## Structure-activity Relationships of Lactacystin, the First Non-protein Neurotrophic Factor

Sir:

Neurotrophic agents such as nerve growth factor (NGF)[1] are required for the survival and function of neurons. In 1991 we reported the isolation and characterization of the first non-protein neurotrophic factor, lactacystin (**1**), a novel sulfur-containing $\gamma$-lactam produced by a culture broth of *Streptomyces* sp. OM-6519.[2,3] Lactacystin induces neuritogenesis and causes a transient increase in the intracellular cAMP level in mouse neuroblastoma cell line Neuro 2A. We have established an economic and versatile total synthesis of **1** involving as key steps stereoselective hydroxymethylation and an asymmetric allylboration which introduces the hydroxyl and methyl substitutes at C(6) and C(7). Our synthetic approach affords **1** in 13% overall yield over 10 steps.[4]

In this report, we have synthesized a variety of analogs to clarify the structure-activity relationships of lactacystin.

Recently, COREY and SCHREIBER reported that several chemical groups of $\gamma$-lactam ring and the hydroxyisobutyl group are very important for neurotrophic activity. These groups may be involed in primary recognition of the target molecules. They also suggested that a related $\beta$-lactone (**4**) may possibly act as key-intermediate in the mechanism inducing neurite outgrowth.[5]

Here, we describe the synthesis of several derivatives modifying *N*-acetylcysteine moiety of lactacystin in order to reveal its significance for activity and cytotoxicity, respectively.

Hydrolysis of $\gamma$-lactam methyl ester (**2**)[4] with 0.1 N NaOH in EtOH gave the dihydroxy acid (**3**)[4] in quantitative yield. By treatment with bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOPCl)[6] and $Et_3N$ in $CH_2Cl_2$, the $\beta$-lactone (**4**) could be formed with a yield of 68%.[7] *Via* the coupling reaction between the related $\beta$-lactone (**4**) and several thiols with $Et_3N$ in $CH_2Cl_2$, the corresponding thioesters could be accomplished (Scheme 1). The structures of these derivatives were determined by NMR analysis and Mass spectrometry. The activity and cytotoxicity of the lactacystin analogs were studied in an *in vitro* assay according to our established method.[2,3] The results are summarized in Table 1.

The activity and cytotoxicity values found for the $\beta$-lactone (**4**) are the same as for lactacystin (**1**), while on the other hand the dihydroxy acid (**2**) as well as the $\gamma$-lactam methyl ester (**3**) are completely inactive. These results indicate the activated esters such as a $\beta$-lactone or thiol esters to be very important for neurotrophic activity. Both compounds (**6**) and (**10**) were about eight times more active than lactacystin, otherwise their cytotoxicity was somehow increased, respectively. The specificity (B/A) level was increased in both cases. When the derivatives became more lipophilic, its neurotrophic activity became stronger. On the other hand, when the derivatives became more hydrophilic such as **5** and **7**, the B/A level in both cases was the same as in **1**.

The most interesting compound is the descarboxylactacystin (**8**). [Analytical data of **8**: HRFAB-MS

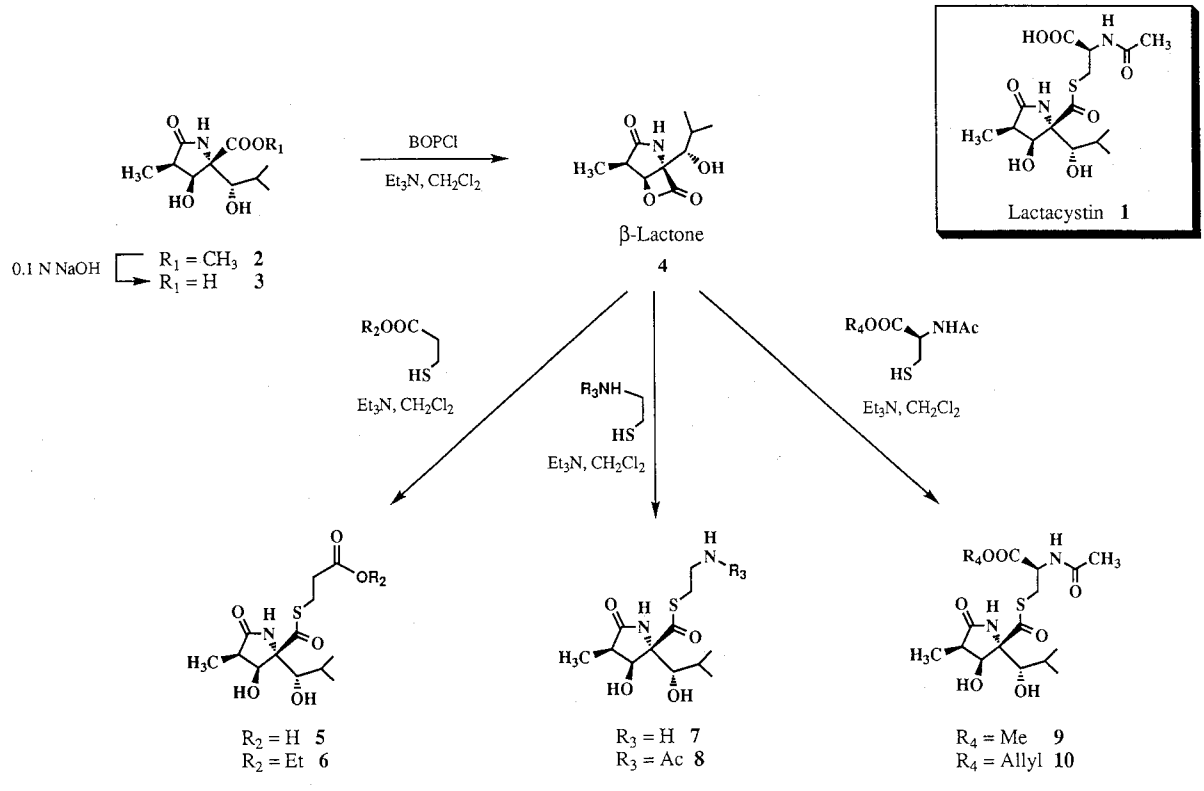

Table 1. Activity of lactacystin analogs in *in vitro* neurite outgrowth assay.

| Compound | Minimum[a] effective dose ($\mu$M) (A) | Cytotoxicity[b] ($\mu$M) (B) | Specificity[c] (B/A) |
|---|---|---|---|
| Lactacystin (**1**) | 1.56 | 12.5 | 8 |
| **2** | n.e.[d] | — | — |
| **3** | n.e.[d] | — | — |
| **4** | 1.56 | 12.5 | 8 |
| **5** | 0.20 | 1.56 | 8 |
| **6** | 0.20 | 3.12 | 16 |
| **7** | 3.12 | 25 | 8 |
| **8** | 0.10 | 12.5 | 125 |
| **9** | 0.78 | 6.25 | 8 |
| **10** | 0.20 | 6.25 | 31 |

Neuro 2A cells were plated at a density of $1 \times 10^4$ cells per $cm^2$ and grown for 24 hours in MEM-H with 10% FBS prior to any treatment. The number of cells with neurites were microscopically counted 24 hours after addition of drug.

[a] Approximately 20% cells with neurites were observed when the drug was added to the culture medium at the indicated concentrations.

[b] No attached Neuro 2A cells were observed when the drug was added to the culture medium at the indicated concentrations.

[c] B/A level was specific for the activity (A: minimum effective dose, B : cytotoxicity).

[d] n.e.; not effective.

333.1497 $(M+1)^+$; Calcd for $C_{14}H_{25}N_2O_5S$: 333.1484. $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 0.87 (3H, d, $J=7.0$ Hz, $(CH_3)CH$), 0.97 (3H, d, $J=7.0$ Hz, $(CH_3)CH$), 1.10 (3H, d, $J=7.5$ Hz, $CH_3CHCHOH$), 1.68 (1H, m, $(CH_3)CH$), 1.92 (3H, s, $NHCOCH_3$), 2.89 (1H, m, $CH_3CHCHOH$), 3.10 (2H, m, $SCH_2CH_2$), 3.31 (2H, m, $SCH_2CH_2$), 3.95 (1H, d, $J=7.0$ Hz, $(CH_3)_2CHCHOH$), 4.54 (1H, d, $J=7.0$ Hz, $CH_3CHCHOH$), $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ 9.1, 19.5, 21.3, 22.6, 29.2, 32.2, 39.8, 42.4, 76.6, 80.3, 81.5, 173.4, 183.2, 202.4]. Its minimum effective dose (0.1 $\mu$M) was about 16 times lower than that of **1**, while its cytotoxicity was the same as that of lactacystin. The high value of the B/A level indicates that its possible use as neurotrophic agent might be much more feasible than lactacystin (**1**). Therefore we are very interested in applying these new results to justify the further development of lactacystin.

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