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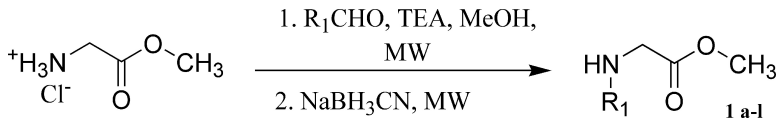

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Efficient Microwave Combinatorial Parallel and Nonparallel Synthesis of N-Alkylated Glycine Methyl Esters as Peptide Building Blocks

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An easy and convenient microwave-assisted synthesis of N-alkylated glycine methyl esters is described. Parallel and nonparallel combinatorial methods are described and compared. The described reactions are reductive alkylations of several aldehydes and glycine methyl ester in the presence of $NaBH_3CN$. Good yields and short reaction times are the main aspects of these procedures.

Introduction

There is a continuing need to develop "novel" peptidomimetics that can provide specific three-dimensional properties inside the target receptor to obtain agonist or antagonist results. In fact, intensive efforts have been made to develop peptidomimetics displaying pharmacological properties more favorable than the prototypes with regard to specific action, resistance to degradation, and bioavailability.[1]

On this basis, an interesting idea was the proposal to shift the amino acid side chains from the α-carbon to the nitrogen, yielding N-substituted glycine derivatives.[2] These amino acid analogues are important building blocks that can be introduced in the syntheses of a great number of peptides, allowing the introduction of pharmacologically relevant functional groups in an unconventional position of the peptide.

A wide variety of reaction conditions have been published for the synthesis of N-alkylated amino acid derivatives in solution and on solid phase; among these, reductive alkylation plays an important role, and several reducing agents have been employed.[3−4]

Knowing the importance of the replacement of the amino acids with the corresponding N-alkyl-substituted glycine derivatives, our attention has turned to improving the syntheses of these modified residues, bearing in mind new synthetic methodologies.[5] In fact, in recent years, our interests have been directed to the advances in the syntheses of peptidomimetics, particularly to the application of microwave irradiation in the field of peptide chemistry.[5−6] Moreover, combinatorial chemistry is recognized as a very powerful tool for the acceleration of the drug discovery process.[7] Starting from these considerations, we developed a small library of N-alkylated glycine methyl esters obtained by microwave flash heating using both parallel and nonparallel combinatorial methods. The application of microwave energy to organic compounds for conducting synthetic reactions at highly accelerated rates has become a well-known technique.[5,8] In fact, in recent years, microwaves have become popular among synthetic organic chemists to improve classical organic reactions, shortening reaction times, improving yields, or both, as well as to promote new reactions.

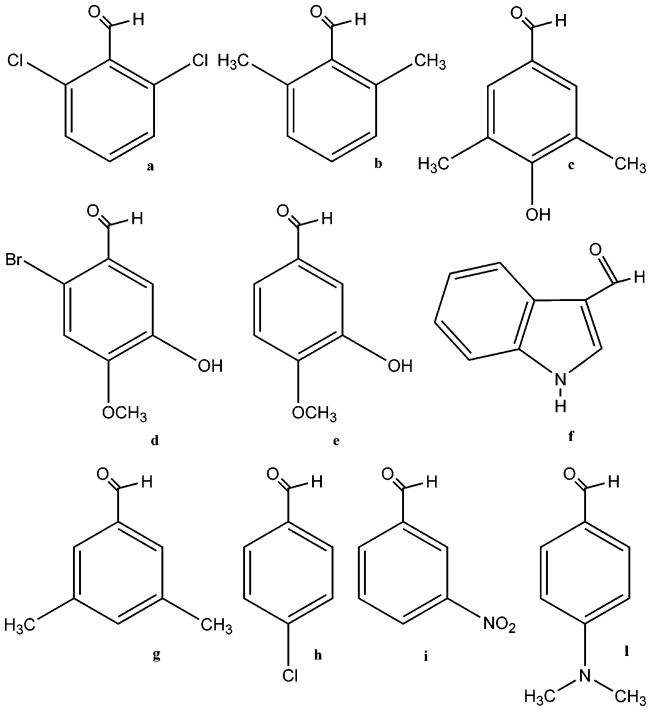

Figure 1. Aldehydes used for the generation of the library.

Results and Discussion

At first, we investigated the parallel reductive alkylation of *N*-glycine methyl ester in the presence of 10 different commercially available aromatic aldehydes (Figure 1) and $NaBH_3CN$; the selected aldehydes provide modified amino acids that can mimic aromatic conventional and unnatural amino acids. Each reaction was performed in a sealed tube fitted in a 36-position multiPREP rotor (Milestone); the other tubes were filled with the same solvent of the reactions

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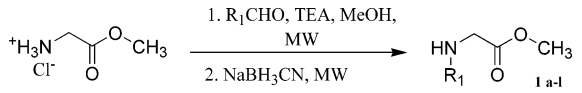
Table 1. *N*-Alkyl-glycine Methyl Esters by Combinatorial Microwave Irradiation



entry	parallel synthesis[c] yield[a] (%)	nonparallel synthesis[d] yield (%)
1a[b]	32.3	29
1b	60.5	42.4
1c	27	21.3
1d	57.2	40.5
1e	28	18.8
1f	45.4	53.5
1g	70.4	57.5
1h	39.4	40.7
1i	63	50.4
1l	34.3	51.2

[a] All the reactions were performed three times, and the reaction yields given are the average values. [b] Conventional heating at 45 °C, 2 h, 16.5% yield. [c] 5 min of irradiation for the formation of the Schiff base and 10 min of irradiation for the reduction. [d] 5 min of irradiation for the formation of the Schiff base and 20 min of irradiation for the reduction.

(methyl alcohol). The synthetic procedure, summarized in Table 1, was performed using a microwave oven (ETHOS 1600, Milestone) especially designed for organic synthesis and following a microwave program which consisted of appropriate ramping and holding steps. The temperature of the stirred reaction mixture was monitored by an IR probe and rotation of the rotor, and irradiation time and power were monitored with the "easyWAVE" software package. The reductive alkylation was performed by two steps of irradiation with 300 W, 45 °C, in methyl alcohol for a total event time of 15 min (Table 1). This condition was found to be the optimized one because higher temperatures, times, or power either gave no increase in the obtained yields or resulted in decomposition of the reagents.

The main advantage to this synthetic route is that a short time of irradiation of the reaction mixtures provided the compounds **1a**−**l** as the major products. After workup, the final compounds were characterized by ESI-MS and ^{1}H NMR. Results obtained are summarized in Table 1, and obtained yields were generally satisfactory, especially if compared to time and yields of the same reactions performed by conventional heating (Table 1).

As a starting point for the nonparallel procedure, a small amount of pure compounds **1a**−**l**, previously obtained, was mixed, and several analytical RP-HPLC elution tables with a Vydac C18-column (5 μm, 4.6 × 250 mm, spherical) were evaluated to optimize a complete separation. The best condition for analytical determination was found to be the following one, carried out by two solvent systems: A, 100% acetonitrile with 0.1% TFA; B, 100% H_2O with 0.1% TFA (linear gradient from 0% A to 50% B over 50 min, UV detection at 220 nm, flow rate 1 mL/min). The obtained chromatographic run showed sufficient separation between the compounds to undergo preparative purification (Figure 2). On the basis of the obtained results, a nonparallel synthetic procedure was developed as a one-pot reaction: *N*-glycine methyl ester was reacted with 10 different commercially available aromatic aldehydes, and $NaBH_3CN$ was added to the mixture; the synthetic procedure was performed using the Milestone microwave oven (ETHOS 1600). We followed the same microwave program of the parallel procedure, and reductive alkylation was performed by two steps of irradiation with 300 W, 45 °C, in methyl alcohol for a total event time of 25 min. The reaction mixture was subjected to an extraction with brine and was purified by preparative RP-HPLC applying the same gradient used for the analytical determinations.

The results obtained are summarized in Table 1 and show that there is not a significant difference between yields obtained in parallel or nonparallel methods; a small reduction in yields is evidenced in nonparallel synthesis, but it can be attributed to the greater complexity of the crude mixture in which the reactivity of the aldehydes plays a dominant role.

In conclusion, we have shown that the application of microwave irradiation and the simultaneous presence of $NaBH_3CN$ improve the yields and significantly reduce reaction times in the synthesis of N-alkylated glycine methyl esters, important building blocks that can be introduced in the syntheses of a great number of peptides, in either parallel or non parallel combinatorial methods.

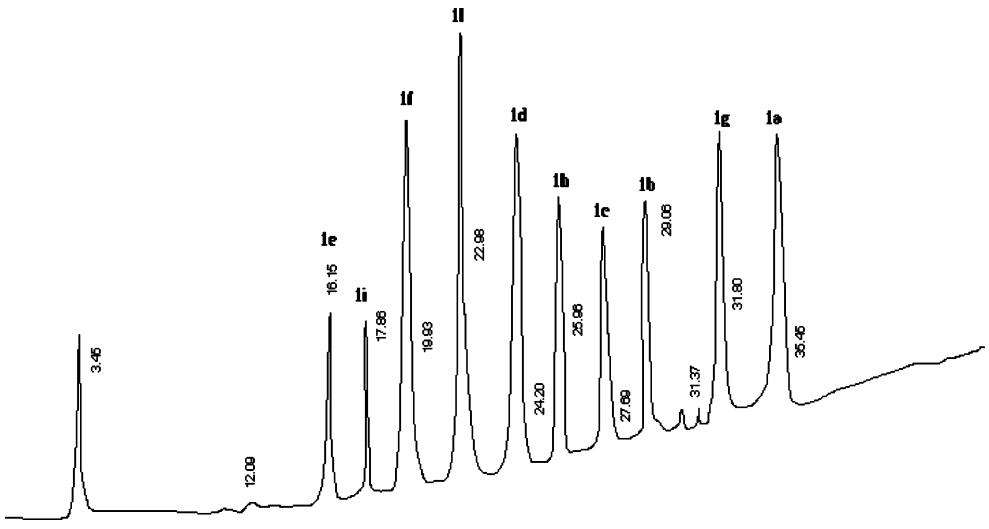


Figure 2. HPLC records of separation of N-alkylated glycine methyl esters.

Experimental Section

The aldehydes, used as starting reagents, are commercially available (Aldrich). All reactions were performed in standard Pyrex glassware or in sealed tubes using a microwave oven (ETHOS 1600, Milestone) especially designed for organic synthesis.

Thin-layer chromatography was performed on Macherey−Nagel silica gel 50 plates with fluorescent indicator, and the plates were visualized with UV light (254 nm). Kieselgel 60 was used for column chromatography. All synthetic reagents were purchased from the Aldrich-Sigma Chemical Company and were used without purification. Solvents were analytical reagent grade or higher purity and were used as supplied. A Buchi R-114 rotavapor was utilized for the removal of the solvents in vacuo. Analytical RP-HPLC was performed with a Vydac C18-column (5 μm, 4.6 × 250 mm, spherical). Preparative RP-HPLC was performed by the same gradient as that used for the analytical determinations. The operational flow rate was 30 mL/min.

The structures were verified spectroscopically by proton ^{1}H NMR, C^{13} NMR, and MS. Spectra were recorded on Varian Mercury Plus 400 instrument. Chemical shifts are referred to Me_4Si as internal standard. Mass spectra of the final products were performed on an Applyed Biosystem API 2000 mass spectrometer.

General Parallel Synthetic Procedure. Using a multiPREP rotor (Milestone) with 36 reaction tubes, glycine methyl ester hydrochloride (2.39 mmol) and the appropriate aldehyde (2.39 mmol) were added to each reaction tube and were dissolved in methyl alcohol. An equimolar amount of triethylamine was added, and then the tubes were closed. The reactions were heated in a Milestone microwave oven at 45 °C using 300 W of power for 5 min. After 1 min of ventilation, the tubes were opened, and an excess of $NaBH_3CN$ (4.78 mmol) was added to each reaction mixture, followed by a new step of microwave heating at 45 °C using 300 W of power for 10 min. Each reaction was evaporated, and the crude mixture was taken up in ethyl acetate. Each organic phase was transferred into a separating funnel and washed with a 10% aqueous solution of citric acid. The aqueous phase was alkalinized by NaOH, 1 N, for compounds **1a**−**1b** and **1f**−**1l** and reextracted with dichloromethane. When aldehydes were substituted with a phenolic ring (comp. **1c**−**1e**), the extractions were avoided. The solvent was removed, and the mixture was purified by silica gel column chromatography to obtain the pure compounds **1a**−**l**.

General Nonparallel Synthetic Procedure. Glycine methyl ester hydrochloride (23.9 mmol) and 10 commercially available aldehydes (2.39 mmol each) were dissolved in a two-neck flask in methyl alcohol. An equimolar amount of triethylamine (23.9 mmol) was added, and then the reaction was transferred to the microwave oven. The reaction was heated and stirred for 5 min at 45 °C using 300 W of power. After 1 min of ventilation, an excess of $NaBH_3CN$ (47.8 mmol) was added, and the mixture was heated further for 20 min under the same conditions. After heating, the solvent was evaporated, and the mixture was washed with brine to remove the excess of $NaBH_3CN$. The crude mixture was dried over Na_2SO_4 anhydrous and evaporated to dryness. Purification by preparative RP-HPLC afforded the pure compounds **1a**−**l**.

(2,6-Dichlorobenzylamino)-acetic Acid Methyl Ester (1a). ESI: 250 (MH^+). ^{1}H NMR ($CDCl_3$): δ 7.31 (d, 2H, J = 7.7 Hz), 7.29 (t, 1H, J = 7.7 Hz), 4.13 (s, 2H), 3.69 (s, 3H), 3.46 (s, 2H), 2.18 (s, NH). C^{13} NMR ($CDCl_3$): δ 172.7, 136.35, 135.14, 129.41, 28.64, 52.08, 49.89, 47.96.

(2,6-Dimethylbenzylamino)-acetic Acid Methyl Ester (1b). ESI: 208 (MH^+). ^{1}H NMR ($CDCl_3$): δ 7.06 (t, 1H, J = 6.5 Hz), 7.03 (d, 2H, J = 6.5 Hz), 3.78 (s, 2H), 3.75 (s, 3H), 3.48 (s, 2H), 2.42 (s, 3H), 1.62 (s, NH). C^{13} NMR ($CDCl_3$): δ 173.41, 137.54, 136.1, 128.46, 127.44, 51.94, 50.80, 47.55, 19.70.

(4-Hydroxy-3,5-dimethylbenzylamino)-acetic Acid Methyl Ester (1c). ESI: 224 (MH^+). ^{1}H NMR ($CDCl_3$): δ 6.93 (s, 2H), 3.73 (s, 3H), 3.65 (s, 2H), 3.41 (s, 2H), 2.23 (s, 3H), 1.62 (s, NH). C^{13} NMR ($CDCl_3$): δ 173.31, 137.84, 136.54, 128.62, 128.01, 52.03, 51.12, 47.97, 19.23.

(2-Bromo-5-hydroxy-4-methoxybenzylamino)-acetic Acid Methyl Ester (1d). ESI: 304 (MH^+). ^{1}H NMR ($CDCl_3$): δ 6.95 (s, 1H), 6.92 (s, 1H), 3.83 (s, 3H), 3.76 (s, 2H), 3.71 (s, 3H), 3.45 (s, 2H). C^{13} NMR ($CDCl_3$): δ 172.74, 146.92, 145.43, 136.23, 116.95, 115.36, 113.02, 56.44, 52.70, 52.07, 49.78.

(3-Hydroxy-4-methoxybenzylamino)-acetic Acid Methyl Ester (1e). ESI: 226 (MH^+). ^{1}H NMR ($CDCl_3$): δ 6.89 (d, 1H, J = 7.8), 6.79 (d, 1H, J = 7.8), 6.78 (s, 1H), 3.86 (s, 2H), 3.72 (s, 3H), 3.70 (s, 3H), 3.40 (s, 2H). C^{13} NMR ($CDCl_3$): δ 173.12, 145.92, 145.56, 133.14, 120.04, 114.89, 110.81, 56.21, 52.96, 52.05, 49.85.

[(1*H*-Indol-3-ylmethyl)-amino]acetic Acid Methyl Ester (1f). ESI: 219 (MH^+). ^{1}H NMR ($CDCl_3$): δ 7.69 (d, 1H, J = 7.2), 7.36 (d, 1H, J = 7.2), 7.19 (t, 1H, J = 7.2), 7.11 (t, 1H, J = 7.2), 7.10 (s, 1H), 4.00 (s, 2H), 3.71 (s, 3H), 3.48 (s, 2H), 1.84 (s, NH). C^{13} NMR ($CDCl_3$): δ 172.99, 137.95, 127.56, 123.37, 122.41, 119.88, 119.00, 111.47, 51.00, 49.88, 44.26.

(3,5-Dimethylbenzylamino)-acetic Acid Methyl Ester (1g). ESI: 208 (MH^+). ^{1}H NMR ($CDCl_3$): δ 6.98 (s, 2H), 6.93 (s, 1H), 3.72 (s, 3H), 3.44 (s, 2H), 3.41 (s, 2H), 2.29 (s, 3H), 2.95 (s, NH). C^{13} NMR ($CDCl_3$): δ 173.41, 139.54, 138.28, 129.10, 126.36, 53.38, 52.07, 50.01, 21.46.

(4-Chlorobenzylamino)-acetic Acid Methyl Ester (1h). ESI: 214 (MH^+). ^{1}H NMR ($CDCl_3$): δ 7.27 (d, 2H, J = 2.2 Hz), 7.26 (d, 2H, J = 2.2 Hz), 3.76 (s, 2H), 3.72 (s, 3H), 3.39 (s, 2H). C^{13} NMR ($CDCl_3$): δ 172.89, 137.23, 136.65, 127.52, 124.46, 52.23, 50.06, 22.11.

(3-Nitrobenzylamino)-acetic Acid Methyl Ester (1i). ESI: 225 (MH^+). ^{1}H NMR ($CDCl_3$): δ 8.20 (s, 1H), 8.09 (d, 1H, J = 8.1 Hz), 7.67 (d, 1H, J = 8.1 Hz), 7.48 (t, 1H, J = 8.1 Hz), 3.89 (s, 2H), 3.72 (s, 3H), 3.44 (s, 2H), 2.81 (s, NH). C^{13} NMR ($CDCl_3$): δ 173.89, 170.15, 141.95, 134.56, 129.62, 122.53, 52.51, 50.01, 22.87.

(4-Dimethylaminobenzylamino)-acetic Acid Methyl Ester (1l). ESI: 223 (MH^+). ^{1}H NMR ($CDCl_3$): δ 7.19 (d, 2H, J = 8.8 Hz), 6.70 (d, 2H, J = 8.8 Hz), 3.72 (s, 3H), 3.69 (s, 2H), 3.40 (s, 2H), 2.92 (s, 6H), 1.83 (s, NH).

C^{13}NMR ($CDCl_3$): δ 173.33, 140.75, 135.16, 128.67, 122.43, 53.47, 52.31, 50.07, 22.87.

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