Arch Pharm Res Vol 27, No 1, 25-30, 2004

Archives of Pharmacal Research
http://apr.psk.or.kr

Identification of Homoserine Lactone Derivatives Using the Methionine Functionalized Solid Phase Synthesis by Gas Chromatography/Mass Spectrometry

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(Received October 20, 2003)

Combinatorial homoserine lactone mixtures and individual products were obtained from the methionine-functionalized resin in solid-phase synthesis. The four-step process consisting of a coupling step of an *N*-Fmoc-L-methionine, deprotection of *N*-Fmoc group, *N*-coupling with a carboxylic acid, and cleavage reaction through a polymer supported strategy is described. Gas chromatography-mass selective detector (GC-MSD) techniques provide the most powerful methods for identifying both the combinatorial mixtures and individual products.

Key words: Combinatorial homoserine lactones, Methionine-functionalized resin, Solid-phase synthesis, GS-MSD

INTRODUCTION

The synthesis of small molecule using polymer-supported synthetic routes has been the most important feature of the combinatorial technology in the recent years. In addition, polymer-supported synthesis is emerging as an important tool in the development of new synthetic strategies in organic chemistry because of purification and reutilization advantages (Kaldor *et al.*, 1996; Flynn *et al.*, 1997). The scaffold of lactones is common in certain biological analogs including immunosuppressant, antiallergy, and antineoplastic agents (Bycroft *et al.*, 1995). In particular, *N*-acyl homoserine lactones (AHL) are biologically very interesting in the intercellular communication and group behavior in bacteria (Suga *et al.*, 2003; Leadbetter *et al.*, 2000).

As our methodology development program for the lactone library synthesis purposes, we investigated efficient routes to the synthesis of homoserine lactone libraries. In this work process, we have developed a polymer-supported synthesis of a methionine-functionalized resin for the lactone libraries (Ko *et al.*, 1998). In our efforts towards, the lactone derivatives can serve as a good scaffold for obtaining diverse lactone derivatives. Analytical methods specific to combinatorial libraries have been discussed in reviews (Thompson *et al.*, 1996). An accurate identification of sample combinatorial libraries was investigated using liquid chromatography-electrospray ionization-mass spectroscopy (LC-ESI-MS) and related tandem experiments (Haap *et al.*, 1997). Combinatorial chemists are more familiar with electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI) because combinatorial libraries exhibit polar properties, e.g. peptides, whereas the application range of gas chromatography (GC) is restricted to volatile compounds of limited molecular mass (Trautwein *et al.*, 1998). Less commonly used analytical techniques can also be successfully used for the analysis of libraries generated by combinatorial synthesis. This was demonstrated by the group of Kurth who utilized GC-MS to examine the β-mercaptoketones libraries (Chen *et al.*, 1997).

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MATERIALS AND METHODS

Reagents and Instruments

All reactions were carried out under positive pressure of nitrogen. Anhydrous solvent was distilled before use and the reagents were typically purchased from Aldrich Co. The resin for the solid-phase synthesis was commercially available aminomethyl polystyrene resin (1% crosslinked; 0.9 mmol/g, 100-200 mesh, Advanced ChemTech., USA). ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded with a Bruker ARX instrument in $CDCl_3$ using

tetramethylsilane (TMS) as internal standard. Infrared (IR) spectra were recorded in potassium bromide (KBr) pellets on a Bruker IFS-66 FTIR spectrometer. GC-MSD analyses were performed on a Hewlett-Packard (HP) 5890A using HP ultra-1 (crosslinked methyl silicone gum; 25 m×0.2 mm×0.3 mm) fused silica capillary column. Mass spectra were obtained with a HP-5970B (He, 15 psi, 200-300°C, and 10°C/min) and electron impact (EI) mode. HP 5890A GC was coupled with HP 5970B mass selective detector. Ionization potential was 70 eV and transfer line temperature was 300°C.

Synthesis

Homoserine lactone mixture libraries (3a~3i)

The methionine-functionalized resin (0.9 mmol/g, 500 mg, 0.450 mmol) was swollen in 5 mL of dry DMF with butyric acid (8.8 mg, 0.1 mmol), ethyl hydrogen malonate (13.2 mg, 0.1 mmol), cyclopentylacetic acid (13.2 mg, 0.1 mmol), *N*-Cbz-L-Valine (25.1 mg, 0.1 mmol), *N*-Cbz-L-phenylalanine (29.9 mg, 0.1 mmol), *N*-(*p*-toluenesulfonyl) phenylalanine (31.9 mg, 0.1 mmol), (3,4-methylenedioxy) phenyl propionic acid (19.4 mg, 0.1 mmol), 4-methoxyphenylacetic acid (16.6 mg, 0.1 mmol), 4-biphenyl carboxylic acid (19.8 mg, 0.1 mmol), 1-hydroxybenzotriazole (HOBt, 122 mg, 0.9 mmol), and *N*-ethylmorpholine (115 μL, 0.98 mmol). 1,3-diisopropyl carbodiimide (DIC, 141 μL, 0.98 mmol) was added to the resin mixture and the reaction mixture was slowly stirred at room temperature for 24 h. After the coupling reaction, the resin mixture was washed with DMF (10 mL×2 times), acetone (10 mL×3 times), methanol (10 mL×2 times), chloroform (10 mL×3 times), and then dried completely by vacuum system. The amide-fused resin was obtained (**2**). The resin was stirred in the mixture solvent (chloroform/water = 5 mL/2 mL, respectively) and cyanogen bromide (572 mg, 5.4 mmol) was added. Two-drop of 99% trifluoroacetic acid (TFA) was added, and the reaction mixture was stirred at room temperature for 24 h in the fume hood. After the cleavage reaction, filtration gave homoserine lactone mixtures of using the excess amount of chloroform. The chloroform solvent was removed by rotary evaporator and the residue was dried completely by vacuum system. Finally, homoserine lactone mixtures were obtained (mean molecular weight: MW=197.2, 40.82 mg, 46% from **1**) without the further purification. Capillary gas chromatographic analyses were carried out using a Hewlett-Packard 5890 GC instrument equipped with a ultra **1** (crosslinked methyl silicone gum; 25 m×0.2 mm×0.33 mm) fused silica capillary column. Mass spectra using GC-MSD was obtained with a HP-5970B (He, 15 psi, 200-300°C,10°C/min, and isothermal method). GC R_t (**3a**) = 2.64 min, MS *m/z* (M^+) 170.95; R_t (**3b**) = 3.37 min, MS *m/z* (M^+) 215.05; R_t (**3c**) = 3.96 min; R_t (**3d**) = 8.18 min, MS *m/z* (M^+) 334.10; R_t (**3e**) = 7.47 min; R_t (**3f**) = 15.95 min; R_t (**3g**) = 7.05 min, MS *m/z* (M^+) 277.05; R_t (**3h**) = 5.70 min, MS *m/z* (M^+) 249.10; R_t (**3i**) = 9.16 min, MS *m/z* (M^+) 281.05.

N-(Butanoyl) homoserine lactone (3a)

The methionine-functionalized resin (0.9 mmol/g, 250 mg, 0.225 mmol) was swollen in 5 mL of dry DMF with butyric acid (49.6 mg, 0.56 mmol), HOBt (75.8 mg, 0.56 mmol), and *N*-ethylmorpholine (71.2 μL, 0.56 mmol). DIC (87.9 μL, 0.56 mmol) was added to the resin mixture and the reaction mixture was slowly stirred at room temperature for 24 h. After the coupling reaction, the resin mixture was washed with DMF (10 mL×2 times), acetone (10 mL×3 times), methanol (10 mL×2 times), chloroform (10 mL×3 times), and dried completely by vacuum system. The butyric amide fused resin was obtained (**2a**). The resin was dissolved in 5mL of chloroform and 2 mL of water and cyanogens bromide (358 mg, 3.38 mmol) was added. Two-drop of 99% TFA was added to the resin at room temperature for 24 h in the fume hood. After the cleav- age reaction, filtration gave a corresponding homoserine lactone of using the excess amount of chloroform and also aminomethyl resin was recovered. The chloroform solvent was removed by rotary evaporator and the residue was dried completely by vacuum system. Finally, *N*-butanoyl homoserine lactone was obtained (**3a**: 18.9 mg, 49% from **1**). IR (KBr) 2918, 1774, 1640 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.07-5.98 (bs, 1H), 4.62-4.54 (m, 1H), 4.52-4.47 (t, *J* = 9.1Hz, 1H), 4.35-4.28 (m, 1H), 2.94-2.88 (m, 1H), 2.28-2.25 (t, *J* = 7.4, 2H), 2.20-2.13 (m, 1H), 1.74-1.67 (q, *J* = 7.5 Hz, 2H), 1.01-0.97 (t, *J* = 7.4 Hz, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 175.7, 173.8, 66.3, 49.5, 38.3, 30.9, 19.1. 13.9; GC R_T = 3.527 min [25 m×0.2 mm×0.3 μm, 200°C (10°C/min) to 270°C and then isothermal method].

N-(Malonyl ethyl ester) homoserine lactone (3b)

The product (19.6 mg, 44%) was obtained through cleavage reaction with the cyanogens bromide after the coupling reaction. IR (KBr) 2922, 1774, 1737, 1697, 1634 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.74 (br, 1H), 4.60-4.58 (m, 1H), 4.50-4.46 (t, *J* = 7.8 Hz, 1H), 4.31-4.2 (m, 3H), 3.38 (s, 2H), 2.84-2.80 (m, 1H), 2.40-2.20 (m, 1H), 1.32-1.28 (t, *J* = 7.2 Hz, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 174.7, 169.1, 165.7, 65.9, 61.9, 49.1, 40.6, 30.6, 14.1; GC R_t = 4.676 min [25 m×0.2 mm×0.3 μm, 200°C (10°C/min) to 270°C and then isothermal method].

N-(Cyclopentylethanoyl) homoserine lactone (3c)

The product (21.4 mg, 45%) was obtained through cleavage reaction with the cyanogens bromide after the coupling reaction. IR(KBr) 3035, 1777, 1642 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 5.93-5.90 (br, 1H), 4.53-4.49 (m, 1H), 4.49-4.47 (dd, *J* = 9.1, 8.8Hz, 1H), 4.30-4.25 (m, 1H),

2.90-2.85 (m, 1H), 2.25 (s, 3H), 2.15-2.09 (m, 1H), 1.89-1.81 (m, 2H), 1.65-1.57 (m, 3H), 1.21-1.13 (m, 4H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 175.7, 173.6, 66.3, 49.5, 42.6, 37.2, 32.8, 32.7, 31.0, 29.9, 25.1; GC R_t = 5.525 min [25 m×0.2 mm×0.3 μm, 200°C (10°C/min) to 270°C and then isothermal method].

_N_-(_N_-Cbz-L-valine) homoserine lactone (3d)

The product (25.9 mg, 43%) was obtained through cleavage reaction with the cyanogens bromide after the coupling reaction. IR (KBr) 3288, 2922, 1776, 1689, 1642 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.41-7.34 (m, 5H), 6.86-6.80 (bs. 1H), 5.47-5.45 (bs, 1H), 5.13 (s, 2H), 4.58-4.56 (m, 1H), 4.49-4.45 (t, J = 9.0Hz, 1H), 4.31-4.25 (m, 1H), 4.11-4.07 (m, 1H), 2.78-2.75 (m, 1H), 2.22-2.15 (m, 2H), 1.03-0.89 (dd, J = 13.8, 7.0, 6H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 175.1, 172.2, 140.5, 136.3, 128.8, 128.4, 128.2, 72.8, 67.4, 66.2, 60.5, 49.3, 31.2, 30.0, 22.9, 19.4, 18.0; GC R_t = 12.853 min [25 m×0.2 mm×0.3 μm, 200°C (10°C/min) to 270°C and then isothermal method].

_N_-(_N_-Cbz-phenylalanine)homoserine lactone (3e)

The product (23 mg, 32%) was obtained through cleavage reaction with the cyanogens bromide after the coupling reaction. IR (KBr) 3297, 2919, 1777, 1691 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.35-7.18 (m, 10H), 6.4 (bs, 1H), 5.26 (bs, 1H), 5.08 (s, 2H), 4.45-4.35 (m, 3H), 4.27-4.23 (m, 1H), 3.12-3.09 (t, J = 6.4Hz, 2H), 2.80-2.70 (m, 1H), 2.10-2.0 (m, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 184.8, 174.6, 171.7, 136.1, 129.5, 129.1, 128.8, 128.5, 127.5, 67.5, 66.1, 49.4, 30.0, 29.9, 14.3; GC R_t = 11.341 min [25 m×0.2 mm×0.3 μm, 200°C (10°C/min) to 270°C and then isothermal method].

_N_-[(_N_-_p_-Toluenesulfonyl)phenylalanine] homoserine lactone (3f)

The product (26 mg, 36%) was obtained through cleavage reaction with the cyanogens bromide after the coupling reaction. IR (KBr) 3288, 2958, 1773, 1654 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.48-7.46 (d, J = 8.3, 2H), 7.15-7.09 (m, 5H), 6.94-6.92 (d, J = 7.2 Hz, 1H), 6.88 (d, J = 9.4 Hz, 2H), 5.0-4.99 (d, J = 7.2 Hz, 1H), 4.40 (t, J = 7.6 Hz, 1H), 4.30-4.22 (m, 1H), 4.20-4.17 (m, 1H), 3.88-3.86 (q, J = 6 Hz, 1H), 2.91-2.86 (m, 2H), 2.61-2.56 (m,1H), 2.35 (s, 3H), 2.18-2.09 (m, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 174.5, 171.2, 144.2, 135.8, 135.0, 130.1, 129.5, 129.2, 127.6, 127.4, 66.2, 57.7, 49.6, 38.1, 32.1, 22.9; GC R_t = 32.594 min [25 m×0.2 mm×0.3 μm, 200°C (10°C/min) to 270°C and then isothermal method].

_N_-[_N_-3,4-Methylenedioxyphenyl]propionyl] homoserine lactone (3g)

The product (27 mg, 47%) was obtained through cleavage reaction with the cyanogens bromide after the coupling reaction. IR (KBr) 3308, 2819, 1773, 1653, 1640 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.74-6.69 (m, 3H), 5.96-5.93 (bs, 1H), 5.93 (s, 2H), 4.56-4.51 (m, 1H), 4.51-4.46 (m, 1H), 4.30-4.25 (m, 1H), 2.92-2.88 (t, J = 7.4 Hz, 2H), 2.88-2.85 (m, 1H) 2.54-2.48 (m, 2H), 2.16-2.04 (m, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 194.5, 175.3, 172.6, 147.7, 146.1, 134.2, 121.2, 108.8, 108.3, 100.9, 66.1, 49.3, 38.2, 31.9, 31.2; GC R_t = 10.705 min [25 m×0.2 mm×0.3 μm, 200°C (10°C/min) to 270°C and then isothermal method].

_N_-(4-Methoxyphenylacetonyl)homoserine lactone (3h)

The product (22.6 mg, 42% mole) was obtained through cleavage reaction with the cyanogens bromide after the coupling reaction. IR (KBr) 3291, 2918, 1773, 1652 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.14-7.10 (d, J = 7.5 Hz, 2H), 6.84-6.80 (d, J = 9.4 Hz, 2H), 5.86 (bs, 1H), 4.44-4.41 (m, 1H), 4.40-4.33 (dd, J = 7.9, 7.8 Hz, 1H), 4.19-4.14 (m, 1H), 3.73 (s, 3H), 3.50 (s, 2H), 2.78-2.61 (m, 1H), 2.03-1.97 (m, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 175.2, 172.2, 159.3, 130.7, 126.1, 114.8, 66.2, 55.5, 49.5, 42.6, 29.9; GC R_t = 8.294 min [25 m×0.2 mm×0.3 μm, 200°C (10°C/min) to 270°C and then isothermal method].

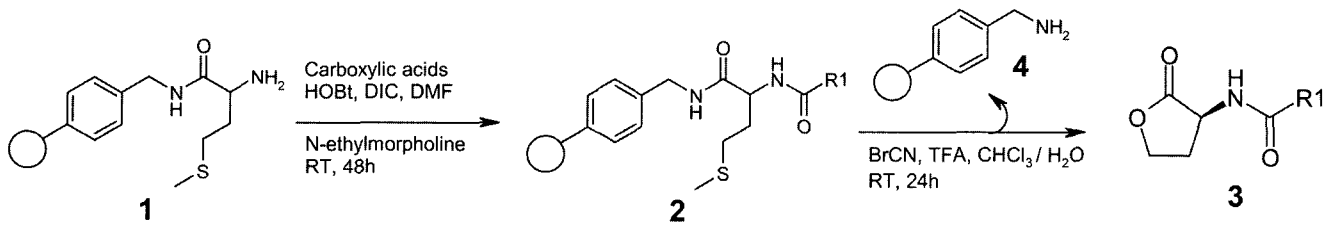
_N_-(_N_-4-Biphenylcarboxyl) homoserine lactone (3i)

The product (32.2 mg, 53%) was obtained through cleavage reaction with the cyanogens bromide after the coupling reaction. IR(KBr) 3058, 2957, 1773, 1718, 1700, 1640 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.89-7.87 (d, J = 9 Hz, 2H), 7.67-7.65 (d, J = 9 Hz, 2H), 7.62-7.60 (d, J = 7,2 Hz, 2H), 7.59-7.39 (m, 3H), 6.84-6.83 (bs, 1H), 4.82-4.78 (m, 1H), 4.57-4.52 (t, J = 8Hz, 1H), 4.40-4.36 (m, 1H), 3.02-2.96 (m, 1H), 2.35-2.24 (m, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 175.9, 167.7, 145.2, 140, 131.8, 129.1, 128.3, 127.9, 127.4, 66.5, 50, 30.9; GC R_t = 14.941 min [25 m×0.2 mm×0.3 μm, 200°C (10°C/min) to 270°C and then isothermal method].

RESULTS ANS DISCUSSION

Mass spectrometry techniques provide the most generally useful methods for characterizing combinatorial mixtures and individual products solid-phase synthesis. In this paper, we set out to identify the compounds separated by GC-MSD (Oprean *et al.*, 2001) from the homoserine lactone libraries generated by combinatorial chemistry. Recently, it was reported that the methionine-functionalized resin was synthesized from the aminomethyl polystyrene resin through the coupling step and deprotection (Ko *et al.*, 1998).

Coupling reaction of the resin (**1**) and selected carboxylic acids in DMF for 24 h gave the amide derivatives (**2**, Scheme 1). Treatment of each amide derivative bound to

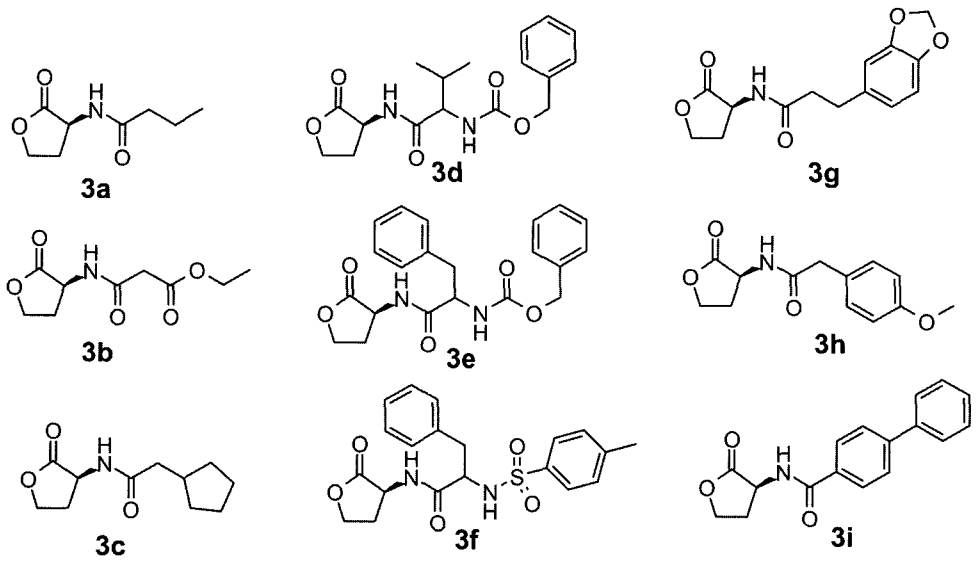


Scheme 1. Homoserine lactone derivatives by the methionine functionalized resin

the resin with 15 equimolar amount of cyanogens bromide and two drops of 99% trifluoroacetic acid in the mixed solvent (chloroform/water = 5 mL/2 mL, respectively) at room temperature for 24 h generated the corresponding homoserine lactone (**3**) with retention of stereochemistry. Aminomethyl polystyrene resin (**4**) was recovered as evidenced by disappearance of the amide band at 1640 cm^{-1}. We obtained homoserine lactones in 32~53% overall yield from the aminomethyl polystyrene resin. Finally, we have demonstrated that the molecular diversity of **3a~3i** (46% yield, Scheme 2) could efficiently provide the nine homoserine lactones in a one-pot system, each lactone corresponded with the starting acid as evidenced by spectroscopic methods and GC analysis.

Fig. 1 shows the full scan spectra of the nine homoserine lactones (top panel) and mass spectra (bottom panel; **3a~3i**). The total ion chromatogram and mass spectra were obtained by EI mode of GC/MS. Here, we observed that the six compounds (**3a**, **3b**, **3d**, **3g**, **3h**, and **3i**) among the nine homoserine lactone mixtures were confirmed by EI mode of mass spectrograph, but the remainder compounds were not confirmed. The molecular weight ion (M^+) of peaks **3a**, **3b**, **3d**, **3g**, **3h**, and **3i** were correspondingly confirmed to be *m/z* 170.95, 215.05, 334.10, 277.05, 249.10 and 281.05. The molecular weight ion (M^+) of peaks **3c**, **3e**, and **3f** were not detected because of the high polarity or non-volatile properties and instability at high temperature. We have to identify the compounds that remain without being confirmed by mass spectrograph. Thus, we synthesized the each compound and confirmed the compounds (**3c**, **3e**, and **3f**) by ^1H-, ^{13}C-NMR and IR spectrometry. A gas chromatograph (HP 5890A) equipped with a HP capillary column ultra-1 (crosslinked methyl silicone gum; 25 m×0.2 mm×0.3 μm) was used for analysis of the individual lactone and the mixture lactones. The flow rate of carrier gas (He) was 1.0 mL/min. The split ratio was 30:1 and head pressure was 15 psi. The oven temperature was increased from 200°C (10°C/min) to 270°C and then isothermal method.

In conclusion, the methionine functionalized resin was used a mild cleavage strategy to provide a combinatorial library of homoserine lactones through the coupling and cleavage step. Moreover, we demonstrated that chemically diverse homoserine lactones are easily obtained. In particular, we demonstrated that the GC-MSD analytical techniques can also be successfully used for the



Scheme 2. Combinatorial libraries of Homoserine lactones

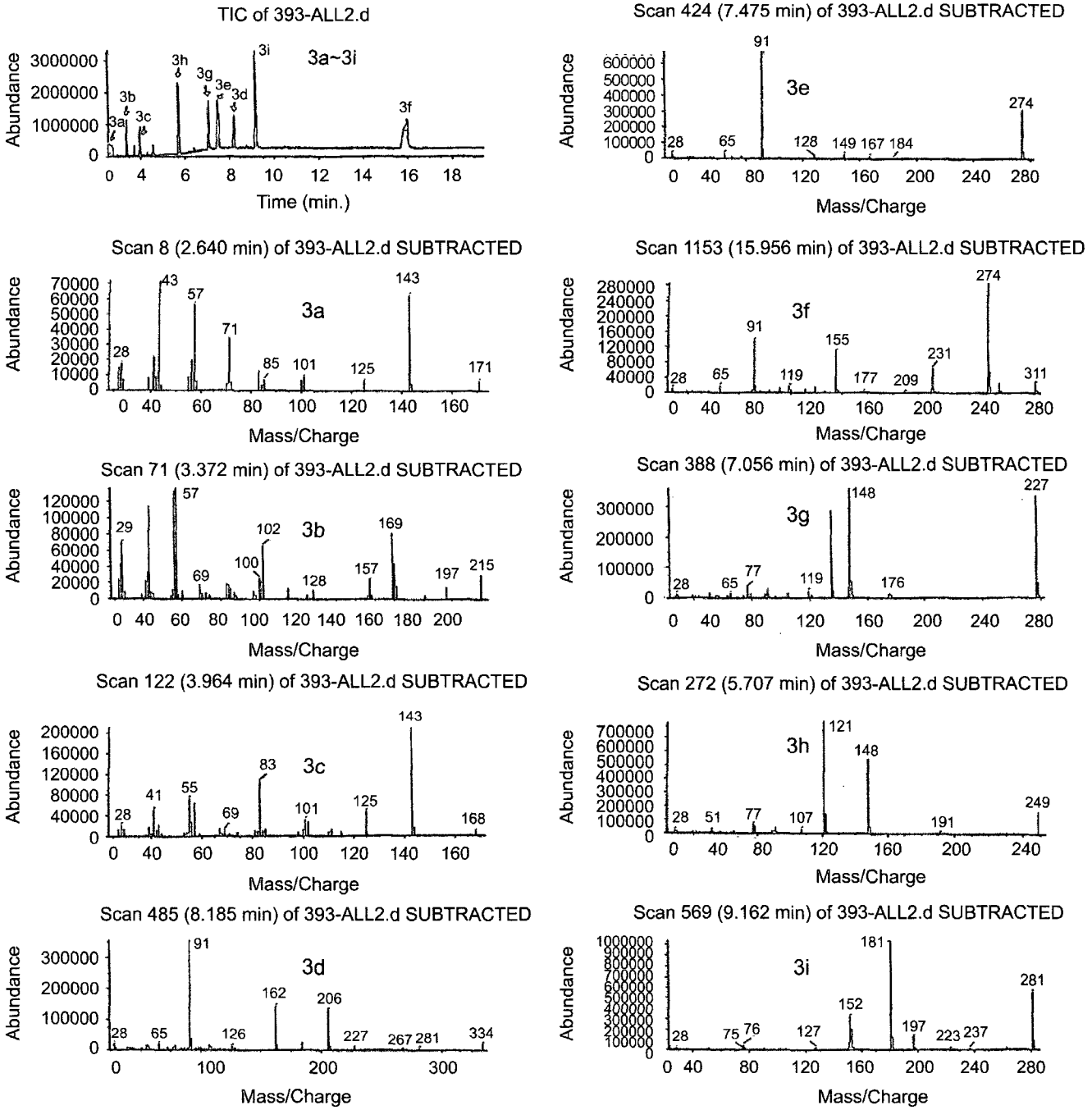


Fig. 1. Top panel: full scan spectra of the nine homoserine lactones by EI (70 eV); Bottom panel: The molecular weight ion (M^+) of the each peak was found to be m/z (M^+) from the interpretation of total ion chromatogram.

analysis of mixture libraries generated by combinatorial chemistry. As demonstrated, the high resolving power of gas chromatography and a high content of structural information due to molecule fragments resulting from EI-ionization are major advantages of this method. Even though the GC-MS technique of the library analysis never achieved the wide-spread importance of LC-ESI techniques.

ACKNOWLEDGEMNENTS

The author would like to thank Dr. Dong-Hyun Ko, Mr. Chun Seon Lyu and Mr. In Ki Min for stimulating discussions and supports.

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