Received: 8 April 2019 | Revised: 10 October 2019 | Accepted: 15 October 2019

DOI: 10.1002/psc.3228

RESEARCH ARTICLE

WILEY Journal of PeptideScience

# Synthesis and evaluation of in vivo anti-hypothermic effect of all stereoisomers of the thyrotropin-releasing hormone mimetic: Rovatirelin Hydrate

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We discovered the orally active thyrotropin-releasing hormone (TRH) mimetic: (4*S*,5*S*)-5-methyl-*N*-{(2*S*)-1-[(2*R*)-2-methylpyrrolidin-1-yl]-1-oxo-3-(1,3-thiazol-4-yl)propan-2-yl}-2-oxo-1,3-oxazolidine-4-carboxamide **1** (rovatirelin). The central nervous system (CNS) effect of rovatirelin after intravenous (iv) administration is 100-fold higher than that of TRH. As **1** has four asymmetric carbons in its molecule, there are 16 stereoisomers. We synthesized and evaluated the anti-hypothermic effect of all stereoisomers of **1**, which has the (4*S*),(5*S*),(2*S*),(2*R*) configuration from the N-terminus to the C-terminus, in order to clarify the structure−activity relationship (SAR) of stereoisomers. The (4*R*),(5*R*),(2*R*),(2*S*)-isomer **16** did not show any anti-hypothermic effect. Only the (4*S*),(5*S*),(2*S*),(2*S*)-isomer **10**, which has the (2*S*)-2-methylpyrrolidine moiety at the C-terminus showed the anti-hypothermic effect similar to **1**. Stereoisomers, which have the (5*R*) configuration of the oxazolidinone at the N-terminus and the (2*R*) configuration at the middle-part, showed a much lower anti-hypothermic effect than that of **1**. On the other hand, stereoisomers, which have the (4*R*) configuration of the oxazolidinone at the N-terminus or the (2*S*) configuration of the C-terminus, have little influence on the anti-hypothermic effect.

**KEYWORDS**

TRH, TRH mimetic, rovatirelin, stereoisomers, anti-hypothermic effect, intravenous administration

## 1 | INTRODUCTION

We previously reported the structure−activity relationship (SAR) studies of thyrotropin-releasing hormone (TRH) mimetic. We evaluated the anti-hypothermic effect, physicochemical properties, pharmacokinetic (PK) properties of each compound and finally, we discovered the orally effective TRH mimetic, rovatirelin hydrate. Rovatirelin hydrate is in a phase III clinical trial (NCT02889302) for the treatment of spinocerebellar degeneration (SCD).[1-5]

TRH is a neuropeptide composed of three amino acids. The chemical structure of TRH was revealed to be L-pyroglutamyl−L-histidyl−L-prolinamide (L-pGlu−L-His−L-Pro-$NH_2$; Figure 1).[6-8] TRH indicates mainly two biological effects via the central nervous system (CNS) and endocrine system. The effects on CNS are expressed by the action

**Abbreviations:** TRH, thyrotropin-releasing hormone; CNS, central nervous system; SCD, spinocerebellar degeneration; TSH, thyroid stimulating hormone; SAR, structure−activity relationship; PK, pharmacokinetic; CPPs, CNS-permeable prodrugs; BBB, blood-brain barrier; iv, intravenous; MeOH, methanol; EtOH, ethanol; THF, tetrahydrofuran; DMSO, dimethylsulfoxide; DMF, *N*,*N*′-dimethylformamide; $Et_3N$, triethylamine; TFA, trifluoroacetic acid; TsOH·$H_2O$, *p*-toluenesulfonic acid monohydrate; TsOH, *p*-toluenesulfonic acid; $Ph_2CHN_2$, diphenyldiazomethane; $Ac_2O$, acetic anhydride; Cbz, benzyloxycarbonyl; Boc, *tert*-butoxycarbonyl; Ms, methanesulfony; Bz, benzoyl; DCC, 1,3-dicyclohexylcarbodiimide; HOBt, *N*-hydroxybenzotriazole; HOSu, *N*-hydroxysuccinimide; TLC, thin-layer chromatography

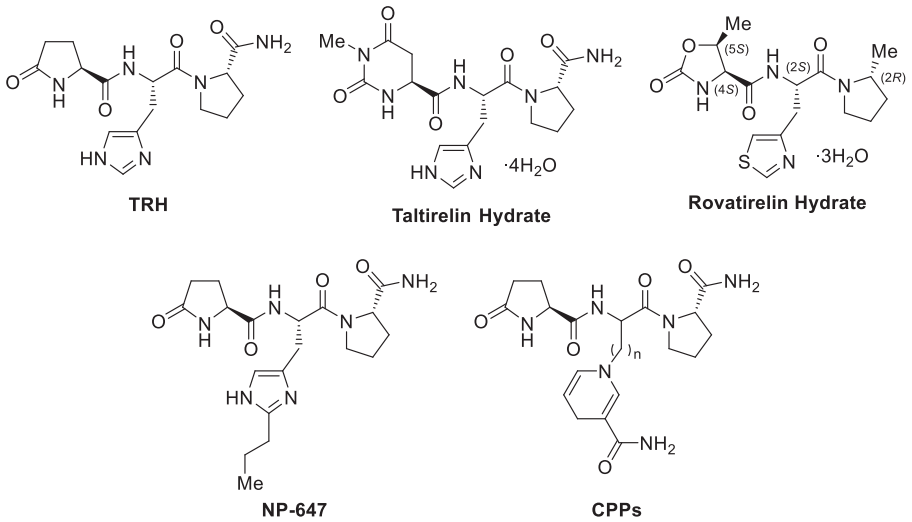



**FIGURE 1** Chemical structure of TRH, taltirelin hydrate, rovatirelin hydrate, NP-647 and CPPs.

of TRH as a neurotransmitter or a neuromodulator.[9-11] The effects on the endocrine system are expressed by stimulating the secretion of thyroid stimulating hormone (TSH) and prolactin from the anterior pituitary.[12] The effects on CNS of TRH have been applied the treatment of CNS disorders in clinical setting.[13,14] Prior to 2000, a large number of TRH analogues have been reported.[15-40] In particular, taltirelin hydrate (Figure 1) has high CNS effects separate from endocrine effects in order to avoid side effects caused by TSH releasing. Around the same time, we discovered rovatirelin hydrate (Figure 1).

Two subtypes of TRH receptor (TRH receptor type 1: TRH-R1[41] and TRH receptor type 2: TRH-R2[42,43]) are reported. TRH-R1 and TRH-R2 are relevant to endocrine effects and CNS effects, respectively.[44] In human, it has been reported that a single type of TRH receptor is expressed and revealed the activities similar to TRH-R1.[45] After 2000, TRH analogue NP-647 (Figure 1), which bound selectively to the TRH receptor subtype, have been reported and showed only CNS effects.[46-50] Another line of research has been the application of a prodrug approach to TRH analogues, for example, CNS-permeable prodrugs (CPPs) (Figure 1) have been synthesized in order to increase the penetration of biological membranes including the blood-brain barrier (BBB), and successively obtained a high CNS effect.[51-55]

Regarding the biological activities of the stereoisomers of TRH and TRH analogues, the configurations of the N-terminus and the C-terminus were found to be important for the biological activities resulting from evaluation of the TSH releasing activity or CNS effect.[15-19,56]

As rovatirelin: (4*S*,5*S*)-5-methyl-*N*-{(2*S*)-1-[(2*R*)-2-methylpyrrolidin-1-yl]-1-oxo-3-(1,3-thiazol-4-yl)propan-2-yl}-2-oxo-1,3-oxazolidine-4-carboxamide **1** has four asymmetric carbons in its molecule, we hypothesized that the existing stereochemistry of **1** was essential to show the highest anti-hypothermic effect and other stereoisomers would not show the anti-hypothermic effect compare with **1**.

Then, we synthesized all stereoisomers of **1**, which has the (4*S*), (5*S*),(2*S*),(2*R*) configurations from the N-terminus to the C-terminus (Figure 1), in order to clarify the SAR of stereoisomers by evaluating the anti-hypothermic effect. The chemical structures and their absolute configurations of TRH mimetics **1–16** are shown in Table 1.

In this paper, we report the synthesis of TRH mimetics **1–16** and the evaluation of their anti-hypothermic effect, which is one of the CNS effects of TRH, based on their reserpine-induced hypothermia in mice.[15-19]

## 2 | RESULTS AND DISCUSSION

### 2.1 | Chemistry

TRH mimetics **1–16** were synthesized by combining four N-terminus fragments (**17–20**), two middle-part fragments (**21**, **22**) and two C-terminus fragments (**23**, **24**) (Figure 2).

### 2.2 | N-Terminus fragments

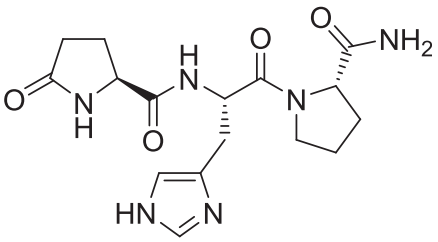
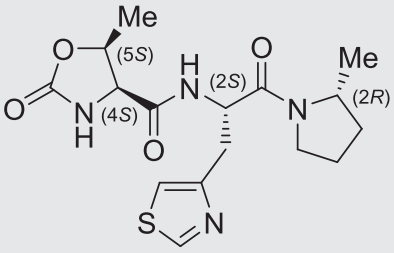
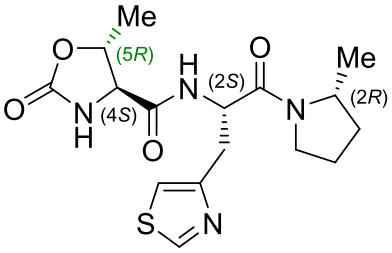
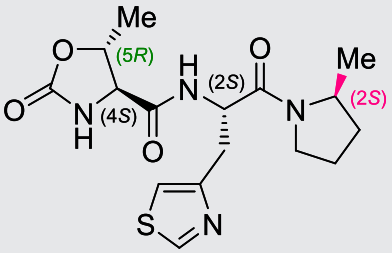
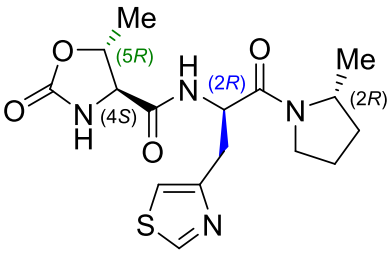
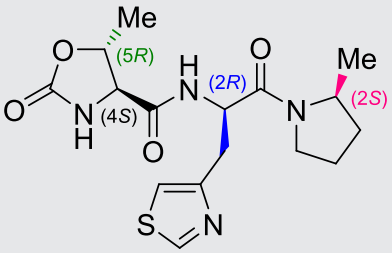
Four N-terminus fragments **17–20** were synthesized from readily available L-threonine **25** or D-threonine **26**. Among them, **19** and **20** were synthesized via *allo*-L-threonine hydrochloride **27** and *allo*-D-threonine hydrochloride **28**, respectively based on previous reports (Scheme 1).[57-61] The detailed synthetic method, yields and physical data of all intermediates are shown in the Supporting Information.

Reagents and conditions: (a) CbzCl, $NaHCO_3$, 1,4-dioxane-$H_2O$, 0 °C to rt; (b) (1) aq. NaOH, MeOH; (2) aq. HCl, MeOH, 29–71%; (c) (1) $SOCl_2$, MeOH, 0 °C; (2) BzCl, $NaHCO_3$, 1,4-dioxane-$H_2O$, 0 °C; (d) $SOCl_2$, 0 °C; (e) aq. HCl, reflux, 60–71%.

### 2.3 | Middle-part fragments

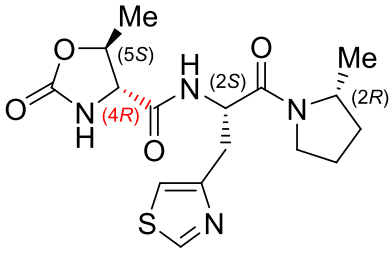
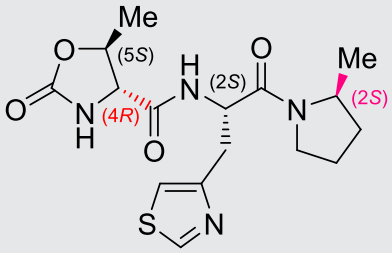
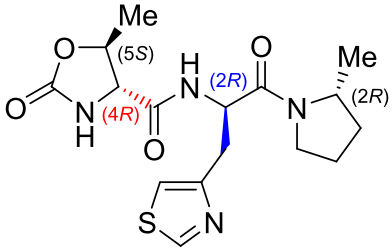
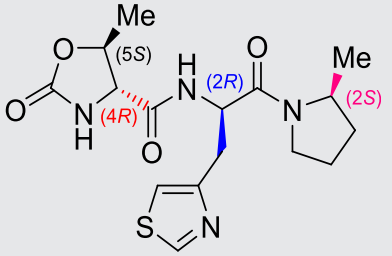
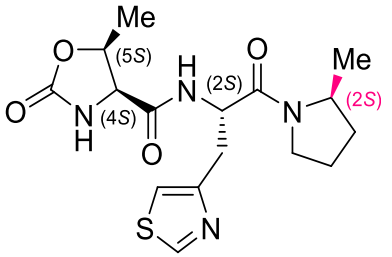
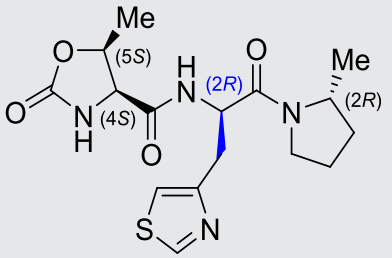
Two middle-part fragments **21** and **22** were synthesized from (2*S*)-2-amino-3-(thiazol-4-yl)propanoic acid **30** and (2*R*)-2-amino-3-(thiazol-

**TABLE 1** Chemical structures and anti-hypothermic effect of TRH, TRH mimetics **1–16** after iv administration to reserpine-induced hypothermia in mice. The absolute configurations are shown near the asymmetric carbons of the chemical structures.

| compounds | chemical structures and absolute configurations | dose (iv) (μmol/kg) | $\Delta AUC_{0-7h}$ (°C·h) |
|---|---|---|---|
| **TRH** |  | 50 | 35.4 ± 3.7 |
| **1** (rovatirelin) |  | 0.50 | 45.7 ± 0.1 |
| **2** |  | 0.50 | 7.8 ± 2.8 |
| **3** |  | 0.50 | −1.8 ± 1.6 |
| **4** |  | 0.50 | 6.0 |
| **5** |  | 0.50 | 1.6 ± 3.8 |

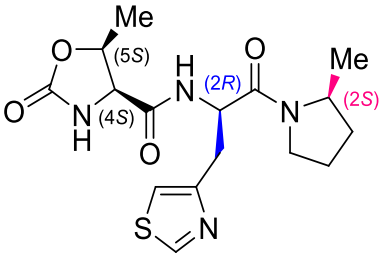
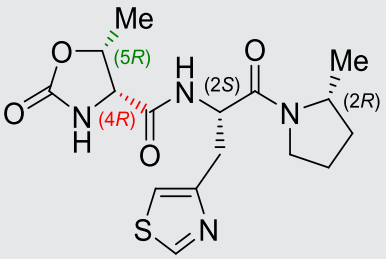
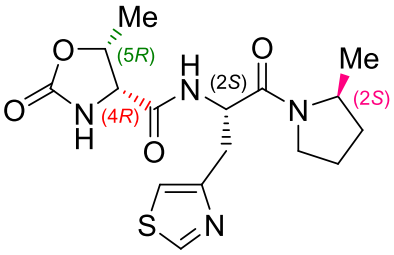
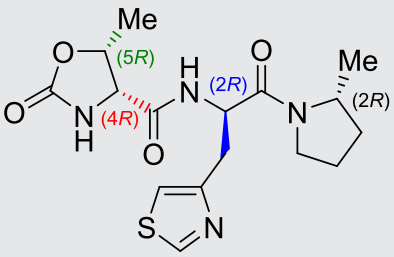
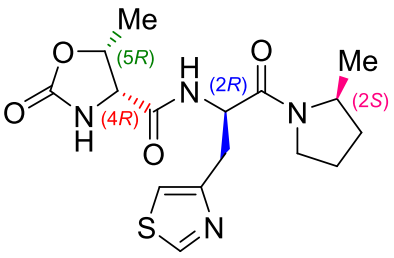
(Continues)

**TABLE 1** (Continued)

| compounds | chemical structures and absolute configurations | dose (iv) (μmol/kg) | $\Delta AUC_{0-7h}$ (°C·h) |
|---|---|---|---|
| 6 |  | 0.50 | 11.1 ± 16.4 |
| 7 |  | 0.50 | 9.0 ± 2.5 |
| 8 |  | 0.50 | 14.4 ± 11.0 |
| 9 |  | 0.50 | 7.2 ± 9.6 |
| 10 |  | 0.50 | 39.1 ± 4.5 |
| 11 |  | 0.50 | 6.8 ± 6.1 |

(Continues)

**TABLE 1** (Continued)

| compounds | chemical structures and absolute configurations | dose (iv) (μmol/kg) | $\Delta AUC_{0-7h}$ (°C·h) |
|---|---|---|---|
| 12 |  | 0.50 | 23.4 ± 2.5 |
| 13 |  | 0.50 | 17.7 ± 2.3 |
| 14 |  | 0.50 | 22.8 ± 0.9 |
| 15 |  | 0.50 | 9.2 ± 0.4 |
| 16 |  | 0.50 | −3.2 ± 0.6 |

Each value represents the mean ± SD of at least three animals (except for two animals for compound **4**).

The formula for computation is as follows:

$AUC_{0-7h}$: area under the rectal temperature-time curve for 7 h,

$\Delta AUC_{0-7h}$ = $\Delta AUC_{0-7h}$ of rectal temperature (compound) − $\Delta AUC_{0-7h}$ of rectal temperature (saline).

Anti-hypothermic effect: administration dose (50 or 0.50 μmol/kg) × 7 h/$\Delta AUC_{0-7h}$.

The detailed calculation method is shown in the Supporting Information.

4-yl)propanoic acid **31**, respectively. Optically pure amino acids **30** and **31** were synthesized from 2-amino-3-(thiazol-4-yl)propanoic acid dihydrochloride **29** based on previous reports.[62-66] Treatment of the amino acids **30** and **31** with *p*-toluenesulfonic acid monohydrate ($TsOH{\cdot}H_2O$) afforded amino acid *p*-toluenesulfonate **32** and **33**, which were not isolated, respectively. The *p*-toluenesulfonate **32** and **33** were esterified with diphenyldiazomethane ($Ph_2CHN_2$) to give the middle-part fragments **21** and **22**, respectively (Scheme 2). The detailed synthetic method, yields and physical data of all intermediates are shown in the Supporting Information.

Reagents and conditions: (a) $TsOH{\cdot}H_2O$, $H_2O$, not isolated; (b) $Ph_2CHN_2$, MeOH, 0 °C, 97%.

## 2.4 | C-Terminus fragments

Two C-terminus fragments, 2-(2*R*)-methylpyrrolidine *p*-toluenesulfonate **23** and 2-(2*S*)-methylpyrrolidine *p*-toluenesulfonate **24** were synthesized via *N*-Boc-2-(2*R*)-methylpyrrolidine **36** and *N*-Boc-2-(2*S*)-methylpyrrolidine **37** from readily available *N*-Boc-L-proline **34** and *N*-Boc-D-proline **35** as starting materials (Scheme 3).[67-69] The detailed synthetic method, yields and physical data of all intermediates are shown in the Supporting Information.

Reagents and conditions: (a) $BH_3$-THF complex, THF, 0 °C to rt; (b) MsCl, $Et_3N$, THF, 0 °C to rt; (c) $LiEt_3BH$, THF, 0 °C to rt, 42–74%; (d) (1) $HCO_2H$, rt; (2) $TsOH{\cdot}H_2O$, quant.

## 2.5 | TRH mimetics 1–16

All fragments were coupled from the N-terminus to the C-terminus in the presence of peptide coupling agents (Scheme 4).[70] Four N-terminus fragments **17–20** were coupled with two middle-part fragments **21** and **22** using 1,3-dicyclohexylcarbodiimide (DCC) in the presence of *N*-hydroxybenzotriazole (HOBt) and triethylamine to afford dipeptide benzhydryl esters **38–45**, respectively. Deprotection of the benzhydryl group of dipeptide esters **38–45** with trifluoroacetic acid (TFA) in anisole gave dipeptide mimetics **46–53**, respectively. Finally, the dipeptide mimetics **46–53** were coupled with two C-terminus fragments **23** and **24** using DCC in the presence of *N*-hydroxysuccinimide (HOSu) and triethylamine to afford TRH mimetics **1–16**, respectively. The detailed physical data of dipeptide benzhydryl esters, dipeptide mimetics are shown in the Supporting Information.

Reagents and conditions: (a) HOBt, DCC, $Et_3N$, THF, 0 °C to rt, 68%–quant; (b) TFA, anisole, 0 °C, 56–98%; (c) HOSu, DCC, $Et_3N$, THF-DMF, 0 °C to rt, 32–71%.

## Anti-hypothermic effect of TRH mimetics 1–16

The anti-hypothermic effect of TRH and TRH mimetics **1–16** (Table 1) were evaluated by in vivo antagonistic effect on reserpine-induced hypothermia in mice.[15-19] Reserpine depletes catecholamine and serotonin in the brain and causes hypothermia. On the other hand, TRH and TRH analogues promote catecholamine secretion against reserpine and stimulate the sympathetic nervous system. As a result, the body temperature increases.[25] The mice used for the study had rectal temperatures of 30 °C or lower about 18 h after subcutaneous administration of reserpine (3 mg/kg). Rectal temperature was measured by thermistor before and after intravenous (iv) administration of TRH and TRH mimetics up to 7 h at a dose of 50 and 0.50 μmol/kg, respectively. The anti-hypothermic effect of all compounds were evaluated based on the area under the temperature-time curve after dosing ($AUC_{0-7h}$).[4] Also, the anti-hypothermic effect of each compound, which were measured by administration doses (iv) and $\Delta AUC_{0-7h}$ of TRH, TRH mimetics and saline, are shown in Table 1. The rectal temperature-time curves of each compound are shown in the Supporting Information.

Although most of the stereoisomers were inactive, only the (4*S*),(5*S*),(2*S*),(2*S*)-isomer **10** showed high anti-hypothermic effect. Five

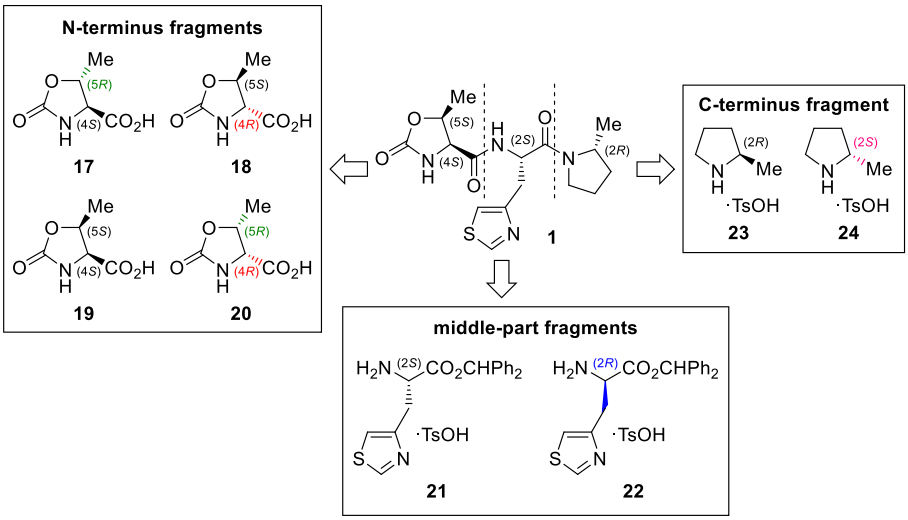
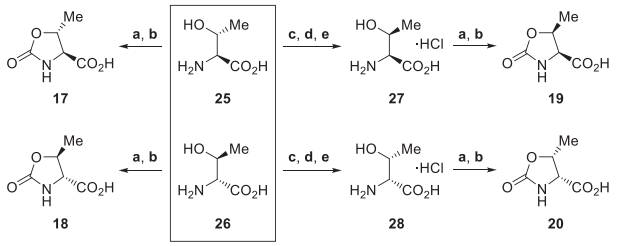



**FIGURE 2** All fragments and their absolute configurations.




**SCHEME 1** Synthesis of N-terminus fragments **17–20**.

stereoisomers **6**, **8** and **12–14** showed about 2- to 5-fold loss of anti-hypothermic effect compared with that of **1**. On the other hand, six stereoisomers **2**, **4**, **7**, **9**, **11** and **15** showed little anti-hypothermic effect. Moreover, three stereoisomers **3**, **5** and **16** showed no anti-hypothermic effect.

As for the N-terminus, the (4*R*),(5*S*),(2*S*),(2*R*)-isomer **6** showed 4-fold loss of the anti-hypothermic effect compared with that of **1**. On the other hand, the (4*S*),(5*R*),(2*S*),(2*R*)-isomer **2** showed approximately 6-fold loss of the anti-hypothermic effect compared with that of **1**. Moreover, the stereoisomers **6–9**, which have the (4*R*),(5*S*) configurations of oxazolidinone tended to show higher anti-hypothermic effect than that of the stereoisomers **2–5**, which have the (4*S*),(5*R*) configurations of oxazolidinone. The stereoisomers **10–12**, which have (4*S*),(5*S*) configurations and **13–15**, which have (4*R*),(5*R*) configurations of the oxazolidinone tended to show higher anti-hypothermic effect than that of **2–5**, which have (4*S*),(5*R*) configurations, and **6–9**, which have (4*R*),(5*S*) configurations. It seemed that the (5*R*) configuration of oxazolidinone strongly affects the anti-hypothermic effect rather than the (4*R*) configuration of oxazolidinone.

As for the middle-part, the (4*S*),(5*S*),(2*R*),(2*R*)-isomer **11** showed 6-fold loss of the anti-hypothermic effect compared with **1**. And the stereoisomers, which have the (5*R*) configuration of oxazolidinone and (2*R*) of the middle-part, in particular, the (4*S*),(5*R*),(2*R*),(2*R*)-isomer **4** and the (4*S*),(5*R*),(2*R*),(2*S*)-isomer **5**, showed little anti-hypothermic effect.

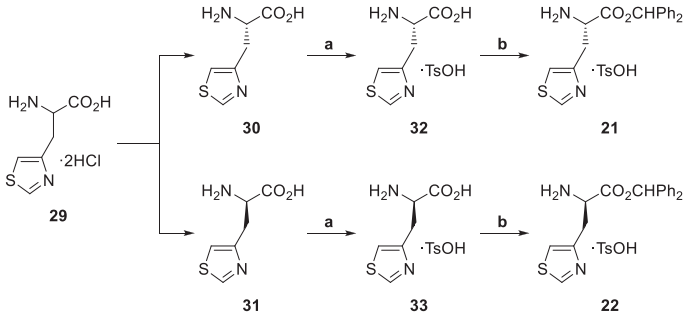
As for the C-terminus, the (4*S*),(5*S*),(2*S*),(2*S*)-isomer **10** showed the highest anti-hypothermic effect among the stereoisomers **2–16**. The stereoisomers showed similar anti-hypothermic effect between the (4*R*),(5*S*),(2*S*),(2*R*)-isomer **6** and the (4*R*),(5*S*),(2*S*),(2*S*)-isomer **7**, between the (4*R*),(5*R*),(2*S*),(2*R*)-isomer **13** and the (4*R*),(5*R*),(2*S*),(2*S*)-isomer **14**. On the other hand, the (4*S*),(5*R*),(2*S*),(2*R*)-isomer **2** and the (4*R*),(5*R*),(2*R*),(2*R*)-isomer **15** showed the anti-hypothermic effect but the (4*S*),(5*R*),(2*S*),(2*S*)-isomer **3** and the (4*R*),(5*R*),(2*R*),(2*S*)-isomer **16** did not show the anti-hypothermic effect at all.

Therefore, the (4*S*),(5*S*),(2*S*),(2*R*)-isomer **1** (rovatirelin) showed the best anti-hypothermic effect among the stereoisomers **1–16** and was selected as the candidate for clinical trials.

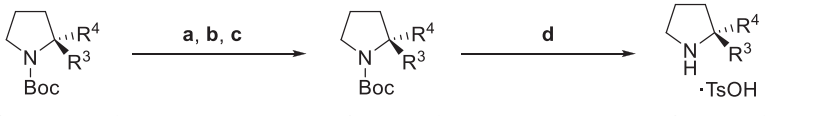
## 3 | SUMMARY AND CONCLUSIONS

The 16 stereoisomers of **1** were synthesized from all stereoisomers of four N-terminus fragments, two middle-part fragments and two C-terminus fragments. Four N-terminus fragments **17**, **19** and **18**, **20** were synthesized from readily available L- or D-threonine. Two middle-part fragments **21** and **22** were synthesized from unnatural amino acids 3-(thiazol-4-yl)-L- and D-alanine **25** and **27**, respectively. Two C-terminus fragments **23** and **24** were synthesized from readily available *N*-Boc-L- and *N*-Boc-D-proline, respectively. All fragments were coupled from the N-terminus to the C-terminus by the usual peptide coupling method to afford TRH mimetics **1–16**.

The in vivo anti-hypothermic effect of all stereoisomers was evaluated based on reserpine-induced hypothermia in mice. It seemed that no significant relationship between stereochemistry and the anti-hypothermic effect was indicated from the results of SAR studies. However, the absolute configuration of the 5-position of the oxazolidinone at the N-terminus and the middle-part seemed to be important for display of the anti-hypothermic effect. In fact, stereoisomers **4**, **5**, **15** and **16**, which have both the (5*R*) configuration of the oxazolidinone at the N-terminus and the (2*R*)

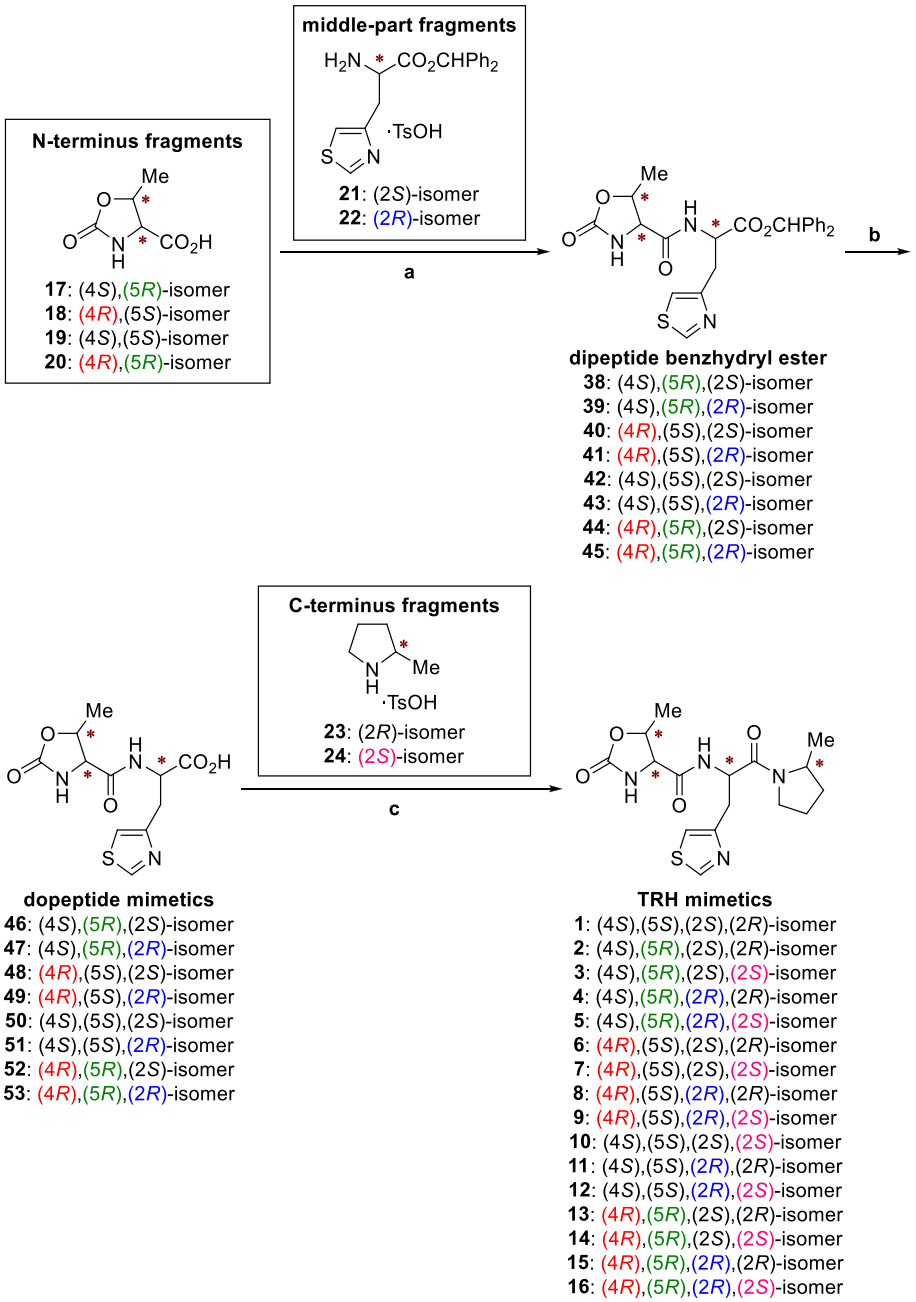



**SCHEME 2** Synthesis of the middle-part fragments **21** and **22**.




**34**: $R^3 = CO_2H$, $R^4 = H$
**35**: $R^3 = H$, $R^4 = CO_2H$
**36**: $R^3 = Me$, $R^4 = H$
**37**: $R^3 = H$, $R^4 = Me$
**23**: $R^3 = Me$, $R^4 = H$
**24**: $R^3 = H$, $R^4 = Me$

**SCHEME 3** Synthesis of C-terminus fragments **23** and **24**.




**SCHEME 4** General synthetic method of TRH mimetics **1**–**16**.

configuration at the middle-part showed much lower anti-hypothermic effect than that of **1**. On the other hand, it seemed that the (4*R*) configuration of the oxazolidinone at the N-terminus or the (2*S*) configuration of the C-terminus has little influence on the anti-hypothermic effect.

Although it was considered that the absolute configurations of TRH and TRH analogues were important to show high biological activities[18,56], our SAR result was incompatible with that of previous reports. As a result of the SAR study, our hypothesis that the (4*S*),(5*S*),(2*S*),(2*R*) stereochemistry of **1** was correct. Thus, this result could be useful when designing new CNS effective compounds around rovatirelin.

## 4 | EXPERIMENTAL SECTION

### 4.1 | General

All solvents and reagents were obtained from commercial sources and were used as received. Melting points (mp) were measured with a Yanagimoto melting point apparatus and are uncorrected. $^{1}$H and $^{13}$C NMR spectra were taken with a Varian VXR-200 or Gemini-200 300 FT-NMR spectrometer using tetramethylsilane as an internal standard. Infrared spectra were recorded on a Nicolet 20SXB FT-IR spectrometer. Elemental analyses were performed by Shionogi Research Laboratories (Shionogi & Co., Ltd. 3-1-1, Futaba-cho,

Toyonaka-shi, Osaka 561-0825, Japan). Optical rotations were determined with a Perkin-Elmer 430 polarimeter. For silica gel column chromatography, Kiesel gel 60 (0.063–0.20 mm, Merck) was employed for the purification. For gel filtration chromatography, MCI GEL CHP-20P (75–150 gm, Mitsubishi Chemical Industries) was utilized with aqueous MeOH as an eluent. Thin-layer chromatography (TLC) was carried out with Merck silica gel 603–254 plates mainly with use of the following two solvent systems: (a) $CHCl_3$/MeOH (9/1), (b) $CHCl_3$/MeOH/$H_2O$ (32/6/0.5) and (c) $CHCl_3$/MeOH/$H_2O$ (6/4/1). The spots were detected under ultraviolet irradiation at 254 nm and by the use of phosphomolybdic acid in ethanol solution and ninhydrin sprays.

## 4.2 | General procedure for preparing the N-terminus fragments 17–20

(4*S*,5*R*)-5-Methyl-2-oxooxazolidine-4-carboxylic acid (17).

To an ice cooled solution of *N*-benzyloxycarbonyl-L-threonine (**54**) (20.0 g, 79.0 mmol) in methanol (160 mL), 1 M aqueous sodium hydroxide solution (160 mL, 160 mmol) was added and the mixture was stirred for 15 min at the same temperature. After the ice bath was removed, the mixture was stirred continuously for 3.5 h. Ethyl acetate (100 mL) was added to the mixture and extracted. The aqueous layer was cooled in an ice bath, then 1 M aqueous hydrochloric acid solution (160 mL, 160 mmol) was added. The mixture was concentrated under reduced pressure. To the residue, chloroform (500 mL) was added and extracted. The organic layer was dried over anhydrous magnesium sulfate and concentrated in vacuo. The residue was crystallized with chloroform to give the title compound 17 (3.29 g, 29%) as a colorless solid.

mp 133–135 °C.

IR (KBr): 3303, 3245, 2714, 2600, 2507, 1744, 1727, 1673, 1227, 1207 $cm^{-1}$.

$^1H$ NMR (200 MHz, DMSO-$d_6$): δ 8.07 (brs, 1H), 4.58 (m, 1H), 3.95 (d, *J* = 5.2 Hz, 1H), 1.38 (d, *J* = 6.2 Hz, 3H).

$[\alpha]_D^{25}$ +40.9° (c 1.0, $H_2O$).

Anal. Calcd for $C_5H_7NO_4 \cdot 0.1H_2O$: C, 40.88; H, 4.94; N, 9.53. Found: C, 40.84; H, 4.90; N, 9.68.

lit.[57] mp 139.8–140.2 °C., $[\alpha]_D^{29.8}$ +41.8° (c 2.7, $H_2O$).

In a similar manner, 5-methyl-2-oxooxazolidine-4-carboxylic acid **18–20** were prepared.

### 4.2.1 | (4*R*,5*S*)-5-Methyl-2-oxooxazolidine-4-carboxylic acid (18)

The cyclization of *N*-benzyloxycarbonyl-D-threonine (**55**) (9.00 g, 35.5 mmol) in methanol (71.0 mL) with 1 M aqueous sodium hydroxide solution (71.0 mL, 71.0 mmol) yielded the title compound **18** (3.51 g, 68%) as colorless crystals.

mp 135–137 °C.

IR (KBr): 3303, 3246, 2714, 2599, 2507, 1744, 1728, 1673, 1227, 1207 $cm^{-1}$.

$^1H$ NMR (200 MHz, DMSO-$d_6$): δ 8.07 (brs, 1H), 4.58 (m, 1H), 3.96 (dd, *J* = 5.2, 0.8 Hz, 1H), 3.34 (brs, 1H), 1.38 (d, *J* = 6.2 Hz, 3H).

$[\alpha]_D^{22}$ −42.4° (c 1.0, $H_2O$).

Anal. Calcd for $C_5H_7NO_4$: C, 41.38; H, 4.86; N, 9.65. Found: C, 41.20; H, 4.82; N, 9.67.

lit.[57] mp 131–133.5 °C., $[\alpha]_D^{27}$ −37.7° (c 2.7, $H_2O$).

### 4.2.2 | (4*S*,5*S*)-5-Methyl-2-oxooxazolidine-4-carboxylic acid (19)

The cyclization of *N*-benzyloxycarbonyl-*allo*-L-threonine (**60**) (4.73 g, 18.0 mmol) in methanol (36.0 mL) with 1 M aqueous sodium hydroxide solution (36.0 mL, 36.0 mmol) yielded the title compound **19** (1.92 g, 73%) as colorless crystals.

mp 165–168 °C.

IR (KBr): 3363, 2632, 2550, 1746, 1685, 1412, 1224, 1156, 1060 $cm^{-1}$.

$^1H$ NMR (200 MHz, DMSO-$d_6$): δ 7.89 (brs, 1H), 4.85 (m, 1H), 4.27 (d, *J* = 8.4 Hz, 1H), 1.24 (d, *J* = 6.4 Hz, 3H).

$[\alpha]_D^{25}$ −20.7° (c 1.0, $H_2O$).

Anal. Calcd for $C_5H_7NO_4$: C, 41.38; H, 4.86; N, 9.65. Found: C, 41.23; H, 4.75; N, 9.63.

### 4.2.3 | (4*R*,5*R*)-5-Methyl-2-oxooxazolidine-4-carboxylic acid (20)

The cyclization of *N*-benzyloxycarbonyl-*allo*-D-threonine (**61**) (35.5 g, 140 mmol) in methanol (280 mL) with 1 M aqueous sodium hydroxide solution (280 mL, 280 mmol) yielded the title compound **20** (12.9 g, 64%) as colorless crystals.

mp 168–171 °C.

IR (KBr): 3360, 2996, 2931, 1746, 1683, 1413, 1225, 1156, 1061 $cm^{-1}$.

$^1H$ NMR (200 MHz, DMSO-$d_6$): δ 7.87 (brs, 1H), 4.84 (m, 1H), 4.27 (d, *J* = 8.6 Hz, 1H), 1.24 (d, *J* = 6.2 Hz, 3H).

$[\alpha]_D^{25}$ +19.3° (c 1.0, $H_2O$).

Anal. Calcd for $C_5H_7NO_4$: C, 41.38; H, 4.86; N, 9.65. Found: C, 41.54; H, 4.94; N, 9.75.

## 4.3 | General procedure for preparing the middle-part fragments 21 and 22

Benzhydryl (2*S*)-2-amino-3-(thiazol-4-yl)propanoate *p*-toluenesulfonate (21).

To a solution of (2*S*)-2-amino-3-(thiazol-4-yl)propanoic acid (**30**) (13.8 g, 80.0 mmol) in water (150 mL), *p*-toluenesulfonic acid monohydrate (15.2 g, 80.0 mmol) was added. The mixture was concentrated in vacuo to give 29.8 g of crude (2*S*)-2-amino-3-(thiazol-4-yl)propanoic acid *p*-toluenesulfonate (**32**). To an ice cooled solution of compound **32** in methanol (400 mL), diphenyldiazomethane (54.3 g, 278 mmol) was added portionwise and the mixture was stirred for 1 h at the same temperature. Acetic acid (100 mL) was added and the mixture was concentrated in vacuo. To the residue,

diethyl ether (700 mL) was added and the precipitate was collected by filtration to give the title compound **21** (39.5 g, 97%) as a colorless solid.

mp 139–140 °C.

IR (KBr): 1753, 1602, 1512, 1496, 1260, 1224, 1171, 1124, 1036, 1012 $cm^{-1}$.

$^1H$ NMR (200 MHz, $CD_3OD$): δ 8.92 (d, *J* = 2.0 Hz, 1H), 7.70 (m, 2H), 7.40–7.20 (m, 13H), 6.91 (s, 1H), 4.62 (t, *J* = 5.8 Hz, 1H), 3.47 (d, *J* = 5.8 Hz, 2H), 2.36 (s, 3H).

$[\alpha]_D^{23}$ −34.7° (c 1.0, $CHCl_3$).

Anal. Calcd for $C_{26}H_{26}N_2O_5S_2$: C, 61.16; H, 5.13; N, 5.49; S, 12.56. Found: C, 61.14; H, 5.32; N, 5.41; S, 12.46.

In a similar manner, (*R*)-isomer **22** was prepared.

Benzhydryl (2*R*)-2-amino-3-(thiazol-4-yl)propanoate *p*-toluenesulfonate (22).

The esterification of (2*R*)-2-amino-3-(thiazol-4-yl)propanoic acid (**31**) (13.6 g, 78.7 mmol) with *p*-toluenesulfonic acid monohydrate (15.0 g, 78.7 mmol) and diphenyldiazomethane (30.6 g, 157 mmol) yielded the title compound **22** (39.1 g, 97%) as a colorless solid.

mp 139–140 °C.

IR (KBr): 3437, 3236, 1753, 1603, 1512, 1496, 1260, 1223, 1171, 1124, 1036, 1011 $cm^{-1}$.

$^1H$ NMR (200 MHz, $CD_3OD$): δ 8.92 (d, *J* = 1.8 Hz, 1H), 7.70 (m, 2H), 7.40–7.20 (m, 13H), 6.91 (s, 1H), 4.62 (t, *J* = 6.0 Hz, 1H), 3.47 (d, *J* = 6.0 Hz, 2H), 2.36 (s, 3H).

$[\alpha]_D^{25}$ +33.1° (c 1.0, $CHCl_3$).

Anal. Calcd for $C_{26}H_{26}N_2O_5S_2$: C, 61.16; H, 5.13; N, 5.49; S, 12.56. Found: C, 60.99; H, 5.19; N, 5.28; S, 12.29.

## 4.4 | General procedure for preparing the C-terminus fragments 23 and 24

### 4.4.1 | *N-tert*-Butoxycarbonyl-(2*R*)-2-methylpyrrolidine (36)

To an ice cooled 1 M lithium triethylborohydride in THF (1.20 L, 1.20 mol), *N-tert*-butoxycarbonyl-(2*S*)-2-[(methylsulfonyl)oxymethyl]pyrrolidine (**68**) (161 g, 580 mmol) in THF (380 mL) was added dropwise for 2 h. The ice bath was removed, and the mixture was stirred for 2 h at room temperature. The mixture was poured into ice cooled water (1.00 L) and stirred for 1 h. Toluene (1.50 L) was added and extracted. The organic layer was washed with aqueous sodium hydrogen carbonate solution (1.00 L) and water (1.00 L × 2). The organic layer was dried over anhydrous magnesium sulfate and concentrated in vacuo. The residue was distilled under reduced pressure to afford the title compound **36** (90.7 g, 85%) as a colorless oil.

bp 56–58 °C (3 mmHg).

IR ($CHCl_3$): 1681, 1477, 1454, 1403, 1366, 1170 $cm^{-1}$.

$^1H$ NMR (300 MHz, $CDCl_3$): δ 3.87 (brs, 1H), 3.35 (brs, 2H), 2.10 −1.70 (m, 3H), 1.60–1.40 (m, 1H), 1.47 (s, 9H), 1.16 (d, *J* = 6.0 Hz, 3H).

$[\alpha]_D^{25}$ −33.2° (c 1.7, $CHCl_3$).

Anal. Calcd for $C_{10}H_{19}NO_2$: C, 64.83; H, 10.34; N, 7.56. Found: C, 64.61; H, 10.34; N, 7.47.

In a similar manner, (*S*)-isomer **37** was prepared.

### 4.4.2 | *N-tert*-Butoxycarbonyl-(2*S*)-2-methylpyrrolidine (37)

The reduction of *N-tert*-butoxycarbonyl-(2*R*)-2-[(methylsulfonyl)oxymethyl]pyrrolidine (**69**) (2.50 g, 8.95 mmol) with 1 M lithium triethylborohydride in THF (18.0 mL, 18.0 mmol) yielded the title compound **37** (1.24 g, 75%) as a colorless oil.

bp 55–56 °C (3 mmHg).

IR ($CHCl_3$): 1681, 1478, 1454, 1404, 1366, 1170 $cm^{-1}$.

$^1H$ NMR (300 MHz, $CDCl_3$): δ 3.87 (brs, 1H), 3.35 (m, 2H), 2.05 −1.40 (m, 4H), 1.46 (s, 9H), 1.16 (d, *J* = 6.3 Hz, 3H).

$[\alpha]_D^{24}$ +33.6° (c 1.6, $CHCl_3$).

Anal. Calcd for $C_{10}H_{19}NO_2$: C, 64.83; H, 10.34; N, 7.56. Found: C, 64.65; H, 10.31; N, 7.52.

### 4.4.3 | (2*R*)-2-Methylpyrrolidine *p*-toluenesulfonate (23)

*N-tert*-Butoxycarbonyl-(2*R*)-2-methylpyrrolidine (**36**) (8.79 g, 47.4 mmol) was dissolved in formic acid (48.0 mL, 1.27 mol) and stirred for 20 h at room temperature. *p*-Toluenesulfonic acid monohydrate (9.01 g, 47.4 mmo) in water (50.0 mL) was added to the solution. The mixture was concentrated in vacuo to give the title compound **23** (12.4 g, quant) as a colorless amorphous powder, which was used without further purification.

$^1H$ NMR (300 MHz, $CD_3OD$): δ 7.70 (d, *J* = 8.7 Hz, 2H), 7.23 (d, *J* = 8.7 Hz, 2H), 3.63 (m, 1H), 3.28 (m, 2H), 2.37 (s, 3H), 2.30–1.90 (m, 3H), 1.62 (m, 1H), 1.37 (d, *J* = 6.9 Hz, 3H).

In a similar manner, (*S*)-isomer **24** was prepared.

### 4.4.4 | (2*S*)-2-Methylpyrrolidine *p*-toluenesulfonate (24)

The deprotection of *N-tert*-butoxycarbonyl-(2*S*)-2-methylpyrrolidine (**37**) (0.371 g, 2.00 mmol) with formic acid (2.00 mL, 53.0 mmol) and *p*-toluenesulfonic acid monohydrate (0.380 g, 2.00 mmol) yielded the title compound **24** (0.516 g, quant) as a colorless amorphous powder, which was used without further purification.

$^1H$ NMR (300 MHz, $CD_3OD$): δ 7.70 (d, *J* = 8.7 Hz, 2H), 7.23 (d, *J* = 8.7 Hz, 2H), 3.63 (m, 1H), 3.28 (m, 2H), 2.37 (s, 3H), 2.30–1.90 (m, 3H), 1.62 (m, 1H), 1.38 (d, *J* = 6.9 Hz, 3H).

## 4.5 | General procedure for preparing the TRH mimetics 1–16

### 4.5.1 | (4*S*,5*S*)-5-Methyl-*N*-{(2*S*)-1-[(2*R*)-2-methylpyrrolidin-1-yl]-1-oxo-3-(1,3-thiazol-4-yl) propan-2-yl}-2-oxo-1,3-oxazolidine-4-carboxamide (1)

To an ice cooled solution of (2*S*)-2-[(4*S*,5*S*)-5-methyl-2-oxooxazolidine-4-carboxamido]-3-(thiazol-4-yl)propanoic acid (**50**)

(9.00 g, 30.1 mmol) and HOSu (3.63 g, 31.6 mmol) in THF (320 mL)-DMF (40.0 mL), DCC (6.51 g, 31.6 mmol) was added and the mixture was stirred for 4 h at the same temperature. To this solution, (2*R*)-2-methylpyrrolidine *p*-toluenesulfonate (**23**) (7.75 g, 30.1 mmol) and triethylamine (8.40 mL, 60.1 mmol) were added and the mixture was stirred for 1 h at 0 °C. The ice bath was removed, and the mixture was stirred for 24 h. The precipitate was filtered off and the filtrate was concentrated in vacuo. The residue was purified by gel filtration chromatography (MCI GEL CHP-20P, 600 mL, eluent: $H_2O$/MeOH) and by silica gel column chromatography (eluent: $CHCl_3$/MeOH). The purified fractions were concentrated in vacuo and the residue was lyophilized to afford the title compound **1** (6.22 g, 57%) as a white amorphous powder.

IR (KBr): 3279, 2972, 2877, 1763, 1655, 1626, 1548, 1456, 1384, 1231 $cm^{-1}$.

$^1H$ NMR (300 MHz, $CD_3OD$): δ 8.97 and 8.96 (d each, *J* = 2.1 Hz, total 1H), 7.34 and 7.33 (d each, *J* = 2.1 Hz, total 1H), 5.19 and 5.05 (t each, *J* = 7.5 Hz, total 1H), 4.92 (dq, *J* = 8.7, 6.6 Hz, 1H), 4.37 and 4.35 (d each, *J* = 8.7 Hz, total 1H), 4.07 and 3.92 (m each, total 1H), 3.75 (m, 1H), 3.41 (m, 1H), 3.22 (m, 2H), 2.00–1.50 (m, 4H), 1.28 and 1.22 (d each, *J* = 6.6 Hz, total 3H), 1.21 and 1.02 (d each, *J* = 6.6 Hz, total 3H).

$[\alpha]_D^{22}$ −4.4° (c 1.0, MeOH).

Anal. Calcd for $C_{16}H_{22}N_4O_4S{\cdot}H_2O$: C, 49.99; H, 6.29; N, 14.57; S, 8.34. Found: C, 49.79; H, 6.22; N, 14.55; S, 8.25.

In a similar manner, other stereoisomers **2–16** were prepared.

### 4.5.2 | (4*S*,5*R*)-5-Methyl-*N*-{(2*S*)-1-[(2*R*)-2-methylpyrrolidin-1-yl]-1-oxo-3-(1,3-thiazol-4-yl) propan-2-yl}-2-oxo-1,3-oxazolidine-4-carboxamide (2)

The condensation of (2*S*)-2-[(4*S*,5*R*)-5-methyl-2-oxooxazolidine-4-carboxamido]-3-(thiazol-4-yl)propanoic acid (**46**) (0.600 g, 2.00 mmol) and (2*R*)-2-methylpyrrolidine *p*-toluenesulfonate (**23**) (0.515 g, 2.00 mmol) yielded the title compound **2** (0.500 g, 68%) as a white amorphous powder.

IR (KBr): 3276, 3093, 2973, 2031, 2875, 1762, 1677, 1625, 1540, 1516, 1447, 1384, 1227, 1067 $cm^{-1}$.

$^1H$ NMR (300 MHz, $CD_3OD$): δ 8.97 and 8.96 (d each, *J* = 2.1 Hz, total 1H), 7.33 (m, 1H), 5.11 and 4.97 (t each, *J* = 7.5 Hz, total 1H), 4.50 (m, 1H), 4.09 and 3.94 (m each, total 1H), 3.94 (d, *J* = 5.4 Hz, 1H), 3.70 and 3.42 (m each, 2H), 3.22 (m, 2H), 2.00–1.50 (m, 4H), 1.47 (d, *J* = 6.6 Hz, 3H), 1.26 and 1.04 (d each, *J* = 6.3 Hz, total 3H).

$[\alpha]_D^{25}$ +34.0° (c 1.0, $H_2O$).

Anal. Calcd for $C_{16}H_{22}N_4O_4S{\cdot}0.9H_2O$: C, 50.22; H, 6.27; N, 14.64; S, 8.38. Found: C, 50.23; H, 6.34; N, 14.88; S, 8.45.

### 4.5.3 | (4*S*,5*R*)-5-Methyl-*N*-{(2*S*)-1-[(2*S*)-2-methylpyrrolidin-1-yl]-1-oxo-3-(1,3-thiazol-4-yl) propan-2-yl}-2-oxo-1,3-oxazolidine-4-carboxamide (3)

The condensation of (2*S*)-2-[(4*S*,5*R*)-5-methyl-2-oxooxazolidine-4-carboxamido]-3-(thiazol-4-yl)propanoic acid (**46**) (0.600 g, 2.00 mmol) and (2*S*)-2-methylpyrrolidine *p*-toluenesulfonate (**24**) (0.515 g, 2.00 mmol) yielded the title compound **3** (0.489 g, 67%) as a white amorphous powder.

IR (KBr): 3338, 3051, 2971, 1754, 1661, 1638, 1537, 1438, 1372, 1329, 1224, 1110, 1072 $cm^{-1}$.

$^1H$ NMR (300 MHz, $CD_3OD$): δ 8.94 (d, *J* = 2.1 Hz, 1H), 7.33 (d, *J* = 2.1 Hz, 1H), 4.99 (m, 1H), 4.50 and 4.05 (m each, total 1H), 4.45 (m, 1H), 3.93 and 3.91 (d each, *J* = 5.1 Hz, total 1H), 3.72 and 3.42 (m each, 2H), 3.40–3.10 (m, 2H), 2.10–1.50 (m, 4H), 1.45 and 1.44 (d each, *J* = 6.3 Hz, total 3H), 1.16 and 1.08 (d each, *J* = 6.3 Hz, total 3H).

$[\alpha]_D^{25}$ +44.3° (c 1.0, $H_2O$).

Anal. Calcd for $C_{16}H_{22}N_4O_4S{\cdot}0.7H_2O$: C, 50.70; H, 6.22; N, 14.78; S, 8.46. Found: C, 50.75; H, 6.26; N, 15.05; S, 8.29.

### 4.5.4 | (4*S*,5*R*)-5-Methyl-*N*-{(2*R*)-1-[(2*R*)-2-methylpyrrolidin-1-yl]-1-oxo-3-(1,3-thiazol-4-yl) propan-2-yl}-2-oxo-1,3-oxazolidine-4-carboxamide (4)

The condensation of (2*R*)-2-[(4*S*,5*R*)-5-Methyl-2-oxooxazolidine-4-carboxamido]-3-(thiazol-4-yl)propanoic acid (**47**) (0.800 g, 2.67 mmol) and (2*R*)-2-methylpyrrolidine *p*-toluenesulfonate (**23**) (0.688 g, 2.67 mmol) yielded the title compound **4** (0.577 g, 59%) as a white amorphous powder.

IR (KBr): 3272, 3087, 2972, 2931, 2875, 1752, 1683, 1625, 1542, 1517, 1442, 1229, 1068 $cm^{-1}$.

$^1H$ NMR (300 MHz, $CD_3OD$): δ 8.95 and 8.94 (d each, *J* = 2.1 Hz, total 1H), 7.33 (d, *J* = 2.1 Hz, 1H), 5.08 and 5.02 (dd each, *J* = 8.1, 6.6 Hz, total 1H), 4.52 and 4.06 (m each, total 1H), 4.37 (m, 1H), 3.92 and 3.89 (d each, *J* = 5.4 Hz, total 1H), 3.73 and 3.43 (m each, total 2H), 3.30–3.10 (m, 2H), 2.10–1.50 (m, 4H), 1.41 and 1.39 (d each, *J* = 6.3 Hz, total 3H), 1.16 and 1.11 (d each, *J* = 6.3 Hz, total 3H).

$[\alpha]_D^{24}$ +10.4° (c 1.0, $H_2O$).

Anal. Calcd for $C_{16}H_{22}N_4O_4S{\cdot}0.2H_2O$: C, 51.93; H, 6.10; N, 15.14; S, 8.66. Found: C, 52.04; H, 6.12; N, 15.21; S, 8.58.

### 4.5.5 | (4*S*,5*R*)-5-Methyl-*N*-{(2*R*)-1-[(2*S*)-2-methylpyrrolidin-1-yl]-1-oxo-3-(1,3-thiazol-4-yl) propan-2-yl}-2-oxo-1,3-oxazolidine-4-carboxamide (5)

The condensation of (2*R*)-2-[(4*S*,5*R*)-5-methyl-2-oxooxazolidine-4-carboxamido]-3-(thiazol-4-yl)propanoic acid

(**47**) (0.800 g, 2.67 mmol) and (2*S*)-2-methylpyrrolidine *p*-toluenesulfonate (**24**) (0.688 g, 2.67 mmol) yielded the title compound **5** (0.391 g, 40%) as a white amorphous powder.

IR (KBr): 3273, 3089, 2973, 2931, 2875, 1752, 1683, 1626, 1542, 1517, 1447, 1229, 1068 $cm^{-1}$.

$^1H$ NMR (300 MHz, $CD_3OD$): δ 8.98 and 8.97 (d each, *J* = 1.8 Hz, total 1H), 7.34 (d, *J* = 1.8 Hz, 1H), 5.13 and 5.00 (t each, *J* = 7.5 Hz, total 1H), 4.44 (m, 1H), 4.09 and 3.97 (m each, total 1H), 3.94 (d, *J* = 5.4 Hz, 1H), 3.71 and 3.43 (m each, total 2H), 3.30–3.10 (m, 2H), 2.00–1.50 (m, 4H), 1.43 (d, *J* = 6.3 Hz, 3H), 1.28 and 1.04 (d each, *J* = 6.6 Hz, total 3H).

$[\alpha]_D^{24}$ +20.7° (c 1.0, $H_2O$).

Anal. Calcd for $C_{16}H_{22}N_4O_4S{\cdot}0.2H_2O$: C, 51.93; H, 6.10; N, 15.14; S, 8.66. Found: C, 51.80; H, 6.10; N, 15.35; S, 8.69.

### 4.5.6 | (4*R*,5*S*)-5-Methyl-*N*-{(2*S*)-1-[(2*R*)-2-methylpyrrolidin-1-yl]-1-oxo-3-(1,3-thiazol-4-yl)propan-2-yl}-2-oxo-1,3-oxazolidine-4-carboxamide (6)

The condensation of (2*S*)-2-[(4*R*,5*S*)-5-methyl-2-oxooxazolidine-4-carboxamido]-3-(thiazol-4-yl)propanoic acid (**48**) (0.500 g, 1.67 mmol) and (2*R*)-2-methylpyrrolidine *p*-toluenesulfonate (**23**) (0.430 g, 1.67 mmol) yielded the title compound **6** (0.240 g, 39%) as colorless crystals.

mp 88–90 °C.

IR (KBr): 3271, 3088, 2973, 2931, 2875, 1751, 1676, 1626, 1543, 1518, 1449, 1230, 1068 $cm^{-1}$.

$^1H$ NMR (300 MHz, $CD_3OD$): δ 8.98 and 8.97 (d each, *J* = 1.8 Hz, total 1H), 7.34 (d, *J* = 1.8 Hz, 1H), 5.13 and 5.00 (t each, *J* = 7.2 Hz, total 1H), 4.45 (m, 1H), 4.09 and 3.97 (m each, total 1H), 3.94 (d, *J* = 4.8 Hz, 1H), 3.70 and 3.43 (m each, total 2H), 3.29 (m, 2H), 2.10–1.50 (m, 4H), 1.43 (d, *J* = 6.3 Hz, 3H), 1.28 and 1.04 (d each, *J* = 6.6 Hz, total 3H).

$[\alpha]_D^{24}$ −20.2° (c 1.0, $H_2O$).

Anal. Calcd for $C_{16}H_{22}N_4O_4S{\cdot}0.5H_2O$: C, 51.19; H, 6.17; N, 14.92; S, 8.54. Found: C, 51.22; H, 6.17; N, 15.11; S, 8.72.

### 4.5.7 | (4*R*,5*S*)-5-Methyl-*N*-{(2*S*)-1-[(2*S*)-2-methylpyrrolidin-1-yl]-1-oxo-3-(1,3-thiazol-4-yl)propan-2-yl}-2-oxo-1,3-oxazolidine-4-carboxamide (7)

The condensation of (2*S*)-2-[(4*R*,5*S*)-5-methyl-2-oxooxazolidine-4-carboxamido]-3-(thiazol-4-yl)propanoic acid (**48**) (0.660 g, 2.21 mmol) and (2*S*)-2-methylpyrrolidine *p*-toluenesulfonate (**24**) (0.569 g, 2.21 mmol) yielded the title compound **7** (0.505 g, 62%) as a white amorphous powder.

IR (KBr): 3272, 3086, 2973, 2875, 1751, 1677, 1624, 1542, 1517, 1442, 1407, 1384, 1345, 1229, 1067 $cm^{-1}$.

$^1H$ NMR (300 MHz, $CD_3OD$): δ 8.95 and 8.94 (d each, *J* = 2.1 Hz, total 1H), 7.33 (d, *J* = 2.1 Hz, 1H), 5.08 and 5.02 (dd each, *J* = 8.1, 6.6 Hz, total 1H), 4.52 and 4.06 (m each, total 1H), 4.36 (m, 1H), 3.93 and 3.89 (d each, *J* = 5.4 Hz, total 1H), 3.74 and 3.42 (m each, total 2H), 3.40–3.10 (m, 2H), 2.10–1.50 (m, 4H), 1.41 and 1.39 (d each, *J* = 6.6 Hz, total 3H), 1.16 and 1.11 (d each, *J* = 6.6 Hz, total 3H).

$[\alpha]_D^{25}$ −10.4° (c 1.0, $H_2O$).

Anal. Calcd for $C_{16}H_{22}N_4O_4S{\cdot}H_2O$: C, 49.99; H, 6.29; N, 14.57; S, 8.34. Found: C, 50.03; H, 6.35; N, 14.86; S, 8.37.

### 4.5.8 | (4*R*,5*S*)-5-Methyl-*N*-{(2*R*)-1-[(2*R*)-2-methylpyrrolidin-1-yl]-1-oxo-3-(1,3-thiazol-4-yl)propan-2-yl}-2-oxo-1,3-oxazolidine-4-carboxamide (8)

The condensation of (2*R*)-2-[(4*R*,5*S*)-5-methyl-2-oxooxazolidine-4-carboxamido]-3-(thiazol-4-yl)propanoic acid (**49**) (0.800 g, 2.67 mmol) and (2*R*)-2-methylpyrrolidine *p*-toluenesulfonate (**23**) (0.688 g, 2.67 mmol) yielded the title compound **8** (0.595 g, 61%) as a white amorphous powder.

IR (KBr): 3274, 3108, 2973, 2931, 2875, 1763, 1676, 1626, 1540, 1517, 1442, 1384, 1227, 1067 $cm^{-1}$.

$^1H$ NMR (300 MHz, $CD_3OD$): δ 8.94 and 8.93 (d each, *J* = 2.1 Hz, total 1H), 7.32 (d, *J* = 2.1 Hz, 1H), 5.02 (m, 1H), 4.45 (m, 1H), 4.44 and 4.05 (m each, total 1H), 3.93 and 3.91 (d each, *J* = 5.4 Hz, total 1H), 3.72 and 3.43 (m each, total 2H), 3.30–3.10 (m, 2H), 2.10–1.50 (m, 4H), 1.45 and 1.44 (d each, *J* = 6.6 Hz, total 3H), 1.16 and 1.08 (d each, *J* = 6.6 Hz, total 3H).

$[\alpha]_D^{24}$ −45.3° (c 1.0, $H_2O$).

Anal. Calcd for $C_{16}H_{22}N_4O_4S{\cdot}0.2H_2O$: C, 51.93; H, 6.10; N, 15.14; S, 8.66. Found: C, 51.76; H, 6.10; N, 15.40; S, 8.64.

### 4.5.9 | (4*R*,5*S*)-5-Methyl-*N*-{(2*R*)-1-[(2*S*)-2-methylpyrrolidin-1-yl]-1-oxo-3-(1,3-thiazol-4-yl)propan-2-yl}-2-oxo-1,3-oxazolidine-4-carboxamide (9)

The condensation of (2*R*)-2-[(4*R*,5*S*)-5-methyl-2-oxooxazolidine-4-carboxamido]-3-(thiazol-4-yl)propanoic acid (**49**) (0.800 g, 2.67 mmol) and (2*S*)-2-methylpyrrolidine *p*-toluenesulfonate (**24**) (0.688 g, 2.67 mmol) yielded the title compound **9** (0.510 g, 52%) as a white amorphous powder.

IR (KBr): 3282, 3101, 2973, 2931, 2875, 1762, 1676, 1625, 1540. 1516, 1447, 1384, 1227, 1067 $cm^{-1}$.

$^1H$ NMR (300 MHz, $CD_3OD$): δ 8.97 and 8.96 (d each, *J* = 2.1 Hz, total 1H), 7.33 (d, *J* = 2.1 Hz, 1H), 5.11 and 4.98 (d each, *J* = 7.2 Hz, total 1H), 4.49 (m, 1H), 4.10 and 3.94 (m each, total 1H), 3.95 (d, *J* = 5.4 Hz, 1H), 3.69 and 3.41 (m each, total 2H), 3.30–3.10 (m, 2H), 2.00–1.50 (m, 4H), 1.47 (d, *J* = 6.3 Hz, 3H), 1.26 and 1.04 (d each, *J* = 6.6 Hz, total 3H).

$[\alpha]_D^{24}$ −35.9° (c 1.0, $H_2O$).

Anal. Calcd for $C_{16}H_{22}N_4O_4S{\cdot}0.2H_2O$: C, 51.93; H, 6.10; N, 15.14; S, 8.66. Found: C, 51.92; H, 6.12; N, 15.34; S, 8.66.

### 4.5.10 | (4*S*,5*S*)-5-Methyl-*N*-{(2*S*)-1-[(2*S*)-2-methylpyrrolidin-1-yl]-1-oxo-3-(1,3-thiazol-4-yl)propan-2-yl}-2-oxo-1,3-oxazolidine-4-carboxamide (10)

The condensation of (2*S*)-2-[(4*S*,5*S*)-5-methyl-2-oxooxazolidine-4-carboxamido]-3-(thiazol-4-yl)propanoic acid (**50**) (0.599 g, 2.00 mmol) and (2*S*)-2-methylpyrrolidine *p*-toluenesulfonate (**24**) (0.515 g, 2.00 mmol) yielded the title compound **10** (0.288 g, 39%) as colorless crystals.

mp 231 °C (decomp.)

IR (KBr): 3326, 3053, 2969, 1746, 1709, 1654, 1635, 1538, 1436, 1384, 1339, 1241 $cm^{-1}$.

$^1H$ NMR (300 MHz, $CD_3OD$): δ 8.95 and 8.93 (d each, *J* = 2.1 Hz, total 1H), 7.32 (d, *J* = 2.1 Hz, 1H), 5.08 (m, 1H), 4.91 (m, 1H), 4.60 and 4.03 (m each, total 1H), 4.34 and 4.30 (d each, *J* = 8.4 Hz, total 1H), 3.83 (m, 1H), 3.42 (m, 2H), 3.40–3.10 (m, 2H), 2.10–1.50 (m, 4H),

1.21 and 1.15 (d each, $J$ = 6.6 Hz, total 3H), 1.13 and 1.04 (d each, $J$ = 6.6 Hz, total 3H).

$[\alpha]_D^{24}$ +8.2° (c 1.0, MeOH).

Anal. Calcd for $C_{16}H_{22}N_4O_4S$: C, 52.44; H, 6.05; N, 15.29; S, 8.75. Found: C, 52.17; H, 6.06; N, 15.30; S, 8.82.

### 4.5.11 | (4*S*,5*S*)-5-Methyl-*N*-{(2*R*)-1-[(2*R*)-2-methylpyrrolidin-1-yl]-1-oxo-3-(1,3-thiazol-4-yl) propan-2-yl}-2-oxo-1,3-oxazolidine-4-carboxamide (11)

The condensation of (2*R*)-2-[(4*S*,5*S*)-5-methyl-2-oxooxazolidine-4-carboxamido]-3-(thiazol-4-yl)propanoic acid (**51**) (0.500 g, 1.67 mmol) and (2*R*)-2-methylpyrrolidine *p*-toluenesulfonate (**23**) (0.430 g, 1.67 mmol) yielded the title compound **11** (0.197 g, 32%) as colorless crystals.

mp 170–172 °C.

IR (KBr): 3340, 3130, 3094, 2985, 2967, 2873, 1749, 1661, 1637, 1538, 1442, 1382, 1340, 1235, 1098 $cm^{-1}$.

$^1H$ NMR (300 MHz, $CD_3OD$): δ 8.95 and 8.94 (d each, $J$ = 2.1 Hz, total 1H), 7.35 and 7.34 (d each, $J$ = 2.1 Hz, total 1H), 5.15 and 5.08 (dd each, $J$ = 8.1, 6.6 Hz, total 1H), 4.90–4.70 (m, 1H), 4.62 and 4.05 (m each, total 1H), 4.31 and 4.26 (d each, $J$ = 8.7 Hz, total 1H), 3.81 and 3.40 (m each, total 2H), 3.30–3.10 (m, 2H), 2.10–1.50 (m, 4H), 1.14 and 1.12 (d each, $J$ = 6.3 Hz, total 3H), 1.07 and 0.99 (d each, $J$ = 6.6 Hz, total 3H).

$[\alpha]_D^{22}$ −20.1° (c 1.0, $H_2O$).

Anal. Calcd for $C_{16}H_{22}N_4O_4S$: C, 52.44; H, 6.05; N, 15.29; S, 8.75. Found: C, 52.40; H, 5.98; N, 15.19; S, 8.77.

### 4.5.12 | (4*S*,5*S*)-5-Methyl-*N*-{(2*R*)-1-[(2*S*)-2-methylpyrrolidin-1-yl]-1-oxo-3-(1,3-thiazol-4-yl) propan-2-yl}-2-oxo-1,3-oxazolidine-4-carboxamide (12)

The condensation of (2*R*)-2-[(4*S*,5*S*)-5-Methyl-2-oxooxazolidine-4-carboxamido]-3-(thiazol-4-yl)propanoic acid (**51**) (0.599 g, 2.00 mmol) and (2*S*)-2-methylpyrrolidine *p*-toluenesulfonate (**23**) (0.515 g, 2.00 mmol) yielded the title compound **12** (0.306 g, 42%) as a white amorphous powder.

IR (KBr): 3398, 3274, 3090, 2972, 2875, 1748, 1683, 1626, 1542, 1517, 1446, 1383, 1342, 1230, 1099 $cm^{-1}$.

$^1H$ NMR (300 MHz, $CD_3OD$): δ 8.97 and 8.96 (d each, $J$ = 2.1 Hz, total 1H), 7.35 (d, $J$ = 2.1 Hz, 1H), 5.08 and 5.02 (dd each, $J$ = 8.1, 6.6 Hz, total 1H), 4.86 (m, 1H), 4.33 and 4.32 (d each, $J$ = 8.7 Hz, total 1H), 4.08 and 3.98 (m each, total 1H), 3.78 and 3.45 (m each, total 2H), 3.18 (m, 2H), 2.00–1.50 (m, 4H), 1.30 and 1.10 (d each, $J$ = 6.6 Hz, total 3H), 1.10 and 1.04 (d each, $J$ = 6.6 Hz, total 3H).

$[\alpha]_D^{25}$ −2.5° (c 1.0, $H_2O$).

Anal. Calcd for $C_{16}H_{22}N_4O_4S{\cdot}H_2O$: C, 49.99; H, 6.29; N, 14.57; S, 8.34. Found: C, 49.82; H, 6.31; N, 14.84; S, 8.26.

### 4.5.13 | (4*R*,5*R*)-5-Methyl-*N*-{(2*S*)-1-[(2*R*)-2-methylpyrrolidin-1-yl]-1-oxo-3-(1,3-thiazol-4-yl) propan-2-yl}-2-oxo-1,3-oxazolidine-4-carboxamide (13)

The condensation of (2*S*)-2-[(4*R*,5*R*)-5-methyl-2-oxooxazolidine-4-carboxamido]-3-(thiazol-4-yl)propanoic acid (**52**) (0.535 g, 1.79 mmol) and (2*R*)-2-methylpyrrolidine *p*-toluenesulfonate (**23**) (0.460 g, 1.79 mmol) yielded the title compound **13** (0.462 g, 71%) as a white amorphous powder.

IR (KBr): 3274, 3089, 2972, 2875, 1749, 1684, 1626, 1543, 1517, 1448, 1383, 1343, 1230, 1099 $cm^{-1}$.

$^1H$ NMR (300 MHz, $CD_3OD$): δ 8.97 and 8.96 (d each, $J$ = 2.1 Hz, total 1H), 7.35 (d, $J$ = 2.1 Hz, 1H), 5.21 and 5.06 (dd each, $J$ = 8.1, 6.6 Hz, total 1H), 4.90–4.70 (m, 1H), 4.33 and 4.31 (d each, $J$ = 8.7 Hz, total 1H), 4.20–3.99 (m, 1H), 3.77 and 3.45 (m each, total 2H), 3.20 (m, 2H), 2.10–1.50 (m, 4H), 1.30 and 1.10 (d each, $J$ = 6.6 Hz, total 3H), 1.10 and 1.04 (d each, $J$ = 6.6 Hz, total 3H).

$[\alpha]_D^{26}$ +1.2° (c 1.0, $H_2O$).

Anal. Calcd for $C_{16}H_{22}N_4O_4S{\cdot}H_2O$: C, 49.99; H, 6.29; N, 14.57; S, 8.34. Found: C, 49.83; H, 6.17; N, 14.71; S, 8.53.

### 4.5.14 | (4*R*,5*R*)-5-Methyl-*N*-{(2*S*)-1-[(2*S*)-2-methylpyrrolidin-1-yl]-1-oxo-3-(1,3-thiazol-4-yl) propan-2-yl}-2-oxo-1,3-oxazolidine-4-carboxamide (14)

The condensation of (2*S*)-2-[(4*R*,5*R*)-5-methyl-2-oxooxazolidine-4-carboxamido]-3-(thiazol-4-yl)propanoic acid (**52**) (0.535 g, 1.79 mmol) and (2*S*)-2-methylpyrrolidine *p*-toluenesulfonate (**24**) (0.460 g, 1.79 mmol) yielded the title compound **14** (0.455 g, 70%) as a white amorphous powder.

IR (KBr): 3273, 3086, 2972, 2875, 1749, 1682, 1625, 1542, 1518, 1442, 1384, 1342, 1230, 1099 $cm^{-1}$.

$^1H$ NMR (200 MHz, $CD_3OD$): δ 8.96 and 8.94 (d each, $J$ = 2.0 Hz, total 1H), 7.35 (d, $J$ = 2.0 Hz, 1H), 5.10 (m, 1H), 4.90–4.70 (m, 1H), 4.61 and 4.05 (m each, total 1H), 4.31 and 4.26 (d each, $J$ = 8.6 Hz, total 1H), 3.81 and 3.40 (m each, total 2H), 3.27 and 3.13 (dd each, $J$ = 14.2, 8.4 Hz, total 2H), 2.10–1.50 (m, 4H), 1.14 and 1.13 (d each, $J$ = 6.4 Hz, total 3H), 1.06 and 0.99 (d each, $J$ = 6.4 Hz, total 3H).

$[\alpha]_D^{26}$ +18.4° (c 1.0, $H_2O$).

Anal. Calcd for $C_{16}H_{22}N_4O_4S{\cdot}0.9H_2O$: C, 50.22; H, 6.27; N, 14.64; S, 8.38. Found: C, 50.06; H, 6.21; N, 14.84; S, 8.31.

### 4.5.15 | (4*R*,5*R*)-5-Methyl-*N*-{(2*R*)-1-[(2*R*)-2-methylpyrrolidin-1-yl]-1-oxo-3-(1,3-thiazol-4-yl)propan-2-yl}-2-oxo-1,3-oxazolidine-4-carboxamide (15)

The condensation of (2*R*)-2-[(4*R*,5*R*)-5-methyl-2-oxooxazolidine-4-carboxamido]-3-(thiazol-4-yl)propanoic acid (**53**) (0.800 g, 2.67 mmol) and (2*R*)-2-methylpyrrolidine *p*-toluenesulfonate (**23**) (0.688 g, 2.67 mmol) yielded the title compound **15** (0.452 g, 46%) as colorless needles.

mp 232 °C (decomp.)

IR (KBr): 3431, 3327, 3054, 2969, 1746, 1654, 1636, 1538, 1437, 1384, 1241 $cm^{-1}$.

$^1H$ NMR (300 MHz, $CD_3OD$): δ 8.95 and 8.93 (d each, *J* = 2.1 Hz, total 1H), 7.32 (d, *J* = 2.1 Hz, 1H), 5.08 (m, 1H), 4.91 (m, 1H), 4.34 and 4.31 (d each, *J* = 8.7 Hz, total 1H), 3.92 (m, 1H), 3.42 (m, 2H), 3.30 −3.10 (m, 2H), 2.10−1.50 (m, 4H), 1.21 and 1.15 (d each, *J* = 6.6 Hz, total 3H), 1.13 and 1.04 (d each, *J* = 6.6 Hz, total 3H).

$[\alpha]_D^{25}$ −10.0° (c 1.0, MeOH).

Anal. Calcd for $C_{16}H_{22}N_4O_4S$: C, 52.44; H, 6.05; N, 15.29; S, 8.75. Found: C, 52.44; H, 5.99; N, 15.34; S, 8.78.

### 4.5.16 | (4*R*,5*R*)-5-Methyl-*N*-{(2*R*)-1-[(2*S*)-2-methylpyrrolidin-1-yl]-1-oxo-3-(1,3-thiazol-4-yl) propan-2-yl}-2-oxo-1,3-oxazolidine-4-carboxamide (16)

The condensation of (2*R*)-2-[(4*R*,5*R*)-5-methyl-2-oxooxazolidine-4-carboxamido]-3-(thiazol-4-yl)propanoic acid (**53**) (0.800 g, 2.67 mmol) and (2*S*)-2-methylpyrrolidine *p*-toluenesulfonate (**24**) (0.460 g, 1.79 mmol) yielded the title compound **16** (0.542 g, 55%) as a white amorphous powder.

IR (KBr): 3274, 2971, 2875, 1760, 1676, 1627, 1540, 1516, 1448, 1232, 1097 $cm^{-1}$.

$^1H$ NMR (300 MHz, $CD_3OD$): δ 8.97 and 8.96 (d each, *J* = 2.1 Hz, total 1H), 7.34 (m, 1H), 5.19 and 5.04 (t each, *J* = 7.5 Hz, total 1H), 4.92 (m, 1H), 4.37 and 4.35 (d each, *J* = 8.7 Hz, total 1H), 4.07 (m, 1H), 3.77 (m, 2H), 3.30−3.10 (m, 2H), 2.00−1.50 (m, 4H), 1.28 and 1.22 (d each, *J* = 6.3 Hz, total 3H), 1.22 and 1.02 (d each, *J* = 6.3 Hz, total 3H).

$[\alpha]_D^{24}$ +2.3° (c 1.0, $H_2O$).

Anal. Calcd for $C_{16}H_{22}N_4O_4S{\cdot}0.2H_2O$: C, 51.93; H, 6.10; N, 15.14; S, 8.66. Found: C, 51.81; H, 6.10; N, 15.40; S, 8.66.

## 4.6 | Experiments Using Laboratory Animals

All experiments using laboratory animals were conducted in accordance with the guideline by the Animal Care and Use Committee in Shionogi.

## 4.7 | Anti Reserpine-Induced Hypothermia Effect in Mice

Male ddY mice were purchased from SLC Japan Inc. at the age of 6 weeks. After quarantine for 1 week, the mice were placed in animal compartments with a controlled room temperature of approximately 25 °C and relative humidity of approximately 60%, and a light cycle time of 12 h [light (8:00−20:00)/dark (20:00−8:00)]. Reserpine-induced hypothermia was conducted by the following method.[15-19] The mice used had rectal

temperatures of 30 °C or lower about 18 h after subcutaneous administration of reserpine (3 mg/kg, 1 mg/mL reserpine injection; Daiichi, Tokyo, Japan). TRH and TRH mimetics were dissolved in saline. Rectal temperature was measured with a thermistor (MGA-III, Nihon Kohden) before and after iv administration of TRH and TRH mimetics up to 7 h after a dose of 50 and 0.5 μmol/kg/mL, respectively. The antagonistic effect of the test drugs on reserpine-induced hypothermia was evaluated based on the area under the temperature−time curve after dosing ($AUC_{0-7h}$). All of the mice used in the experiments were killed immediately after the last measurement.

Supporting Information

Details are given of the synthetic procedure and analytical data for the intermediates (N-terminus: **27**, **28** and **54**–**61**; middle-part: **30**, **31** and **63**–**65**; C-terminus: **66**–**69**; dipeptide benzhydryl esters: **38**–**45**, dipeptide mimetics: **46**–**53**).

## FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## ACKNOWLEDGEMENTS

We thank R. Nishimura, K. Yoshino, K. Kuwano and R. Ikenishi for the elemental analysis, M. Sugita for the IR spectroscopy and optical rotation data, Y. Takayama and J. Kikuchi for the NMR spectroscopy data. T. Kihara, Y. Fujimura and H. Nakai for discussions concerning this work.

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## SUPPORTING INFORMATION

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**How to cite this article:** Kobayashi N, Sato N, Sugita K, et al. Synthesis and evaluation of in vivo anti-hypothermic effect of all stereoisomers of the thyrotropin-releasing hormone mimetic: Rovatirelin Hydrate. *J Pep Sci*. 2019;e3228. https://doi.org/10.1002/psc.3228