. Avenoza A, Pereprine J.: Tetrahedron 58, 10107 (2002).
14. Grzonka Z., Palacz Z., Baran L., Przegalinski E., Kupryszewski G.: Pol. J. Chem. 55, 1025 (1981).
15. Kawasaki K., Maeda M., Tamura, H., Ohashi S., Yoshimura T., Hatayama E.: Chem. Pharm. Bull. 32, 1717 (1984).
16. Kolesinska B., Kaminski Z.J.: Org. Lett., 11, 765 (2009).
17. a) Kolesinska B., Kasperowicz K., Sochacki M., Mazur A., Jankowski S., Kaminski Z.J.: Tetrahedron Lett. 51, 20 (2010); b) Kolesinska B., Kasperowicz-Frankowska K., Fraczyk J., Kaminski Z.J.: Helv. Chim. Acta 95, 2084 (2012); c) Kaminski, Z.J.; Kolesinska, B. Patent EPO 09001796,3-1211, priority PL/04. 02. 08/ PLA 384377608.
18. Kaminski Z. J, Kolesinska B, Kolesinska J, Jastrzabek K.: EP-05006473.2-2117 PCT, C07D 251/45, 18. 08. 2005.
19. da Silva R. A., Estevamb I. H. S., Bieber L. W.: Tetrahedron Lett. 48, 7680 (2007).
20. Hassner A., Maurya R.: J. Org. Chem. 56, 2775 (1991).