Supporting Information

for

Menthylation esterification allows chiral resolution for the synthesis of artificial glutamate analogs

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Stereochemical analysis of TKM-38 by the PGME amide method, as a support for the original determination of the configuration of menthyl esters 21 and 21* based on NOESY spectra and CONFLEX calculations
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Stereochemical analysis of TKM-38 by the PGME method, as a support for original determination of the configuration of the menthyl esters 21 and 21* performed based on NOESY spectra and CONFLEX calculations

From rac-19, diastereomeric (R)-PGME amides SVII (t_R = 16.2 min on HPLC) and SVII* (t_R = 10.8 min) were prepared in 26% and 28% yield, respectively (Scheme SVII-1). We then attempted to determine the structures, based on the empirical rule named “the PGME method” [1].

However, the PGME method is generally applied to α,α-disubstituted, α,α,α-trisubstituted, or β,β-disubstituted chiral carboxylic acids [1, 2]. The substrates, SVII and SVII* used in this study are β,β,β-trisubstituted carboxylic acids, and there has been only one example of application to such a substrate [3]. However, in that study, it was shown that the PGME method could be applied by independently analyzing the conformation of the PGME plane in the prepared PGME amides by ROESY. A related discussion can also be found in the original paper by Kusumi et al [1]. Therefore, in this study, the conformational analysis of the PGME plane was first attempted independently by ROESY before conducting the analysis by the PGME method. The results are shown in Figures SVII-1 ~ SVII-3. It was found that NOESY gave better spectra than ROESY for SVII and SVII*. In the NOESY spectra of SVII (Figures SVII-1A, 1B, 1C), the presence of five correlations from NH other than NH/PGME(α) supports that the O-C_3-C_12-C(=O)-N moiety is in a zigzag conformation (Figure SVII-1D) [4]. Another correlation between PGME aryl proton(s) and CO_2Me is also remarkable to indicate that CO_2Me is positioned at one side of the plane defined by (R)-PGME [1, 2]. From the NOESY correlations summarized in Figure SVII-1D, it was also suggested that SVII is the (2R)-isomer (Figure SVII-3A). Since these seven NOESY correlations are satisfied in the conformation shown in Figure SVII-3A, SVII is considered to exist mainly in this conformation [5].

In the NOESY spectra of diastereomeric SVII* (Figures SVII-2A and SVII-2B), the correlations at NH/H_{12a}, NH/H_{12b}, and H_{12a}/H_7(H_8) showed that H_{12a} is positioned at one side of the plane defined by (R)-PGME [1, 2]. These structural requirements seemed to be well satisfied by the (2S)-isomer in
the conformation depicted in Figure SVII-3B; the spatial relationship between NH, H7, H8, H12a, and H12b is consistent with the NOESY correlations observed. Although the absent correlations at NH/H2 and PGME(Ar)/CO2Me may indicate the relative conformation in the O-C3-C12-C(=O)-N moiety is slightly twisted compared to SVII, the NOESY correlations observed would indicate the conformation of SVII* is quasi enantiomeric to that of the (2R)-isomer SVII (Figure SVII-3A). Since no other important NOESY correlations were observed, the structure shown in Figure SVII-3B seems to be the predominant conformation for SVII*.

From NOESY analyses discussed above, it was shown that H12a, H12b, and some protons on the heterotrichyclic skeleton are located on either side of the plane defined by (R)-PGME in both SVII and SVII*, and the protons can be useful for diagnostics to determine the absolute configurations. Thus, from the differences of chemical shift value in 1H NMR between SVII and SVII* (\(\Delta \delta = \delta_{SVII} - \delta_{SVII*}\)) shown in Figure SVII-4, it was determined that SVII is the (2R)-isomer, and SVII* is the (2S)-isomer. It should be also noted that the conclusion is consistent with the configurations shown in Figure SVII-3, proposed based on NOESY correlations.

Using SVII (2R), the protecting groups were attempted to remove (Scheme SVII-1). First, the PMB group was smoothly removed by CAN in 96% yield. However, final hydrolytic deprotection of all other groups under alkaline conditions (1 M KOH, MeOH, 40 °C, 8 d) as used for TKM-38 was not completed, and prolonged reaction (6 d) with higher concentration of KOH (3 M) resulted in decomposition of the substrate.

We next examined acidic hydrolysis (6 M HCl, 110 °C, 1 h) with 0.05 mg (0.09 μmol) of SVII (2R), and in this case the reaction was cleanly completed to give XVII3 as a sole product. HRMS analysis of crude XVII3 showed MH+ (m/z 311.1237) that seemed to correspond to TKM-38, however, XVII3 did not match either (2R)- and (2S)-TKM-38 on chiral HPLC. The observations indicated that the product XVII3 is a constitutional isomer or a diastereomer of TKM-38.

We then attempted to reproduce the same reaction on menthyl esters 21
(0.05 mg, 0.09 μmol) and 21* (0.05 mg, 0.09 μmol). As shown in Scheme SVII-2, acid treatment (6 M HCl, 110 °C, 1 h) of 21 and 21* gave SVII4 and SVII4*, respectively, and both products (SVII4, SVII4*) showed satisfactory MH⁺ ions (Scheme SVII-2). As expected, the behaviors of SVII4 and SVII4* on chiral HPLC were obviously different from those of TKM-38 enantiomers, and retention time of SVII4 was found to be consistent with that of SVII3 (2R) (Figure SVII-5).

From above experiments shown in Schemes SVII-1 and SVII-2, we reasonably concluded that 21 is the enantiomer with 2R configuration, and the conclusion strongly supported our original assignment in this study for the menthyl esters 21 and 21* based on NOESY spectra and CONFLEX calculations.

It should be noted, however, that menthyl esterification is not generally applicable to the configurational analysis of chiral carboxylic acid, from the fact that no other examples have been reported so far. In this study, the bulkiness and the rigidity of the heterotricyclic skeleton of menthyl esters 21 and 21* would have enabled configurational analysis based on NOESY data and CONFLEX calculations.
Scheme SVII-1. Preparation from rac-19, separation, and configurational analysis of (R)-PGME amides SVIII and SVIII*, and sequential deprotection toward (2R)-SVIII3.
Since SVII3 (2R) and SVII4 were identical on chiral HPLC (Figure SVII-S), SVII4 was concluded to bear 2R configuration.

**Scheme SVII-2. Preparation of SVII4 and SVII4* from 21 and 21*, respectively, for comparison with (2R)-SVII3.**
References

[4] A similar zigzag conformation has been observed in the related paper for S-CR1R2-CH2-C(=O)-N. See reference 3.
[5] The conformation would be thermodynamically favored, since the conformation can be reproduced in conformational search by CONFLEX calculation (data not shown).
NOESY analysis of (R)-PGME amides
Figure SVII-1. NOESY spectra of (R)-PGME amide SVIIa
H\textsubscript{12a} denotes one of the geminal protons appearing at the lower field in the \textsuperscript{1}H NMR spectrum, and H\textsubscript{12b} denotes the one appearing at the higher field. The purpose of this NOESY analysis was to show the location of the PGME plane in the whole molecule, and hence assignment of H\textsubscript{12a}/H\textsubscript{12b} was not necessary. H\textsubscript{12a} and H\textsubscript{12b} were, however, later found to be pro-\textit{S} and pro-\textit{R}, respectively, by the PGME method.
Figure SVII-2. NOESY spectra of (R)-PGME amide SVIII*
Figure SVII-3. Key NOE correlations of \((R)\)-PGME derivatives SVIII (A) and SVIII* (B), for presumption of the absolute configurations with predominant conformations.
$\Delta \delta_H$ analysis of (R)-PGME amides

\[ \Delta \delta = \delta_{\text{SVIII}} - \delta_{\text{SVIII}^*} \]

Figure SVII-4. $\Delta \delta$ values obtained for (R)-PGME amides SVIII and SVIII$^*$ (400 MHz, CDCl$_3$)
Chiral LC-MS analysis of SVII3, SVII4, and SVII4*

**Figure SVII-5.** Chiral LC-MS profiles of acid hydrolysates SVII3, SVII4 and SVII4*.  
*Conditions: 4.6 × 250 mm CHIRALPAK ZWIX (+) column, 98% MeOH/H2O + 25 mM HCO2H + 25 mM HCO2NH4, 0.5 mL/min, 40 °C.*
NMR spectra for (R)-PGME amides SVII1, SVII1*, and SVII2