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New aminopropandiol derivatives as orally available and short-acting calcium-sensing receptor antagonists

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ABSTRACT

Synthesis and structure–activity relationship studies on a new aminopropandiol class of derivatives as calcium-sensing receptor antagonists are described. Modification of the phenolic moiety of a calcilytic compound NPS 2143 led to the identification of an orally available compound (*R*,*R*)-**31** which demonstrated a rapid and transient stimulation of PTH release in rats.

Osteoporosis is a bone disease associated with progressive decrease in bone mass and strength, leading to a significant increase in the risk of fracture. More than 75 million people in Japan, US, and Europe suffer from osteoporosis today, resulted in more than 2.3 million osteoporotic fractures every year in the US and Europe.[1] The prevention and treatment for the disease is increasingly linked with the growth of the elderly population in those countries.

The current drug therapies for osteoporosis are mainly bisphosphonates and include other anti-resorptive agents such as estrogen and selective estrogen receptor modulators (SERM).[2]

In recent reports, daily subcutaneous injection of Teriparatide, a recombinant 1–34 amino acid fragment of human parathyroid hormone (PTH 1–34) in postmenopausal women improved bone mineral density in the lumbar spine and reduced fracture rates by 65%.[3] Teriparatide has been approved by the FDA as an anabolic agent.

The search for anabolic agents other than PTH has focused on orally available calcium-sensing receptor (CaSR) antagonists, and has developed into a competitive research area. CaSR is a heptahelical G-protein-coupled receptor expressed on the surface of parathyroid cells and regulates PTH secretion by detecting extracellular calcium ion concentrations. Lower extracellular Ca^{2+} levels attenuate CaSR signaling and lead to a stimulation of PTH release.[4] Antagonists acting on the parathyroid CaSR, known as calcilytics, could mimic low extracellular Ca^{2+} concentrations and stimulate PTH secretion. Although intermittent exposure to PTH by subcutaneous injection stimulates new bone formation and leads to bone mass increase, prolonged exposure to elevated PTH increases bone turnover and results in the loss of bone.[5] Thus, PTH can act both as an anabolic and as a catabolic agent, depending on the pattern of the exposure. The profile needed for an orally available anabolic agent is one, that is, rapidly absorbable and short acting. Pharmaceutical companies have directed much effort toward the discovery of a small molecule CaSR antagonist with these desired characteristics. Several classes of compounds have been reported recently[6] and several compounds including Ronacaleret[7] and JTT-305 (MK-5442)[8] have been advanced to clinical trials.

NPS 2143 (Fig. 1) is one of the first reported orally available small molecule calcilytics which has been shown to stimulate PTH secretion.[9] However, this compound did not exert a net increase in bone mass in ovariectomized (OVX) rats due to the long plasma half-life resulting in sustained high PTH levels and thereby

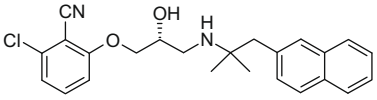

Figure 1. Structure of NPS 2143.

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accelerated bone turnover. We were interested in this molecule and considered that the undesired pharmacokinetic character could be revised by changing the molecular structure. Here, we report a new aminopropandiol class of compounds which evoked rapid and transient increases of plasma PTH levels in rats after oral administration.

The compounds synthesized for this study were tested in the human CaSR reporter gene assay using PC12h cells transfected with zif promoter/luciferase and hCaSR plasmids.[10] We started our investigation from the phenoxy part of the molecule to evaluate if we could change the structure while keeping the high antagonist potency. Our first attempt was to replace the phenyl ether bond with a metabolically unstable ester (compound **1**) or benzyl ether (compound **2**), which resulted in loss of the activity in the ester **1** but maintained an antagonist activity with IC_{50} of 0.56 μM in the benzyl ether **2** (Table 1). Although the potency of compound **2** was ~20-fold weaker compared with that in NPS 2143, it was acceptable as a starting point. An introduction of a Me group (**3**) on the benzylic position slightly increased the potency compared to **2**, while further addition of a second Me group, dimethyl compound **4**, decreased the potency ~10-fold. Lengthening the chain to a phenethyl substituent (compounds **5** and **6**) showed 2- to 4-fold decrease in potency compared with **3**, and the phenyl propyl compound **7** exhibited no improvement in potency. Changing the benzene ring to pyridine (**8**) or naphthyl rings (**9**, **10**) had no effect. Therefore, the benzyloxy derivative was the optimal structure.

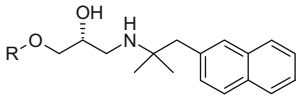
We then focused on the substituent at the benzylic position (Table 2). Addition of an ethyl group (**11**) slightly increased the potency compared to Me (**3**). The vinyl group (**12**), *n*-Pr (**13**), *i*-Pr (**14**) and *n*-Bu (**15**) groups were as potent as Me (**3**), while the more bulky *t*-Bu group (**16**) decreased the potency. Small size cycloalkyl substituents (cyclopropyl **17**, IC_{50} of 0.070 μM and cyclobutyl **18**, IC_{50} of 0.074 μM) showed a threefold increased activity compared to **3** and were about eightfold more potent than the non-substituted benzyloxy derivative **2**. Expansion of the ring size to cyclopentyl (**19**) and cyclohexyl (**20**) decreased the potency in accordance with the ring size. Changing to an aromatic ring, Ph (**21**), also decreased the potency.

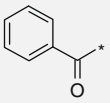
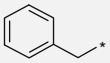
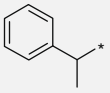
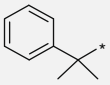
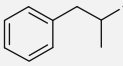
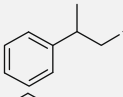
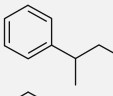
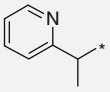
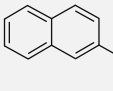
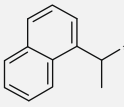
We then examined the substituent on the phenyl ring (Table 3). Introduction of a Cl atom (**22**–**24**) or Me group (**25**–**27**) showed similar potencies regardless of the position on the phenyl ring.

Since it was unknown if these new compounds are orally available at this stage, we tested compounds **22**–**27** for PTH secretion study in rats and compared the outcomes with NPS 2143.[11] Substantial increases of endogenous plasma PTH (PTH 1–34) levels equivalent to the increases in NPS 2143 after oral administration of the compounds were observed only for the 2-Me derivative **25** (Table 3). Then, other substituents on the 2 position were examined. The 2-OMe (**28**) and 2-CN (**29**) derivatives showed similar IC_{50} values, while 2-NO_2 (**30**) decreased the potency. Among these, **28** showed a comparable efficacy as **25** in the PTH secretion study. Given these results, we chose the 2-Me and 2-OMe groups for the substituents on the phenyl ring and synthesized cyclopropyl derivatives at the benzylic position. Both **31** and **32** showed increased antagonist potencies compared to the corresponding methyl versions **25** and **28**, and demonstrated robust increases in PTH levels in rats. We identified compound **31** (IC_{50} = 0.037 μM) as the most potent candidate of all those shown in Table 3.

Compound **31** has two chiral centers and is a diastereomixture of the cyclopropyl group possessing the (*R*)-configuration of the secondary alcohol in the aminopropandiol unit. We prepared all four enantiomers to investigate the effect of the stereochemistry on the antagonist activity (Table 4). Diastereomers having an (*R*)-configuration of the cyclopropyl group, (*R*,*R*)-**31** and (*R*,*S*)-**31**, appeared more potent than the corresponding (*S*)-diastereoisomers, (*S*,*R*)-**31** and (*S*,*S*)-**31**, showing a preference for the (*R*)-con-

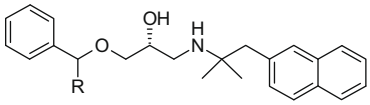
Table 1
In vitro hCaSR antagonist activity of the aminopropandiol derivatives



Compd	R	hCaSR IC_{50}[a] (μM)
NPS 2143		0.026
1		>30
2		0.56
3		0.22
4		2.24
5		0.44
6		0.81
7		0.30
8		0.42
9		0.55
10		0.36

[a] In vitro hCaSR antagonist activity was determined in PC12h cells, as described in Ref. 10. Values are means of two or more experiments.

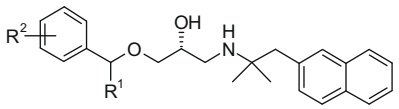
Table 2
In vitro hCaSR antagonist activity of the benzyloxy analogues



Compd	R	hCaSR IC_{50}[a] (μM)
11	Et	0.092
12	vinyl	0.21
13	*n*-Pr	0.15
14	*i*-Pr	0.20
15	*n*-Bu	0.17
16	*t*-Bu	0.57
17	Cyclopropyl	0.070
18	Cyclobutyl	0.074
19	Cyclopentyl	0.20
20	Cyclohexyl	0.61
21	Ph	0.73

[a] Values are means of two or more experiments.

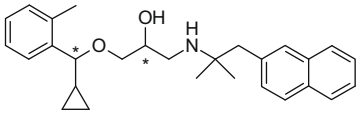
Table 3
In vitro hCaSR antagonist activity and rat in vivo PTH secretion activity of the substituted benzyloxy analogues



Compd	R^1	R^2	hCaSR IC_{50}[a] (μM)	PTH secretion[b]
22	Me	2-Cl	0.14	N.E.
23	Me	3-Cl	0.22	N.E.
24	Me	4-Cl	0.20	—
25	Me	2-Me	0.20	++
26	Me	3-Me	0.17	+
27	Me	4-Me	0.14	N.E.
28	Me	2-OMe	0.12	++
29	Me	2-NO_2	0.54	N.E.
30	Me	2-CN	0.16	+
31	Cyclopropyl	2-Me	0.037	++
32	Cyclopropyl	2-OMe	0.041	++

[a] Values are means of two or more experiments.
[b] Serum PTH 1–34 increases (Δ) from the basal levels at 30 min and 60 min after oral administration of the compounds (100 mg/kg) in rats ($n = 5$) were compared with the increases (10–24 pg/mL) in NPS 2143 (100 mg/kg, po). **++**: >80% of NPS 2143, **+**: 50–80% of NPS 2143, N.E.: <50% of NPS 2143.

Table 4
Effect of the stereochemistry in compound **31** on in vitro hCaSR antagonist activity



Compd	Cyclopropyl	OH	hCaSR IC_{50}[a] (μM)
(*R*,*R*)-**31**	*R*	*R*	0.023
(*S*,*R*)-**31**	*S*	*R*	0.083
(*R*,*S*)-**31**	*R*	*S*	0.31
(*S*,*S*)-**31**	*S*	*S*	0.76

[a] Values are means of two or more experiments.

figuration on the benzylic position. Diastereomers possessing the (*R*)-configuration of the OH group are 10-fold more potent than the corresponding (*S*)-OH diastereomers respectively ((*R*,*R*)-**31** versus (*R*,*S*)-**31**, (*S*,*R*)-**31** versus (*S*,*S*)-**31**). Consequently, of all four isomers, (*R*,*R*)-**31** was found to be the most potent variant with an IC_{50} value of 0.023 μM, and is as potent as NPS 2143.

Compound (*R*,*R*)-**31** was advanced to a PTH secretion time course study in rats. A rapid and transient increase of endogenous plasma PTH 1–84 levels, maximal at 30 min and restored to baseline within 2 h, was observed from a 10 mg/kg oral administration of (*R*,*R*)-**31** (Fig. 2). This result meets the needed PTH response profile for a bone anabolic agent. A pharmacokinetic study of this compound in rats (10 mg/kg, po) showed plasma C_{max} of 0.3 μM, T_{max} of 1 h, and $T_{1/2}$ of 2 h, suggesting a rapid absorption and a short duration of action, although bioavailability was low (13%). Unfortunately, this compound showed a strong inhibition for cytochrome P450 2D6 with an IC_{50} of 0.5 μM.

The syntheses of the compounds tested in this Letter are described in Schemes 1–4.[12] Glycidol was benzoylated to give a glycidyl ester **33**, which was reacted with an amine **34**[10] in EtOH at 60 °C to afford compound **1** (Scheme 1). Compounds **2**–**7**, **9**, **10**, **12**, **14**–**17**, **21**–**24** and **27** were prepared from the corresponding

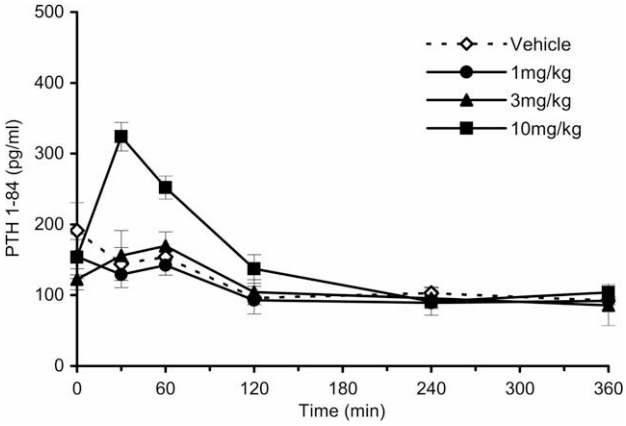
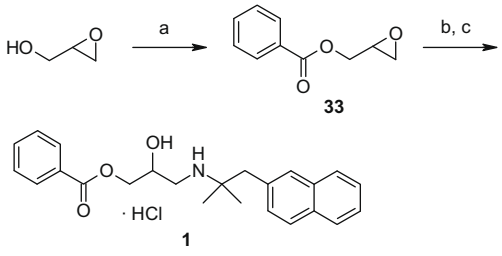
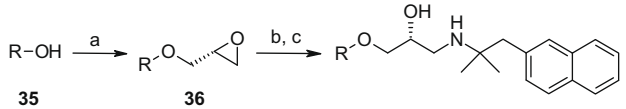


Figure 2. Time courses of PTH 1–84 levels after oral administration of (*R*,*R*)-**31** 1/2 fumarate in rats. Data are expressed as mean values ± standard deviation, $n = 5$.

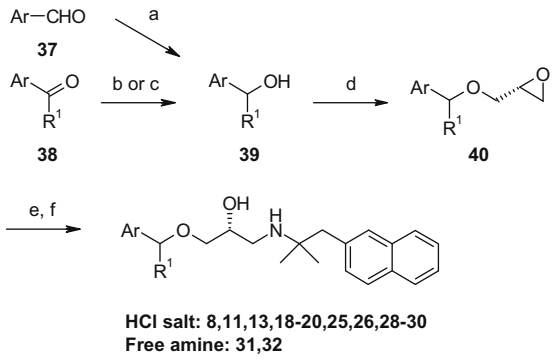
commercially available alcohols **35** by alkylation with (*R*)-(−)-glycidyl 3-nitrobenzenesulfonate in the presence of NaH in DMF to give a glycidyl ether **36**, followed by the epoxide-opening reaction with the amine **34** (Scheme 2). Other compounds in Tables 1–3 were synthesized from the corresponding secondary alcohol **39**, prepared either from the aldehyde **37** by Grignard reaction in THF or the ketone **38** by the reduction with $LiAlH_4$ or $NaBH_4$ (Scheme 3). The alcohol **39** was converted to the glycidyl ether **40** and then to compounds **8**, **11**, **13**, **18**–**20**, **25**, **26**, **28**–**32** by the same method described above. The syntheses of the four stereoisomers of compound **31** are described in Scheme 4. 2-Methylbenzoylchloride was converted to the Weinreb amide and reacted with cyclopropyl magnesium bromide to give a ketone **41**. Hydrogenation (0.5 MPa) of the ketone in the presence of $RuCl_2$[(*S*)-BINAP](dmf)$_n$-(*S*,*S*)-1,2-diphenylethylenediamine[13] and KO*t*-Bu in *i*-PrOH at room temperature gave an (*R*)-alcohol **42** in 74% ee, which was converted to a glycidyl ether **43** by the alkylation with (*R*)-(−)-glycidyl 3-nitrobenzenesulfonate. An epoxy-opening reaction by the amine **34**



Scheme 1. Reagents and conditions: (a) benzoyl chloride, Et_3N, $CHCl_3$; (b) [1,1-dimethyl-2-(2-naphthalenyl)ethyl]amine (**34**), EtOH, 60 °C; (c) 4 N HCl/AcOEt, Et_2O.



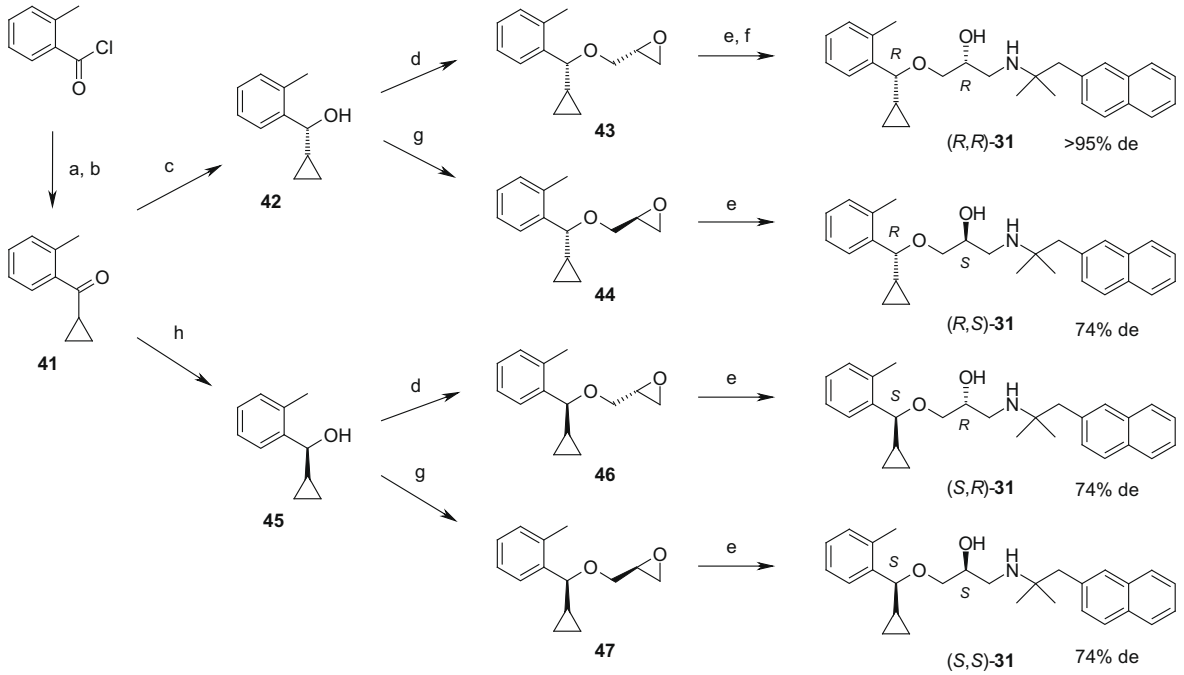
Scheme 2. Reagents and conditions: (a) (*R*)-(−)-glycidyl 3-nitrobenzenesulfonate, NaH, DMF, rt; (b) [1,1-dimethyl-2-(2-naphthalenyl)ethyl]amine (**34**), EtOH, 60 °C; (c) 4 N HCl/AcOEt, Et_2O, (except for **7**, **14**, **17**, **24**).



Scheme 3. Reagents and conditions: (a) R^1MgBr , THF, 0 °C to rt; (b) LAH, THF, 0 °C to rt; (c) $NaBH_4$, MeOH, 0 °C to rt; (d) (*R*)-(−)-glycidyl 3-nitrobenzenesulfonate, NaH, DMF, rt; (e) [1,1-dimethyl-2-(2-naphthalenyl)ethyl]amine (**34**), EtOH, 60 °C; (f) 4 N HCl/AcOEt, Et_2O (except for **31** and **32**).

afforded (*R*,*R*)-**31** in 74% de. Recrystallization from *n*-hexane raised the de to >95%. The absolute configuration was established by single-crystal X-ray diffraction analysis of (*R*,*R*)-**31** HCl salt (Fig. 3).[14] Alkylation of **42** with (*S*)-(+)-glycidyl 3-nitrobenzenesulfonate to give a glycidyl ether **44** followed by the epoxide-opening reaction afforded (*R*,*S*)-**31** (74% de). The (*S*)-alcohol **45** was prepared from the ketone **41** by the hydrogenation using (*R*)-BINAP-Ru-(*R*,*R*)-diamine catalyst in 74% ee, and converted to (*S*,*R*)-**31** and (*S*,*S*)-**31** in 74% de via the glycidyl ethers **46** and **47**, respectively, by the same method described above.

In conclusion, we have identified a new orally available aminopropandiol class of calcilytics. The most potent compound (*R*,*R*)-**31** significantly stimulated endogenous PTH secretion in rats with a rapid and transient increase of plasma PTH levels, which is an needed profile for an anabolic effect. Further study toward the development of a clinical candidate will be reported in due course.



Scheme 4. Reagents and conditions: (a) *N*,*O*-dimethyl hydroxylamine hydrochloride, Et_3N, CH_2Cl_2, rt; (b) cyclopropyl magnesium bromide, THF, 0 °C to rt; (c) H_2 (0.5 MPa), $RuCl_2$[(*S*)-BINAP](dmf)$_n$, (*S*,*S*)-1,2-diphenylethylenediamine, KO*t*-Bu, *i*-PrOH, rt, 74% ee; (d) (*R*)-(−)-glycidyl 3-nitrobenzenesulfonate, NaH, DMF, rt; (e) [1,1-dimethyl-2-(2-naphthalenyl)ethyl]amine (**34**), EtOH, 60 °C; (f) recrystallization from *n*-hexane; (g) (*S*)-(+)-glycidyl 3-nitrobenzenesulfonate, NaH, DMF, rt; (h) H_2 (0.5 MPa), $RuCl_2$[(*R*)-BINAP](dmf)$_n$, (*R*,*R*)-1,2-diphenylethylenediamine, KO*t*-Bu, *i*-PrOH, rt, 74% ee.

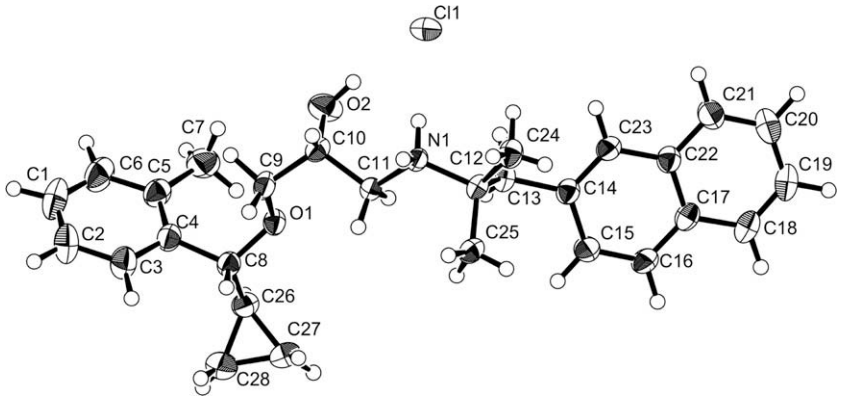


Figure 3. Crystal structure of (*R*,*R*)-**31** HCl salt by single-crystal X-ray diffraction.

Acknowledgments

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11. The endogenous plasma PTH levels after oral administration of the compounds in Sprague–Dawley rats were measured using rat PTH 1–34 ELISA kit (Amersham) or rat PTH 1–84 ELISA kit (Immutopics). See Ref. 10 for the experimental details. We observed PTH 1–34 in the screening stage (Table 3) and PTH 1–84 in the time course study for compound (*R*,*R*)-**31** (Fig. 2).
12. The experimental procedures have been previously disclosed. See Ref. 10. All the tested compounds were characterized by NMR and MS. The diastereomeric excess (de) for the (*R*,*R*)-**31** was determined by reversed-phase HPLC using a ULTRON ES-OVM column (Shinwa Chemical, 0.46 cm × 15 cm, solvent system A: 20 mM K_2HPO_4, 5 mM KPF_6 (pH 7) B: CH_3CN, A:B = 72:28, 1.0 mL/min, 30 °C, t_R: 17.2 min for (*R*,*R*)-**31**, 21.9 min for (*R*,*S*)-**31**, 12.3 min for (*S*,*R*)-**31**, 14.7 min for (*S*,*S*)-**31**). The de for the (*R*,*S*)-**31**, (*S*,*R*)-**31** and (*S*,*S*)-**31** were determined by ^1H NMR. The optical purities for **42** and **45** were analyzed by HPLC using a CHIRALPAK AS column (DAICEL, 0.46 cm × 25 cm, solvent system A: *n*-hexane, B: *i*-PrOH, A:B = 98:2, 1.0 mL/min, 35 °C, t_R: 12.4 min for **42**, 13.6 min for **45**).
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14. The data can be obtained from the Cambridge Crystallographic Data Centre (CCDC 770504).