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TETRAHEDRON: *ASYMMETRY*

Absolute configuration of the four stereoisomers of valnoctamide (2-ethyl-3-methyl valeramide), a potentially new stereospecific antiepileptic and CNS drug

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Abstract

Valnoctamide (2-ethyl-3-methyl valeramide, Nirvanil®, VCD), a mild tranquilizer endowed with anticonvulsant properties, exhibits diastereoselective and enantioselective pharmacokinetics in healthy subjects and epileptic patients. The purpose of this paper is to assign the absolute configuration of the four VCD stereoisomers and to describe the stereoselective synthesis used to prepare two-key VCD stereoisomers. We have synthesized two out of the four stereoisomers, with high diastereomeric excess, by two different synthetic methods. In both methods the (*S*) configuration at C-3 of VCD was fixed by synthesizing (*S*)-3-methyl valeric acid from L-isoleucine. In the first method the diastereomeric mixture (2*RS*,3*S*)-VCD was prepared. This mixture gave one of the diastereomers via repeated crystallizations, and its absolute configuration (2*R*,3*S*)-VCD, was established by X-ray crystallography using a single crystal. The absolute configuration of all four VCD stereoisomers, separated by chiral gas chromatography, was established on the basis of diastereomeric and enantiomeric correlations. In order to assess stereoselective pharmacodynamic properties of VCD, the single stereoisomers have to be synthesized. Stereoselective synthesis of (2*R*,3*S*)-VCD and (2*S*,3*S*)-VCD was accomplished by using chiral oxazolidinone auxiliaries. These diastereomers were obtained in 99.6% diastereomeric excess.

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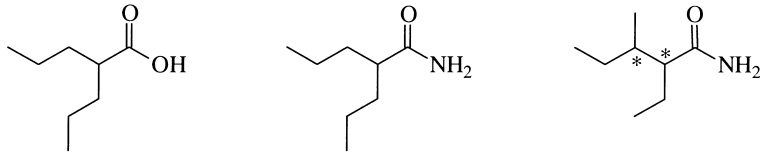
1. Introduction

Valproic acid (VPA) is one of the established and widely used antiepileptic drugs.[1] Valnoctamide (2-ethyl-3-methyl valeramide, Nirvanil®, VCD) is an isomer of valpromide (VPD), the primary amide of VPA (Fig. 1). VCD is used clinically as a mild tranquilizer in several European countries.[2] In addition, VCD is about three times more potent as an anticonvulsant than VPA.[3] Unlike VPA, VCD is not teratogenic in mice[4] and unlike VPD, it is hardly biotransformed to its corresponding acid, valnoctic acid.[5] Therefore, due to better anticonvulsant potency (compared to VPA), metabolic stability and lack of teratogenicity, VCD single stereoisomers have the potential to become new antiepileptic drugs.

VCD contains two stereogenic carbon atoms; therefore, it exists as a mixture of four stereoisomers (Fig. 1). Individual VCD stereoisomers may differ in their pharmacokinetics and/or pharmacodynamics, and consequently may have different profiles.[6,7] Thus, one of the VCD single stereoisomers may have better anticonvulsant or CNS activity with less side effects than the commercially available mixture of four stereoisomers.



valproic acid (VPA) valpromide (VPD) valnoctamide (VCD)

(2S,3S)-VCD, (**12**, **A**) (2R,3R)-VCD, (**C**)

(2R,3S)-VCD, (**7**, **D**) (2S,3R)-VCD, (**B**)

Figure 1. Chemical structures of valproic acid, valpromide, valnoctamide and the individual valnoctamide stereoisomers

We recently demonstrated that VCD exhibits diastereoselective and enantioselective pharmacokinetics in healthy subjects and epileptic patients.[8] Separation between all four VCD stereoisomers was achieved by gas chromatography (GC) with an enantioselective capillary column. The chiral stationary phase consisted of a γ-cyclodextrin derivative, enabling baseline separation of all four VCD stereoisomers (Fig. 2A). Lack of authentic reference standards for each stereoisomer initially, resulted in labelling the four stereoisomers consecutively as **A**, **B**, **C** and **D** on the basis of their respective retention times in the GC chromatogram (Fig. 2A).[8] This paper describes the strategy of assigning the absolute configuration to all four VCD stereoisomers, and the stereoselective synthesis of two single VCD stereoisomers.

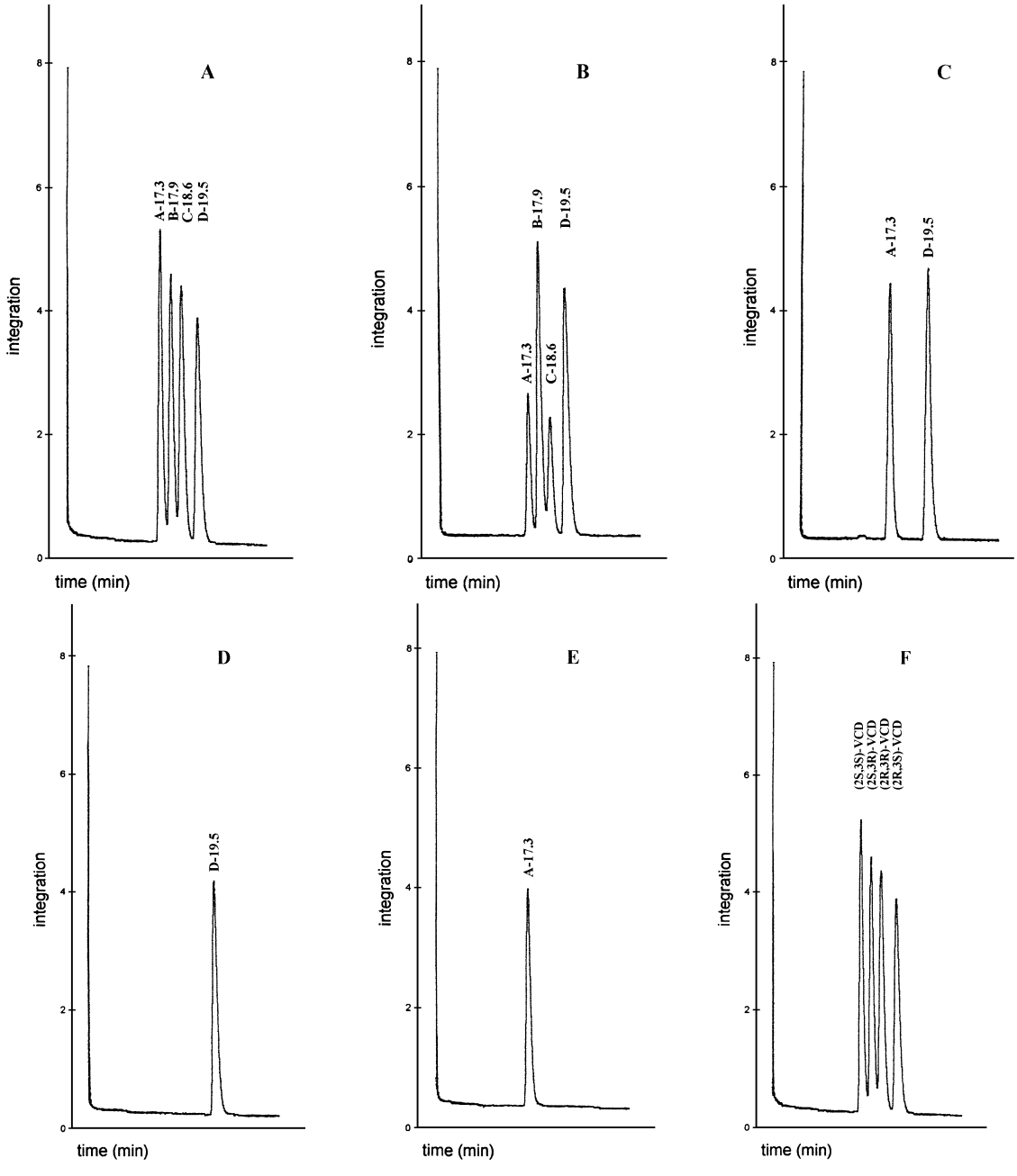


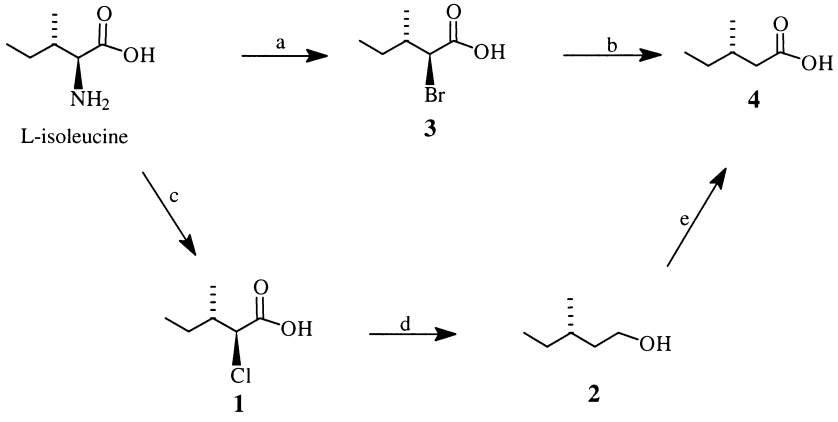
Figure 2. Chromatograms of valnoctamide (VCD) stereoisomers separated by enantiospecific gas chromatography [stationary phase: octakis-(3-*O*-butanoyl-2,6-di-*O*-pentyl)-γ-cyclodextrin; column dimensions: 12 m×0.25 mm, 0.25 μm; temperature: isothermal 120°C] and labelled as A, B, C and D according to their retention time (min). (A) Even mixture of the four VCD stereoisomers; (B) enriched VCD after eight recrystallizations; (C) **6**: (2*RS*,3*S*)-VCD; (D) **7**: (2*R*,3*S*)-VCD; (E) **12**: (2*S*,3*S*)-VCD; (F) absolute configuration of VCD stereoisomers

2. Results and discussion

Quite often, stereoisomers of chiral drugs exhibit pronounced differences in pharmacologic and toxicologic properties.[9] When the racemic mixture of a chiral drug with one asymmetric carbon and one active enantiomer (eutomer) is given to a patient, only half the dose is actually regarded as the active entity.[10] The other half (distomer) is considered as an 'isomeric ballast'.[10] In the case of epilepsy and other chronic diseases which require repetitive drug dosing for many years, this issue is of great importance. Since VCD is a potentially new antiepileptic drug that consists of four stereoisomers, it is essential to test and evaluate the pharmacology of each single stereoisomer.

Enantioselective gas chromatography of VCD enabled baseline separation of all four stereoisomers (Fig. 2A). Since the four stereoisomers occur in an approximate unimolecular ratio of 1:1:1:1, no distinction between enantiomeric and diastereomeric pairs of VCD was possible. Repeated crystallizations led to discrimination between the molecular ratios of the diastereomers, and enabled us to identify the two enantiomeric pairs (Fig. 2B). Thus, the enriched sample revealed that stereoisomers **A** and **C** represent one enantiomeric pair, whereas stereoisomers **B** and **D** form the other pair.

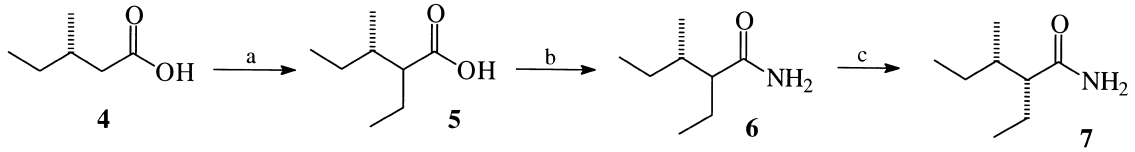
In order to further elucidate the absolute configuration of VCD stereoisomers, we synthesized (3*S*)-methyl valeric acid (**4**, Scheme 1) which was the key intermediate in synthesizing the diastereomeric mixture (2*RS*,3*S*)-VCD (**6**, Scheme 2). Compound **4** was prepared via two different synthetic pathways, both starting from commercially available L-isoleucine that has the (*S*) configuration at C-3 (Scheme 1). In the first pathway we obtained the chloro-acid **1** by diazotating L-isoleucine in HCl.[11] Exhaustive reduction of **1** with LAH for 7–10 days[12] afforded alcohol **2** which was further oxidized by chromic acid to **4**.[13] In order to improve the procedure for obtaining **4**, we utilized a second pathway (Scheme 1) by diazotating L-isoleucine in HBr to the bromo-acid **3**.[14] Reduction of **3** with zinc and sulfuric acid yielded **4**. It is quite clear that the second pathway is superior to the first, since the first pathway requires more synthetic steps. In addition, reduction of **1** to **2** (first pathway) in large quantities is hazardous since it utilizes large amounts of LAH (for a full list of abbreviations, see Appendix A).



Scheme 1. Synthesis of (3*S*)-methyl valeric acid **4** from L-isoleucine via two pathways. (a) $NaNO_2$, HBr; (b) Zn, H_2SO_4; (c) $NaNO_2$, HCl; (d) $LiAlH_4$; (e) $Na_2Cr_2O_7$, H_2SO_4

The subsequent LDA catalyzed alkylation of **4** with ethyl bromide (Scheme 2) as described by Pfeffer et al.[15], furnished the diastereoisomeric mixture of (2*RS*,3*S*)-2-ethyl-3-methyl valeric acid **5**. Conversion of **5** through acid chlorides to their corresponding amides afforded the diastereomeric mixture (2*RS*,3*S*)-2-ethyl-3-methyl valeramide **6**. GC analysis of **6** indicated the presence of two stereoisomers

corresponding to peaks **A** and **D** (Fig. 2C). These peaks represent the two VCD diastereomers with the (*S*) configuration at C-3, since no epimerization at C-3 is observed during the synthetic process.[11]



Scheme 2. Synthesis of (2*RS*,3*S*)-VCD **7** from (3*S*)-methyl valeric acid **4**. (a) C_2H_5Br, diisopropylamine, BuLi, HMPA; (b) $SOCl_2$, NH_4OH; (c) recrystallizations

Assigning the absolute configuration of all four VCD stereoisomers was possible after fractional crystallization of **6**. By using an EtOAc and PE diffusion apparatus (see Experimental section), several single crystals were obtained. These single crystals corresponded to peak **D** in the chromatogram (Fig. 2D). The absolute (*R*) configuration at C-2 belonging to these single crystals was determined by X-ray crystallography, assuming retention of the (*S*) configuration at C-3.[11] Therefore, the absolute configuration of the stereoisomer corresponding to peak **D** in the GC chromatogram was established as (2*R*,3*S*)-VCD **7**. One dimer of the unit cell and the triple position of non-hydrogen atoms are presented in Fig. 3 and Table 1, respectively.

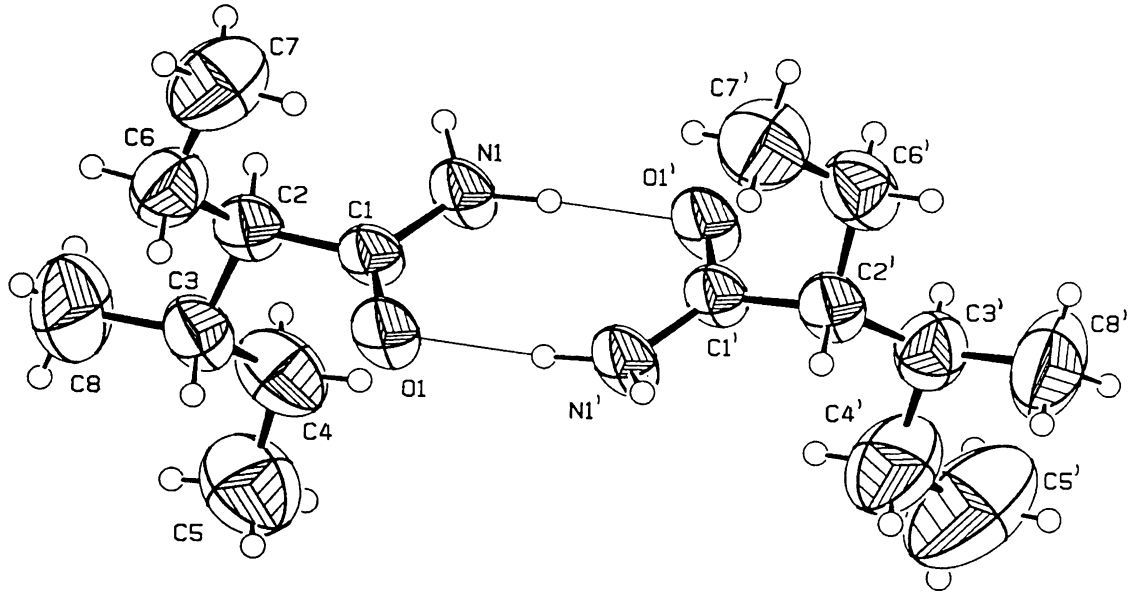


Figure 3. X-Ray crystal structure of (2*R*,3*S*)-VCD **7**

After establishing the absolute configuration of stereoisomer **D**, we were able to assign the absolute configuration of all four stereoisomers: stereoisomers **A** and **D** (Fig. 2C) possess the (*S*) configuration at C-3 since they both originate from L-isoleucine. As stereoisomer **D** is (2*R*,3*S*)-VCD, stereoisomer **A** was assigned as (2*S*,3*S*)-VCD. Since stereoisomer **B** is the enantiomer of stereoisomer **D** (Fig. 2B), its absolute configuration is (2*S*,3*R*)-VCD, and by analogy the absolute configuration of stereoisomer **C** is (2*R*,3*R*)-VCD (Fig. 2F).

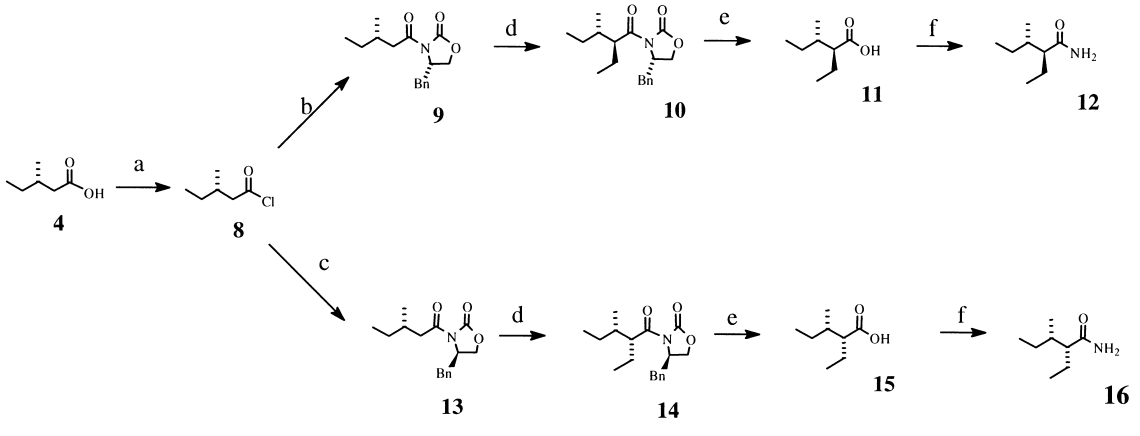
Pharmacodynamic and pharmacokinetic properties of the individual VCD stereoisomers could not be evaluated due to their lack of availability, and by the above described procedures (Schemes 1 and 2) we were able to produce limited amounts of pure **7**. We failed to obtain pure **12** by repetitive crystallizations of **6**, since contamination from **7** was present in all trials.

In order to obtain stereochemically pure **12** and **7** for biological studies, we had to develop a stereoselective synthetic method of both stereoisomers. Stereoselective synthesis of **12** and **7** was

Table 1
Three dimensional positions of non-hydrogen atoms of (2*R*,3*S*)-2-ethyl-3-methyl valeramide (**7**) as obtained from X-ray crystallography

Atom	X	Y	Z	B (eq)
O (1)	0.6928(1)	0.3000	0.8130(2)	5.6 (1)
N (1)	0.7037(1)	0.7385(7)	0.8080(2)	5.1 (1)
C (1)	0.6782(1)	0.5261(7)	0.8325(2)	4.3 (1)
C (2)	0.6318(1)	0.5632(7)	0.8856(2)	4.9 (1)
C (3)	0.5837(1)	0.460(1)	0.8324(3)	6.0 (2)
C (4)	0.5759(2)	0.592(1)	0.7400(3)	8.0 (2)
C (5)	0.5350(2)	0.476(2)	0.6775(4)	10.6 (3)
C (6)	0.6416(2)	0.429(1)	0.9780(3)	6.9 (2)
C (7)	0.6893(2)	0.525(2)	1.0315(3)	9.7 (3)
C (8)	0.5362(2)	0.491(2)	0.8861(4)	11.5 (4)
O (1′)	0.8024(9)	0.6821(5)	0.7193(2)	5.5 (1)
N (1′)	0.7909(1)	0.2446(7)	0.7267(2)	5.4 (1)
C (1′)	0.8184(1)	0.4551(7)	0.7068(2)	4.4 (1)
C (2′)	0.8705(1)	0.4114(8)	0.6710(2)	5.0 (1)
C (3′)	0.8716(1)	0.521(1)	0.5755(3)	6.5 (2)
C (4′)	0.8276(2)	0.403(2)	0.5133(3)	10.4 (4)
C (5′)	0.8190(3)	0.520(3)	0.4248(4)	15.8 (6)
C (6′)	0.9116(1)	0.528(1)	0.7381(3)	6.6 (2)
C (7′)	0.9107(2)	0.425(1)	0.8329(3)	8.7 (3)
C (8′)	0.9229(2)	0.476(2)	0.5370(4)	11.1 (4)

accomplished by using chiral oxazolidinone auxiliaries (Scheme 3).[16] By coupling the chiral auxiliaries (4*S*)- and (4*R*)-benzyl-2-oxazolidinone with **8**, the chiral imides **9** and **13** were obtained. Treating **9** and **13** with LDA at −78°C, formed their respective (*Z*)-lithium enolates,[17] and stereoselective alkylation could then be achieved. Due to low nucleophilicity,[16] compounds **9** and **13** could not be alkylated with ethyl bromide or ethyl iodide (as done with **4**, Scheme 2). Therefore, we were forced to use the highly reactive alkylating agent ethyl triflate (at −30°C with LDA) to convert compounds **9** and **13** to **10** and **14**, respectively (Scheme 3).



Scheme 3. Stereospecific synthesis of (2*S*,3*S*)-VCD **12** and (2*R*,3*S*)-VCD **7** from (3*S*)-methyl valeric acid **4**. (a) Oxalyl chloride, DMF; (b) (4*S*)-benzyl-2-oxazolidinone, BuLi; (c) (4*R*)-benzyl-2-oxazolidinone, BuLi; (d) ethyl triflate, LDA; (e) LiOH, H_2O_2; (f) oxalyl chloride, DMF, NH_4OH

Alkaline hydrolysis of **10** and **14** with lithium hydroperoxide[18] yielded acids **11** and **15** and the

recovered chiral auxiliaries. Converting acids **11** and **15** with oxalyl chloride to their acid chlorides and amidation with ammonium hydroxide yielded stereoisomers **12** and **7**, respectively. Both stereoisomers **12** and **7** are single stereoisomers with diastereomeric excess of 99.6% as determined by GC.

Previous data regarding the stereoselective alkylation of chiral oxazolidinone auxiliaries[17] enabled us to predict the absolute configuration of the synthesized VCD stereoisomers. When **9** [which contains the (4*S*)-benzyl-2-oxazolidinone auxiliary] is transformed to its respective (*Z*)-lithium enolate, alkylation with ethyl triflate favors the (*S*) configuration at C-2′; and when **13** (which contains the (4*R*)-benzyl-2-oxazolidinone auxiliary) is alkylated, the opposite configuration at C-2′ is favored. When stereoisomer **7**, which was predicted as (2*R*,3*S*)-VCD (Scheme 3), is analyzed by the enantioselective GC method, it appears as a single peak corresponding to stereoisomer **D**. Stereoisomer **7** was previously assigned as (2*R*,3*S*)-VCD by X-ray crystallography (chromatogram of **7** via the two synthetic pathways as outlined in the Experimental section are identical, Fig. 2D). As expected, stereoisomer **12** also appears as a single peak in GC, corresponding to stereoisomer **A**, which was assigned as (2*S*,3*S*)-VCD (Fig. 2E).

Pharmacodynamic evaluation of stereoisomers **12** and **7** is currently in progress in order to assess their anticonvulsant activity, teratogenicity, epoxide hydrolase inhibition and pharmacokinetics. Stereoselective synthesis of (2*R*,3*R*)-VCD and (2*S*,3*R*)-VCD is also in progress. If any of these single stereoisomers possess favorable pharmacodynamic and/or pharmacokinetic properties, it would be an excellent candidate as a new antiepileptic and CNS drug.

3. Experimental

3.1. Chemicals

(*S*)-(−)-Benzyl-(2)-oxazolidinone, (*R*)-(+)-benzyl-(2)-oxazolidinone, lithium aluminum hydride, sodium dichromate, L-isoleucine, sodium nitrite, sodium bisulfite, sodium sulfite, butyllithium, dimethylformamide, diisopropylamine, ethyl trifluoromethansulfonate, lithium hydroxide, ethyl bromide, 4 Å molecular sieves, hexamethylphosphoramide and oxalyl chloride were purchased from Aldrich Chemical Company Inc., Milwaukee, WI, USA. Tetrahydrofuran, methanol, hydrogen peroxide, ammonium hydroxide, dichloromethane, sodium hydroxide, diethyl ether, hydrochloric acid, ethyl acetate and petroleum ether, were purchased from Frutarom, Jerusalem, Israel. Ammonium chloride, pentane, hydrobromic acid and sulfuric acid were purchased from J.T. Baker Chemical Co., Philipsburg, NJ, USA. Silica gel (silica gel 60 PF_{254} with gypsum) and zinc powder were purchased from Merck, Darmstadt, Germany. All solvents were of analytical grade.

Anhydrous DCM, HMPA and diisopropylamine were obtained by drying over CaH_2 and distillation. Anhydrous THF was obtained by drying over sodium–benzophenone and distillation. DMF and Et_2O were dried over 4 Å molecular sieves.

3.2. Instruments

^{1}H-NMR and ^{13}C-NMR were measured on a Varian VXR-300S or a Bruker AC-250 spectrophotometer. All chemical shifts of ^{1}H-NMR are reported relative to TMS, whereas chemical shifts of ^{13}C-NMR are reported relative to the solvents used in analysis. The gas chromatography–mass spectrometry (GC–MS) apparatus consisted of a Hewlett–Packard GCD plus 1800B series equipped with an HP-MS 5971 quadrupole mass analyzer and electron impact (EI) source operating at 70 eV and 250°C. The column used was a Hewlett–Packard HP-5 fused silica capillary column (30 m×0.25 mm, 0.25 μm)

coated with a bonded stationary phase (5% phenyl silicone). Carrier gas was helium, flow was set at 0.7 mL/min, injector at 250°C, column at 220°C for compounds **9**, **10**, **13**, **14**; 120°C for compounds **7**, **11**, **12**, and 100°C for compound **4**. Mass spectra (MS) were measured on a Finnigan TSQ 70 operating in the EI mode at 70 eV. Melting points (uncorrected) were measured on a Büchi model 530 apparatus, Büchi, Switzerland. Optical rotation was measured at 22°C with an Autopol® III automatic polarimeter apparatus, Rudolph Research, Flanders, New Jersey, USA. $[\alpha]_D$ values are reported as mean±standard deviation. IR spectra were measured either on a Bruker FT-IR IFS 48 or a Perkin–Elmer 1310 infrared spectrophotometer.

3.3. Enantioselective gas chromatography (GC)

The GC apparatus used to separate VCD stereoisomers consisted of a Hewlett–Packard model 5890 series 2 gas chromatograph equipped with a capillary split injector, FID detector and a Hewlett–Packard model 3396-A integrator. Separation was achieved on a capillary column (12 m×0.25 mm, 0.25 μm) coated with oktakis-(3-*O*-butanoyl-2,6-di-*O*-pentyl)-ɣ-cyclodextrin as the stationary phase. Carrier gas was nitrogen, column head pressure at 35 kPa, split ratio 1:30, injector at 250°C, detector at 300°C and column temperature at 120°C.

3.4. Enrichment

Enrichment of VCD stereoisomers was carried out by repeated crystallizations of racemic VCD from EtOAc. After eight consecutive recrystallizations, the peak area ratio of enantiomers **A** and **C**:**B** and **D** was 1:3, respectively (Fig. 2B), as determined by the enantioselective GC method.

3.5. Thin layer chromatography

Thin layer chromatography was carried out using silica gel F_{254} coated aluminium plates (Merck, Darmstadt, Germany). Chromatograms were developed using varying mixtures of EtOAc and PE depending on the analyte. Spots on the plates were visualized by exposure to UV light (compounds **9**, **10**, **13**, **14**) or iodine.

3.6. Synthetic procedures

*3.6.1. (2S,3S)-2-Chloro-3-methyl valeric acid **1***

To a cold (0°C) solution of L-isoleucine (40 g, 0.305 mol dissolved in 750 mL 5 N HCl) was added dropwise a solution of sodium nitrite (35 g, 0.507 mol in 100 mL water — the reaction mixture turned green). After stirring overnight at room temperature (color changed to yellow) the reaction mixture was extracted with EtOAc (3×100 mL). The combined organic extracts were washed with sodium bisulfite solution, water and saturated brine, then dried with $MgSO_4$, filtered and concentrated. The crude product **1**, yellowish oil was distilled under reduced pressure (5 torr, 111°C) to afford a colorless oil (32 g), 70% yield. ^{1}H-NMR ($CDCl_3$, 250 MHz): δ 11.72 (s, 1H); 4.15 (d, J=6.5 Hz, 2H); 2.08–1.98 (m, 1H); 1.63–1.53 (m, 1H); 1.33–1.21 (m, 1H); 0.99 (d, J=6.8 Hz, 3H); 0.86 (t, J=7.4 Hz, 3H). ^{13}C-NMR ($CDCl_3$, 62.9 MHz): δ 175.8, 62.6, 38.8, 24.9, 15.9, 10.8. Elemental analysis: found (calculated) C, 48.1% (47.8%); H, 7.5% (7.4%); Cl, 23.1% (23.5%). MS (m/z): 153.0 (MH^+), 151.1 (MH^+), 115.1, 96.0, 94.1, 69.0, 57.1, 41.2. $[\alpha]_D$ −1.8±0.2 (c=1.4, MeOH).

*3.6.2. (3S)-Methyl pentanol **2***

To a solution of **1** (66 g, 0.438 mol) dissolved in 2.5 L of dry Et_2O was carefully added powdered LAH (50 g, 1.32 mol) and the reaction mixture refluxed for 7–10 days. Excess LAH was quenched by careful dropwise addition of EtOAc (250 mL) followed by methanol (100 mL), water (100 mL) and 50% sulfuric acid (100 mL). The organic phase was separated and the aqueous phase extracted with EtOAc (3×200 mL). The combined organic extracts were washed with 10% $NaHCO_3$ solution, water and half saturated brine, dried with $MgSO_4$ and then concentrated. The crude product **2**, green oil was purified by vacuum distillation (5 torr, 42°C) to afford a colorless oil (27.3 g), 61% yield. ^{13}C-NMR ($CDCl_3$, 75 MHz): δ 62.0, 40.3, 31.8, 30.6, 19.9, 12.1. Elemental analysis: found (calculated); C, 70.3% (70.5%); H, 14.0% (13.7%). $[\alpha]_D$ 7.4±0.1 (c=1.4, MeOH).

*3.6.3. (2S,3S)-2-Bromo-3-methyl valeric acid **3***

L-Isoleucine (65.6 g, 0.5 mol) was dissolved in 500 mL HBr (6 N) and the solution cooled to 0°C. Sodium nitrite (69 g, 1.0 mol in 130 mL water) was added slowly while maintaining the reaction temperature below 5°C, and the solution stirred overnight at room temperature. The organic layer was separated and the aqueous layer extracted with Et_2O (3×200 mL). The combined organic extracts were washed with saturated sodium bisulfite solution, water and brine; dried with $MgSO_4$ and concentrated in vacuo to give 90.1 g of the crude oily product **3**. Pure **3** (69.2 g) 71% yield, was obtained by distillation under reduced pressure and crystallization from PE. Melting point 39°C. ^{1}H-NMR ($CDCl_3$, 250 MHz): δ 11.29 (s, 1H); 4.10 (d, J=7.9 Hz, 2H); 2.10–1.94 (m, 1H); 1.81–1.65 (m, 1H); 1.40–1.15 (m, 1H); 1.03 (d, J=6.7 Hz, 3H); 0.90 (t, J=7.5 Hz, 3H). ^{13}C-NMR ($CDCl_3$, 62.9 MHz): δ 175.9, 52.4, 38.1, 26.2, 16.2, 10.6. Elemental analysis: found (calculated) C, 36.9% (37.0%); H, 5.7% (5.7%); Br, 41.0% (41.0%). MS (m/z): 197.0 (MH^+), 195.0 (MH^+), 140.0, 138.0, 115.1, 69.1, 57.1, 41.1. IR (neat): 2970, 1717. $[\alpha]_D$ −4.8±0.1 (c=1.5, MeOH).

*3.6.4. (3S)-Methyl valeric acid **4***

First pathway: to a cool (0°C) solution of **2** (37.9 g, 0.371 mol) dissolved in 1.5 L of Et_2O was slowly added 1.2 L of the oxidation reagent [$Na_2Cr_2O_7$ (240 g), 97% H_2SO_4 (177 mL) and water to make 1.2 L]. The reaction mixture was stirred overnight at room temperature, the organic phase separated and the aqueous phase extracted with Et_2O (3×150 mL). The combined organic extracts were washed with 5% HCl solution (3×50 mL) followed by 2 N NaOH solution (3×200 mL). The basic aqueous phase was separated, acidified with concentrated HCl (to pH 2) and extracted with EtOAc (3×100 mL). The combined organic extracts were then dried with $MgSO_4$ and concentrated to give a dark colored oil which was purified by vacuum distillation (5 torr, 55°C) to afford the product **4**, colorless oil (25.2 g), 58% yield.

Second pathway: to a solution of **3** (53.9 g, 0.28 mol) dissolved in sulfuric acid (1 N, 800 mL) was added slowly zinc powder (36 g, 0.55 mol). The reaction mixture was stirred overnight at room temperature, filtered and extracted with Et_2O (3×200 mL). The combined organic extracts were washed with water and saturated brine, then dried with $MgSO_4$ and concentrated. The crude yellowish oil (28.7 g) was purified by vacuum di08 (ddstillation (5 torr, 51–56°C) to afford product **4**, colorless oil (18.5 g) which was stored over 4A molecular sieves, 58% yield.

^{1}H-NMR ($CDCl_3$, 250 MHz): δ 12.16 (s, 1H); 2.30 (dd, J=6.0 Hz, 1H, C_2-Ha); 2.08 (dd, J=8.2 Hz, 1H, C_2-Hb); 1.93–1.74 (m, 1H); 1.43–1.09 (m, 2H); 0.91 (d, J=6.7 Hz, 3H); 0.85 (t, J=7.5 Hz, 3H). ^{13}C-NMR ($CDCl_3$, 62.9 MHz): δ 180.2, 41.2, 31.6, 29.2, 19.1, 11.1. MS (m/z): 117.1 (MH^+), 99.1, 87.1, 60.0, 56.9, 41.2. IR (neat, cm^{-1}): 2964, 2932, 2880, 1709. $[\alpha]_D$ 5.8±0.1 (c=1.4, MeOH).

*3.6.5. (2*RS*,3*S*)-2-Ethyl-3-methyl valeric acid **5***

To cold (−20°C) anhydrous THF (190 mL) and diisopropylamine (42.1 mL, 0.297 mol) was slowly added BuLi (186 mL, 0.297 mol, 1.6 M in *n*-hexane). While maintaining the reaction below 0°C, **4** (15 g, 0.129 mol) was added dropwise followed by HMPA (20 mL) to maintain the homogeneity of the solution. The reaction mixture was slowly warmed to room temperature, stirred for 30 min, cooled to 0°C and ethyl bromide (11.1 mL, 0.148 mol) then added rapidly. The reaction mixture was stirred for 1.5 h at room temperature, then washed with ice-cold 10% HCl (450 mL) and extracted with PE (3×100 mL). The combined organic layers were washed with 10% HCl (3×100 mL), water and saturated brine, then dried with sodium sulfate, filtered and the solvent evaporated. The crude product was distilled under reduced pressure (91°C, ~10 torr) to afford **4**, colorless oil (12.4 g), 67% yield. ^{1}H-NMR ($CDCl_3$, 250 MHz): δ 11.67 (s, 1H); 2.17–2.06 (m, 1H); 1.69–1.28 (m, 4H); 1.22–1.05 (m, 1H); 0.91–0.80 (m, 9H). ^{13}C-NMR ($CDCl_3$, 62.9 MHz); (2*R*,3*S*)-2-ethyl-3-methyl valeric acid: δ 182.6, 52.6, 36.6, 27.3, 21.2, 15.8, 12.1, 11.4; (2*S*,3*S*)-2-ethyl-3-methyl valeric acid: δ 182.2, 52.2, 36.6, 26.6, 22.5, 16.2, 12.1, 11.1. IR (neat, cm^{-1}): 2966, 2935, 2880, 2681, 1707. MS (m/z): 145.2 (MH^+), 127.0, 115.1, 88.0, 73.1, 57.0, 41.1.

*3.6.6. (2*RS*,3*S*)-2-Ethyl-3-methyl valeramide **6***

To cold (0°C) thionyl chloride (6.9 mL, 95 mmol) was added **5** (10.9 g, 76 mmol) over 30 min, and the reaction mixture stirred overnight at room temperature. The crude reaction mixture was distilled under reduced pressure (85–93°C, ~60 torr) to give (2*RS*,3*S*)-2-ethyl-3-methyl valeroyl chloride (8.3 g), 67% yield. (2*RS*,3*S*)-2-Ethyl-3-methyl valeroyl chloride (8.0 g) was slowly added to ice-cold NH_4OH (100 mL, 0.71 mol, 25% solution in water). The crude product was obtained by filtration and extraction with EtOAc and then purified by flash chromatography (EtOAc:PE, 70:30) to afford pure **6** (4.9 g), 70% yield. ^{1}H-NMR (CD_3OD, 250 MHz): δ 1.93–1.82 (m, 1H); 1.54–1.29 (m, 4H); 1.13–0.95 (m, 1H); 0.83–0.77 (m, 9H). ^{13}C-NMR (CD_3OD, 62.9 MHz); (2*R*,3*S*)-2-ethyl-3-methyl valeramide: δ 181.7, 55.5, 38.4, 28.8, 23.7, 16.7, 12.8, 12.0; (2*S*,3*S*)-2-ethyl-3-methyl valeramide: δ 181.5, 54.9, 38.1, 27.8, 24.3, 17.4, 12.8, 11.5. IR (KBr, cm^{-1}): 3381, 3194, 2966, 2932, 2876, 1655. MS (m/z): 143.1 (M^+), 127.1, 115.2, 99.0, 87.7, 72.7, 57.0, 41.0.

*3.6.7. (2*R*,3*S*)-2-Ethyl-3-methyl valeramide **7***

Synthesis from **6**: obtained by recrystallizing **6**, as described in the preparation of single crystals for X-ray crystallography.

Asymmetric synthesis: obtained from **15** by the same procedure as **12**. Product **7** (35 mg) was obtained in 20% yield. Melting point 146–147°C. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 5.65 (s, 1H, NH_a); 5.45 (s, 1H, NH_b); 1.85 (q, 1H); 1.50–1.58 (m, 4H); 1.18–1.22 (m, 1H); 0.86–0.94 (m, 9H, 3×CH_3). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ 54.5, 37.1, 27.4, 22.3, 16.1, 12.3, 11.6. Elemental analysis: found (calculated); C, 67.1% (67.1%); H, 11.7% (12.0%); N, 9.7% (9.8%). GC–MS (m/z) 114, 87, 72, 57, 41. $[\alpha]_D$ −8.0±0.4 (c=1.0, MeOH).

*3.6.8. (3*S*)-Methyl valeroyl chloride **8***

To a cold (0°C) solution of **4** (10 g, 86 mmol) and dry DMF (6.3 g, 87 mmol) dissolved in dry DCM (50 mL) was added dropwise oxalyl chloride (32.7 g, 0.258 mol in 50 mL dry DCM). After stirring the reaction mixture for 1 h, the DCM and excess oxalyl chloride were evaporated by a nitrogen stream. The crude product was rinsed with dry DCM (2×20 mL) which was evaporated by a nitrogen stream, and used without further purification in the next step.

3.6.9. (4S,3′S)-3-(3′-Methyl-1′-oxopentyl)-4-benzyl-2-oxazolidinone ***9***

To a cooled (−78°C) solution of (4*S*)-benzyl-2-oxazolidinone (3.65 g, 20.6 mmol) dissolved in dry THF (50 mL) was added dropwise via cannula BuLi (13 mL, 20.8 mmol, 1.6 M in hexane). After stirring for 30 min, a cooled (−78°) solution of **8** (2.8 g, 20.8 mmol) dissolved in dry THF (20 mL) was added at once via cannula. The reaction was slowly warmed to 0°C, stirred for 2 h and quenched with a saturated NH_4Cl solution. The THF was evaporated and the aqueous phase extracted with DCM (3×50 mL). The combined organic extracts were washed with water and half saturated brine, then dried with $MgSO_4$ and concentrated. Product **9** (3.9 g) was crystallized from 5% EtOAc in PE, yield 69%. Melting point 56°C. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.21–7.37 (m, 5H, aryl-H); 4.70 (m, 1H, C_4-H); 4.16 (m, 2H, C_5-H); 3.30–3.35 (dd, 1H, C_6-Ha); 2.96–3.03 (dd, 1H, $C_{2'}$-H); 2.70–2.78 (m, 2H, $C_{2'}$-Hb, C_6-Hb); 1.99–2.06 (m, 1H, $C_{3'}$-H); 1.39–1.50 (m, 1H, $C_{4'}$-Hb); 1.24–1.36 (m, 1H, $C_{4'}$-Ha); 0.97 (t, 6H, 2×CH_3). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ 172.9, 135.3, 129.4, 129.0, 127.3, 66.1, 55.2, 42.2, 38.0, 31.1, 29.4, 19.2, 11.4. Elemental analysis: found (calculated); C, 69.6% (69.8%); H, 7.6% (7.7%); N, 4.9% (5.1%). GC–MS (m/z) 275 (M^+), 246, 219, 184, 134, 117, 99, 71. $[\alpha]_D$ 104.5±0.4 (c=1.2, MeOH).

3.6.10. (4S,2′S,3′S)-3-(2′-Ethyl-3′-methyl-1′-oxopentyl)-4-benzyl-2-oxazolidinone ***10***

To a cold (−78°C) solution of dry diisopropylamine (1.19 mL, 8.4 mmol) and dry THF (4 mL) was added dropwise BuLi (5.25 mL, 8.4 mmol, 1.6 M solution in hexane). After stirring for 30 min, a cooled (−78°C) solution of **9** (2.1 g, 7.6 mmol in 10 mL THF) was added via cannula. After stirring for 45 min, a cooled (−78°C) solution of ethyl triflate (2.7 g, 15.2 mmol in 11 mL of pentane) was added via cannula. The reaction was slowly warmed to −30°C, stirred for 12 h at −30°C and quenched with saturated NH_4Cl. The THF was evaporated and the aqueous phase extracted with DCM (3×20 mL). The combined organic extracts were washed with water and dried with $MgSO_4$. Purification of the crude yellowish oil (3.1 g) by flash chromatography (silica gel, 0.5–2% EtOAc in PE) afforded 1.15 g of **10**, colorless oil, 50% yield. Product **10** was used in the next step without further purification. GC–MS (m/z): 303 (M^+), 274, 247, 212, 178, 127, 99, 57.

3.6.11. (2S,3S)-2-Ethyl-3-methyl-valeric acid ***11***

To a cold (0°C) solution of **10** (1.15 g, 3.8 mmol) in a 4:1 mixture of THF:water (70 mL) was added H_2O_2 (30%, 2.6 mL, 22.6 mmol) followed by LiOH (0.32 g, 7.6 mmol in 6 mL water). After stirring for 3 h the reaction was slowly warmed to room temperature and stirred overnight. After 24 h the reaction was cooled to 0°C, quenched with sodium sulfite (2.8 g, 22.2 mmol in 10 mL water) and stirred for 1 h. The THF was evaporated and the basic aqueous phase (pH 11) extracted with DCM (3×15 mL) to recover the chiral auxiliary. The aqueous phase was acidified with concentrated HCl (to pH 2) and extracted with EtOAc (3×20 mL). The combined organic extracts were washed with saturated brine, dried with $MgSO_4$ and evaporated to afford 0.21 g of **11**, colorless oil, yield 38%. Product **11** was used in the next step without further purification. GC–MS (m/z): 115, 88, 73, 57, 41.

3.6.12. (2S,3S)-2-Ethyl-3-methyl valeramide ***12***

To a cold (0°C) solution of **11** (0.21 g, 1.46 mmol) dissolved in dry DCM (5 mL) and dry DMF (0.11 g, 1.5 mmol) was added dropwise a solution of oxalyl chloride (1.8 mL, 3.6 mmol, 2.0 M solution in DCM). After stirring for 90 min, the DCM and excess oxalyl chloride were evaporated by a nitrogen stream. The crude product was rinsed with dry DCM (2×2 mL), dissolved in ice-cold dry DCM (5 mL), added via cannula to NH_4OH (1 mL, 7.1 mmol, 25% solution in water) and stirred for 1 h. The reaction mixture was extracted with DCM (3×15 mL) and the combined organic extracts were washed with water and half-saturated brine, dried with $MgSO_4$, filtered and concentrated. Product **12** (50 mg) was crystallized

from 20% EtOAc in PE, 24% yield. Melting point 128°C. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 5.83 (s, 1H, NH_a); 5.55 (s, 1H, NH_b); 1.85–1.92 (m, 1H); 1.50–1.64 (m, 4H); 1.19–1.23 (m, 1H); 0.86–0.95 (m, 9H, 3×CH_3). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ 54.0, 36.7, 26.5, 23.1, 16.6, 12.3, 11.0. Elemental analysis: found (calculated); C, 66.5% (67.1%); H, 11.5% (12.0%); N, 9.3% (9.8%). GC–MS (m/z) 114, 87, 72, 57, 41. $[\alpha]_D$ −9.7±0.4 (c=1.2, MeOH).

*3.6.13. (4*R,*3′*S*)-3-(3′-Methyl-1′-oxopentyl)-4-benzyl-2-oxazolidinone **13***

Synthesized from **8** by the same procedure as **9**, with (4*R*)-benzyl-2-oxazolidinone as the chiral auxiliary. Product **13** (1.74 g) was obtained in 80% yield. GC–MS (m/z) 275 (M^+), 247, 212, 184, 127, 99, 91, 57.

*3.6.14. (4*R,*2′*S,*3′*S*)-3-(2′-Ethyl-3′-methyl-1′-oxopentyl)-4-benzyl-2-oxazolidinone **14***

Synthesized from **13** by the same procedure as **10**. Compound **14** (0.75 g) was obtained in 39% yield. GC–MS (m/z) 303 (M^+), 274, 247, 212, 178, 127, 99, 57.

*3.6.15. (2*R,*3*S*)-2-Ethyl-3-methyl valeric acid **15***

Obtained from **14** by the same procedure as **11**. Compound **15** (0.18 g) was obtained in 50% yield.

3.7. Single crystal for X-ray analysis

Compound **6** (300 mg) was recrystallized twice from a 1:1 ethanol:water solution, to afford stereochemically impure crystals of **7** (15 mg, diastereomeric excess >95% by GC). This material was dissolved in a minimum amount of EtOAc and placed in a small screw cap vial, covered by aluminum foil. This vial was placed in a large closed vial, with PE. Pure single crystals of **7** were formed on slow diffusion of PE into the EtOAc solution.

3.8. Crystallographic analysis

Data were measured on an Enraf–Nonius CAD-4 computer controlled diffractometer. CuK_α (λ=1.54178 Å) radiation with a graphite crystal monochromator in the incident beam was used. The standard CAD-4 centering, indexing, and data collection programs were used.

Crystal data: $C_8H_{17}NO$, FW=143.23, monoclinic, space group C2, *a*=26.1015(4), *b*=5.033(2), *c*=14.811(2) Å, β=94.04(1)°, *V*=1934.4(8), *Z*=8, *D*c=0.98 g/cm^3 and μ(CuK_α)=4.73 cm^{-1}. Intensity data were collected using the θ–2θ technique to a maximum 2θ value of 140°.

Intensities were corrected for Lorentz and polarization effects. All non-hydrogen atoms were found by using the results of the SHELXS-86 direct method analysis. Refinements were carried out on the assumption that atom C(3) retains its (*S*) configuration[11] (details in Section 2). After several cycles of refinements the positions of the hydrogen atoms were calculated, and added to the refinement process. Refinement proceeded to convergence by minimizing the function $\sum w(|F_0| - |F_C|)^2$. A final difference Fourier synthesis map showed several peaks less than 0.14 e/Å^3 scattered about the unit cell without a significant feature. The discrepancy indices $R = \sum \| F_0| - |F_C \| / \sum |F_0|$ and $R_w = \left[\sum w(|F_0| - |F_C|)^2 / \sum w|F_0|^2\right]^{1/2}$ are *R*=0.049, R_w=0.068.

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Appendix A. Abbreviations

BuLi, *n*-butyl lithium; DCM, dichloromethane; DMF, dimethylformamide; Et_2O, diethyl ether; EtOAc, ethyl acetate; ethyl triflate, ethyl trifluoromethansulfonate; HMPA, hexamethylphosphoramide; LAH, lithium aluminum hydride; LDA, lithium diisopropyl amide; PE, petroleum ether; THF, tetrahydrofuran.

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