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Synthesis and activity in enhancing long-term potentiation (LTP) of clausenamide stereoisomers

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ABSTRACT

Clausenamide, isolated from aqueous extract of dry leaves of *Clausena lansium*, a Chinese folk medicine, was found to have potent activity in enhancing LTP and show nootropic activity in animal tests. In order to discovery more potent stereoisomers and to analyze the relationship of structure–activity, the synthesis of 16 (8 pairs) optically pure stereoisomers of clausenamide with four chiral centers was achieved. The results of LTP assay showed that the nootropic activity of the stereoisomers of clausenamide is closely related to the configuration of stereoisomers.

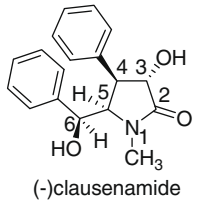
Synaptic plasticity in mammalian brain is one of the most widely studied topics in neuroscience over the last decade. One of the clinical symptoms of Alzheimer's disease (AD) is disorder of memory that has been suggested to be related with synaptic plasticity.[1] LTP is an important form of synaptic plasticity that increases the strength of synapses, and it is a physiologic correlate of memory and a cellular model for study of learning and memory.[2]

The naturally occurring clausenamide is in racemic form and was found to have potent activity in enhancing LTP and show nootropic activity in animal tests.[3] The synthetic single enantiomer (−)-(3*S*,4*R*,5*R*,6*S*)-clausenamide (**1**) (Fig. 1) could improve Aβ induced impairment of spatial discrimination, potentiate basic synaptic transmission and HFS-induced LTP on either anesthetized or freely moving rats, and increase cortical ChAT activity, hippocampal synapses and Mossy fiber sprouting.[4] In addition, **1** was 50–100 times more active than the known nootropic drug, piracetam, and 5–10 times more active than the racemic clausenamide[5], which indicated that the nootropic activity may be related with the configuration of clausenamide.

There are four chiral centers (C3, C4, C5, C6) in clausenamide, which means that 16 (8 pairs) stereoisomers are possible (Fig. 2). In order to discover more potent stereoisomers and to analyze the relationship between structure and activity of the clausenamide stereoisomers, herein, all optically pure stereoisomers of clausenamide were prepared, their activity in enhancing LTP were evaluated, and the primary relationships of structure–activity were analyzed.

A number of synthetic routes are available to build into the lactam core structure in clausenamide.[6] Four optically pure intermediate compounds, (3*S*,4*R*,5*R*)-clausenamidone (**17**), (3*R*,4*S*,5*S*)-clausenamidone (**18**), (3*S*,4*R*,5*S*)-neo-clausenamidone (**19**) and (3*R*,4*S*,5*R*)-neo-clausenamidone (**20**), were obtained through chiral resolution of racemic clausenamidone and neo-clausenamidone from a biomimetic synthetic route,[7] using menthoxylacetic acid as chiral derivatizing reagent.[8] Reduction of **17** with $NaBH_4$ afforded single product **1**, but its C6 isomer, (3*S*,4*R*,5*R*,6*R*) *epi*-clausenamide was not found under various reduction conditions (Scheme 1) due to the steric hindrance of the C4 phenyl group.

Reduction of **20** with sodium boronhydride gave two C6 isomers, (3*R*,4*S*,5*R*,6*S*)-neo-clausenamide (**3**) and (3*R*,4*S*,5*R*,6*R*)-*epi*-neo-clausenamide (**5**) in almost equal yields after column chromatography. However, reduction with Al(*i*-OPr)$_3$/*i*-PrOH afforded predominantly isomer **5** (mol ratio3:**5** = 1:20). Protection of C3–OH with dihydropyran and reduction with L-selectride mainly produced **3** (mol ratio **3**:**5** = 10:1) (Scheme 2).[9]



(-)clausenamide

Figure 1. The structure of (−)clausenamide (**1**).

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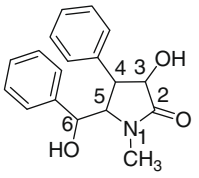
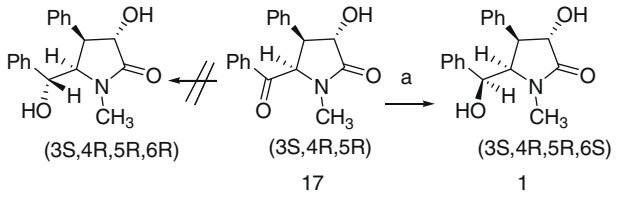


1 (3S,4R,5R,6S) **2** (3R,4S,5S,6R) clausenamide
3 (3R,4S,5R,6S) **4** (3S,4R,5S,6R) neoclausenamide
5 (3R,4S,5R,6R) **6** (3S,4R,5S,6S) epi-neoclausenamide
7 (3R,4R,5R,6S) **8** (3S,4S,5S,6R) cis-clausenamide
9 (3R,4R,5S,6R) **10** (3S,4S,5R,6S) cis-neoclausenamide
11 (3R,4R,5S,6S) **12** (3S,4S,5R,6R) epi-cis-neoclausenamide
13 (3R,4R,5R,6R) **14** (3S,4S,5S,6S) epi-cis-clausenamide
15 (3S,4R,5R,6R) **16** (3R,4S,5S,6S) epi-clausenamide

Figure 2. The configuration of 16 optically active stereoisomers of clausenamide.



Scheme 1. Reagents and condition: $NaBH_4/CH_3OH$ (85%).

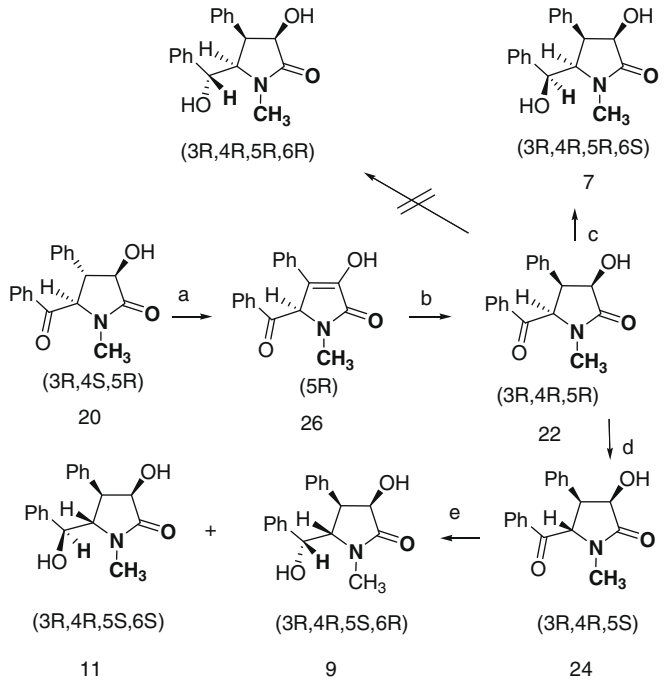
cis-Clausenamide has three substituent groups, C3–OH, C4–Ph, and C5-side chain on the same sides of the lactam ring. The congested structure might encounter difficulty in preparation. Indeed inversion of C3–OH in clausenamidone by Mitsunobu reaction or CsOAc/18-Crown ether failed. An attempt of de novo synthesis using cis epoxy cinnamate ester instead of trans epoxy in the clausenamide synthesis was also unsuccessful. Finally a revised synthetic approach by oxidation of C3–OH of neoclausenamidone for rebuilding the chirality of C4 into the desired configuration was designed and carried out (Scheme 3).

The oxidation of C3–OH of **20** gave a product (5*R*)-3,4-dehydroclausenamidone (**26**). An attempt to reduce the double bond in **26** under catalytic hydrogenation in ethanol gave a complex mixture. While treatment with $NaBH_4$ in the presence of acetic acid in dichloromethane, a single compound (3*R*,4*R*,5*R*)-*cis*-clausenamidone (**22**) was given. Its structure was confirmed by ^1H NMR and NOESY1D, in which strong NOEs between C3–H and C4–H, C4–H and C5–H were observed. The cis relation among C3, C4, C5 was established.

According to the structure of **22**, the reduction of C6=O should favor the Si face to give C6(*S*) configuration, (3*R*,4*R*,5*R*,6*S*)-*cis*-clausenamide, as the Re face is highly hindered by C4 phenyl group. Indeed, the reduction of **22** with $NaBH_4$ in methanol at 0 °C gave a product **7**.

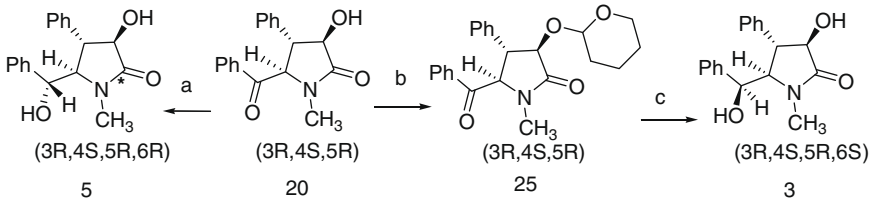
The treatment of (3*R*,4*R*,5*R*)-*cis*-clausenamidone (**22**) with K_2CO_3 in methanol afforded quantitatively a single C5 isomer **24**, which was recrystallized three times in acetone until specific rotation was consistent, giving white crystals with mp: 143–145 °C, $[\alpha]_D^{20}$ +140.5 (*c* 0.56, $CHCl_3$).

Reduction of **24** with $NaBH_4$ in methanol gave a mixture of two C6 isomer **9** and **11** in 80% combined yield, which was separated by column chromatography, with a ratio of **11**:**9** being 2:1. Then they were purified by repeated crystallization, **11** with mp: 248–250 °C, $[\alpha]_D^{22}$ +19.2 (*c* 0.525, CH_3OH), **9** with mp: 164.5–166 °C, $[\alpha]_D^{22}$ +66.7 (*c* 0.46, CH_3OH). Their structure was confirmed by spectra, MS_{FAB} showed: FW = 297, and ^1H NMR data of **11** and **9** showed that the configuration of C6 in **11** is different from that of C6 in **9**, which was inferred from the clear difference between **11** and **9**, $J_{5,6}$ = 2.7 Hz in **11**, $J_{5,6}$ = 5.4 Hz in **9**.



Scheme 3. Reagents and conditions: (a) $Na_2Cr_2O_7/H_2SO_4/H_2O/CH_2Cl_2$, 30–40 °C (76%); (b) $NaBH_4$/HOAc/CH_2Cl_2, 0 °C–rt (79%); (c) $NaBH_4/CH_3OH$ (85%); (d) KOH/CH_3OH, rt (90%); (e) $NaBH_4/CH_3OH$, 0 °C–rt (80%).

The absolute configuration of C6 in **11** could be deduced by comprehensive analysis for the dominant conformation, the reduction mechanism of **24** and the spectra of **11** and **24**. The reduction of C6=O should favor the Si face to give C6(*S*) configuration, (3*R*,4*R*,5*S*,6*S*)-*epi*-*cis*-neoclausenamide (**11**) as a main isomer, which is identical with the result of **11**:**9** = 2:1 (mol ratio). The product **9** should be (3*R*,4*R*,5*S*,6*R*)-*cis*-neo-clausenamide.



Scheme 2. Reagents and conditions: (a) Al(*i*-OPr)$_3$/*i*-PrOH (90%); (b) 3,4-dihydro-2H-pyran/CH_2Cl_2 (85%); (c) L-selectride/THF; TsOH/EtOH, rt (two steps, 75%).

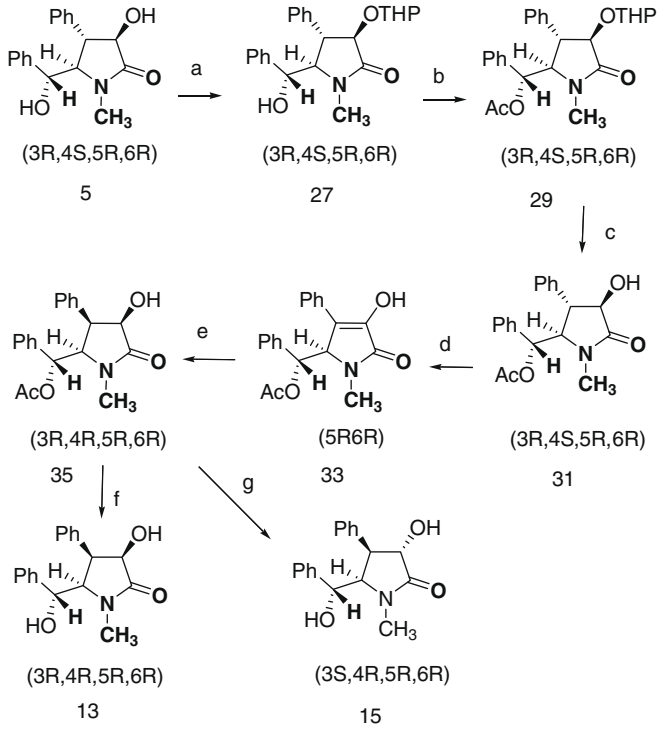
epi-cis-Clausenamide, a C6 isomer of *cis*-clausenamide, could not be produced by reduction of *cis*-clausenamidone. The preparation of (3*R*,4*R*,5*R*,6*R*)-*epi-cis*-clausenamide **13** required a Re reduction of C6=O of (3*R*,4*R*,5*R*) *cis*-clausenamidone **22**. Since the Re reduction of C6=O in the C4/C5 cis series (4*R*,5*R*) is very unfavorable, for the preparation of compound **13**, Scheme 4 was modified by using compound (+)(3*R*,4*S*,5*R*,6*R*)*epi*-neoclausenamide **5**, a compound carrying C6(*R*) configuration as starting material instead of C6=O (Scheme 4).

The C3–OH in **5** was first transferred into the acid labile pyranyl ether **27**. The C6–OH was acetylated to form the acid stable ester **29**. By acid treatment to set free the C3–OH yielded compound **31**, which was ready to build the cis part by the method used for compound **7**. The *epi-cis*-clausenamidyl acetate **35** obtained was then hydrolyzed in basic medium to give compound **13**, (3*R*,4*R*,5*R*,6*R*)*epi-cis*-clausenamide, in 19.5% total yield from starting material **5** through six steps.

epi-Clausenamide, a C6-isomer of clausenamide, could not be obtained from the reduction of clausenamidone above. For its preparation, we also selected a compound carrying C6(*R*) configuration, (3*R*,4*R*,5*R*,6*R*)*epi-cis*-clausenamidyl acetate **35** as starting material. Through inversion of C3–OH and hydrolysis of acetate, (−)(3*S*,4*R*,5*R*,6*R*)*epi*-clausenamide (**15**) was produced.

The preparation of eight optically pure isomers, **1**, **3**, **5**, **7**, **9**, **11**, **13** and **15** were described above. Their enantiomers, **2**, **4**, **6**, **8**, **10**, **12**, **14** and **16** were obtained from the corresponding starting material by the similar corresponding process described above. The physical data of 16 stereoisomers were given as follow (Table 1).

LTP was discovered in the mammalian hippocampus by Terje Lømo in 1966 and has remained a popular subject of neuroscientific research since. LTP is believed to play a critical role in behavioral learning, but its biological mechanisms have not yet been fully determined. (−)clausenamide(**1**) showed potent nootropic activity in many behavioral experiments, and is developed as a promising antidementia drug. The nootropic activity of other stereoisomers of clausenamide were evaluated herein in LTP assays compared with (−)clausenamide (**1**), as a positive control.



Scheme 4. Reagents and conditions: (a) 3,4-dihydro-2H-pyran/CH_2Cl_2, rt (84%); (b) acetic anhydride/pyridine (80%); (c) TsOH/EtOH, rt (78%); (d) $Na_2Cr_2O_7/H_2SO_4/H_2O/CH_2Cl_2$, 30–40 °C (68%); (e) $NaBH_4$/HOAc/CH_2Cl_2, 0 °C–rt (73%); (f) K_2CO_3/CH_3OH (75%); (g) DEAD/PPh_3/$ClCH_2COOH$/toluene, rt; TsOH/CH_3OH, rt (two steps, 18%).

Activity data in enhancing LTP for the all stereoisomers of clausenamide are reported in Table 1. Results showed that 60 min after compounds administration, **7** increased PS amplitude by 105.4%, **16** increased PS amplitude by 107.8%, **9** increased PS amplitude by 92.3% in comparison with before compounds treatment. The increased PS amplitude is over 30% and can maintained for more than 40 min, indicating that the three stereoisomers could increase basal synaptic transmission and induce LTP formation. Moreover, the increased PS amplitude (%) of the three isomers are greater than that of (−)clausenamide (**1**) (58.1%), suggesting that **7**, **9** and **16** had more potent nootropic activity than **1**.

In addition, **7**, **9** and **16** exhibited much more potent nootropic activity than their enantiomers, **8**, **10** and **15**. Stereoisomer **7** was 5 times more active than its enantiomer **8** (with increased PS amplitude by 22.5%), **16** was 6 times more active than its enantiomer **15** (with increased PS amplitude by 17.3%), and **9** was even more times than its enantiomer **10** (with increased PS amplitude by −5.1%).

Results in Table 1 also showed that 60 min after compounds administration, the increased PS amplitudes (%) of 1, 7, 9 and 16 are between 58.1 and 107.8, but other stereoisomers are between −15.4 and 22.5. The configuration range patterns for four chiral carbons in the four stereoisomers that could clearly increase basal synaptic transmission and induce LTP formation are (3*S*,4*R*,5*R*,6*S*)(**1**), (3*R*,4*R*,5*R*,6*S*)(**7**), (3*R*,4*R*,5*S*,6*R*)(**9**) and (3*R*,4*S*,5*S*,6*S*)(**16**), respectively. These results indicated that induction of LTP has the high selectivity for the configuration of clausenamide stereoisomers, and the nootropic activity of stereoisomers of clausenamide is closely related to the configuration of stereoisomers.

We should expect to elucidate the mechanisms of compounds (**1**, **7**, **9**, **16**) enhancing LTP, such as the activation of *N*-methyl-D-aspartate(NMDA) receptor and the production of brain derived neurotrophic factor, to draw a causal link between LTP and behavioral learning, and to find out the effect of compounds (**1**, **7**, **9**, **16**) on Alzheimer's disease and vascular dementia.

In summary, an efficient and economical synthesis of the 16 (8 pairs) optically pure stereoisomers of clausenamide with four chiral carbons was described. Based on a biomimetic synthetic route used for the preparation of clausenamide, two racemic intermediates clausenamidone and neoclausenamidone were produced; through the resolution with natural Menthol, four optically pure intermediates, **17**, **18**, **19** and **20** were obtained as starting chiral material used for the synthesis of all stereoisomers of clausenamide; by the strategy of elimination and regeneration of chiral centers and the invertion of C5 substituent group, four other optically pure intermediates, **22**, **24** and their enantiomers **23**, **25** were prepared; through a variety of reducing agents, 12 stereoisomers of clausenamide (**1–12**) were prepared; in the protecting of C6 configuration, the final 4 stereoisomers of clausenamide (**13–16**) were obtained. Their nootropic activities were evaluated by LTP assay, the result indicated that the increased PS amplitude of clausenamide stereoisomers is strongly related to the configuration of stereoisomers, and three isomers (**7**, **9**, **16**) exhibited more potent nootropic activity than **1**, however their corresponding enantiomers (**8**, **10**, **15**) displayed less potency than **1**. The discovery of three highly active compounds (**7**, **9**, **16**) may afforded more candidates for the developing of new nootropic drugs or antidementia drug. The high stereoselectivity of inducing LTP for the configuration of clausenamide

Table 1
The physical properties and their activity data in enhancing LTP of 16 optically active stereoisomers of clausenamide

No.	Compound	Absolute configuration	Mp (°C)	$[\alpha]_D^{20}$ (*c*, solvent)	Increased PSA%:means ± SD		
					15 min	30 min	60 min
1	(−)Clausenamide	3*S*,4*R*,5*R*,6*S*	161–162	−146 (0.21, MeOH)	31.8 ± 0. 4	38.5 ± 8.9	**58.1 ± 4.2***
2	(+)Clausenamide	3*R*,4*S*,5*S*,6*R*	161–162	+147 (0.19, MeOH)	−9.4 ± 0.3	10.1 ± 13.1	6.4 ± 4.1
3	(+)Neoclausenamide	3*R*,4*S*,5*R*,6*S*	186–187	+89.5 (0.2, MeOH)	−0.7 ± 29.6	−29.1 ± 7.9	−2.5 ± 18.9
4	(−)Neoclausenamide	3*S*,4*R*,5*S*,6*R*	186–187	−88.6 (0.18, MeOH)	−18.2 ± 14.0	−34.5 ± 4.3	
5	(+)*epi*-Neoclausenamide	3*R*,4*S*,5*R*,6*R*	220–222	+36.5 (0.15, MeOH)	4.7 ± 8.0	−5.3 ± 10.3	1.1 ± 28.5
6	(−)*epi*-Neoclausenamide	3*S*,4*R*,5*S*,6*S*	221–222	−37.7 (0.16, MeOH)	−9.4 ± 11.9	−8.1 ± 15.2	18.2 ± 15.4
7	(−)*cis*-Clausenamide	3*R*,4*R*,5*R*,6*S*	196–198	−6.07 (0.675, $CHCl_3$)	26.2 ± 2.3	41.2 ± 8.6	**105.4 ± 24.7***
8	(+)*cis*-Clausenamide	3*S*,4*S*,5*S*,6*R*	197–199	+6.30 (0.46, $CHCl_3$)	−12.7 ± 4.4	2.9 ± 5.9	22.5 ± 14.5
9	(+)*cis*-Neoclausenamide	3*R*,4*R*,5*S*,6*R*	164–166	+66.7 (0.46, MeOH)	35.0 ± 12.9	50.9 ± 19.9	**92.3 ± 22.4***
10	(−)*cis*-Neoclausenamide	3*S*,4*S*,5*R*,6*S*	168–170	−65.3 (0.32, MeOH)	−5.9 ± 6.7	−1.8 ± 6.6	−5.1 ± 17.5
11	(+)*epi-cis*-Neoclausenamide	3*R*,4*R*,5*S*,6*S*	248–250	+19.2 (0.53, MeOH)	−3.0 ± 18.9	10.1 ± 25.7	21.7 ± 37.1
12	(−)*epi-cis*-Neoclausenamide	3*S*,4*S*,5*R*,6*R*	250–252	−20.7 (0.265, MeOH)	−11.5 ± 10.2	−11.9 ± 9.4	−15.4 ± 16.8
13	(−)*epi-cis*-Clausenamide	3*R*,4*R*,5*R*,6*R*	199–202	−38.37 (0.66, $CHCl_3$)	7.2 ± 15.8	0.3 ± 12.3	5.4 ± 24.2
14	(+)*epi-cis*-Clausenamide	3*S*,4*S*,5*S*,6*S*	198–199	+39.7 (0.78, $CHCl_3$)	1.7 ± 5.2	9.7 ± 11.7	9.6 ± 12.6
15	(−)*epi*-Clausenamide	3*S*,4*R*,5*R*,6*R*	107–109	−203 (0.242, MeOH)	−2.2 ± 21.7	−8.8 ± 22.5	17.3 ± 22.5
16	(+)*epi*-Clausenamide	3*R*,4*S*,5*S*,6*S*	108–110	+201 (0.345, MeOH)	36.0 ± 22.8	47.5 ± 15.0	**107.8 ± 12.8***

The average amplitude of the population spikes recorded 30 min before drug administration was defined as 100%. The average amplitude of the population spikes recorded after drug administration was defined as PSA%. The increased PSA% values in table 1 were calculated by the formula: PSA% − 100%. All data are represented as mean ± SD of 5 observations.
* $p < 0.05$.

stereoisomers may contribute to the study on biological mechanisms of LTP.

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