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Novel Water Soluble 2,6-Dimethoxyphenyl Ester Derivatives with Intravenous Anaesthetic Activity

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Abstract—A number of water soluble bis-amino-2,6-dimethoxyphenyl ester derivatives were found to exhibit improved anaesthetic activity in mice relative to propofol **1**. Of the analogues disclosed, **44** was further profiled in rodents and found to be a superior agent to propofol for the induction and maintenance of anaesthesia.

Propofol, a formulation of 2,6-diisopropylphenol **1** (Fig. 1) has gained acceptance as the leading short-acting hypnotic for induction and maintenance of general anaesthesia.[1] It exhibits the desirable characteristics of rapid onset and offset of anaesthesia and can be used for long-term administration without significant accumulation.[2] Propofol is however associated with cardiovascular side effects and pain on injection. We recently reported on the SAR and general anaesthetic activity of a series of α-amino acid phenolic ester derivatives as exemplified by Org 25435 **2** (Fig. 1).[3] The lead optimisation which led to the identification of **2** focussed mainly on structural modifications to the phenolic and amino moieties, though lengthening the alkyl side chain from methyl to *n*-butyl was also investigated. Whilst these changes led to an increase in anaesthetic activity lengthening the alkyl side chain resulted in a concomitant increase in excitatory side effects and decrease in aqueous solubility.

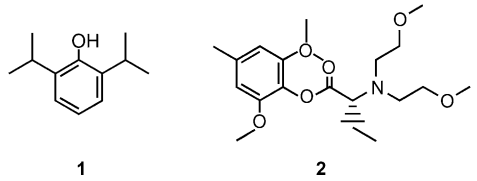

Figure 1. Propofol **1** and Org 25435 **2**.

We thought that incorporation of heteroatoms into the side chain should enhance the aqueous solubility and the increased polarity might also abolish the excitatory side effects previously observed with more lipophilic derivatives. In this paper we report on a series of phenolic ester derivatives containing amino and ether side chains designed to explore this hypothesis (Fig. 2). A number of these novel analogues exhibit better anaesthetic activity and aqueous solubility than either propofol **1** or Org 25435 **2**.

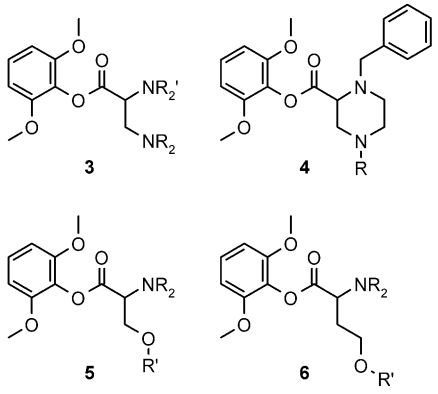

Figure 2. Heteroatom-containing side chain phenolic ester derivatives.

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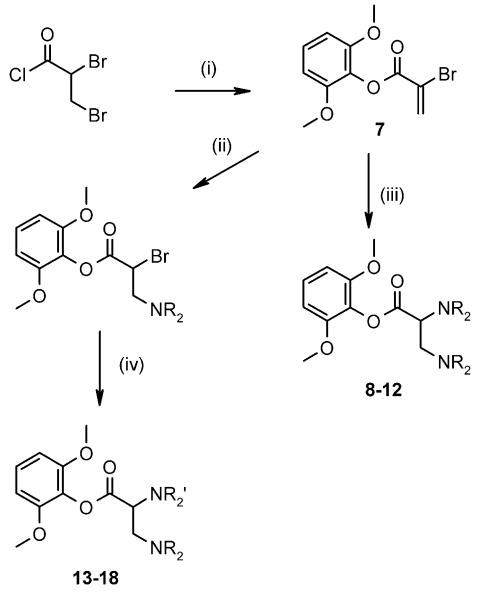
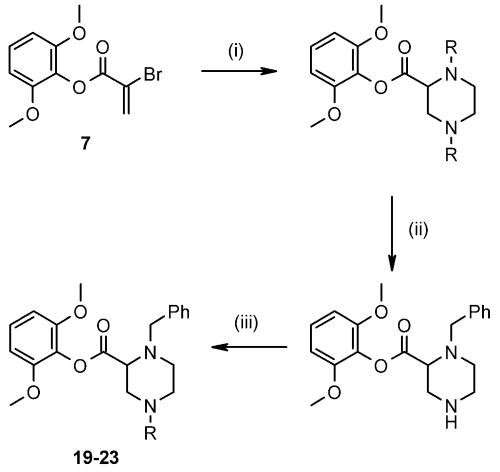
Initially we focussed on synthesising analogues containing a further amino substituent in the side chain. Acyclic and cyclic bis-amino-α-amino acid analogues **3** and **4** were prepared from the versatile bromo acrylate intermediate **7** using the synthetic strategies outlined in Schemes 1 and 2, respectively. All analogues were characterised by NMR, LCMS, HPLC and GC (residual solvent levels) prior to submission for testing.

Many anaesthetics including barbiturates, benzodiazepines, etomidate and propofol **1** are believed to exert their effect by potentiating the effects of GABA at $GABA_A$ receptors via an allosteric interaction. During the optimisation work described herein compounds were tested as $GABA_A$ receptor modulators through determination of their ability to inhibit [^{35}S]TBPS binding to rat whole brain membranes. All in vitro studies were carried out as described by Anderson et al.[4]



Scheme 1. Synthesis of compounds **8–18**: (i) 2,6-Dimethoxyphenol, NEt_3, DCM; (ii) HNR_2, DCM; (iii) HNR_2, NEt_3, DCM; (iv) HNR_2'', NEt_3, DCM.



Scheme 2. Synthesis of compounds **19–23**: (i) *N*,*N*′-Dibenzylethylenediamine, DCM; (ii) ACE-Cl, DCM; (iii) RI, K_2CO_3, acetone.

The in vivo anaesthetic activity of compounds was determined upon intravenous (iv) administration to mice. Propofol was injected as the commercial veterinary product (Rapinovet™, Schering-Plough Animal Health, UK) at 10 mg mL^{-1}. All in vivo studies were carried out as described by Anderson et al.[4] except male MF-1 mice were used instead of male CFLP mice. Compounds were tested as free bases or HX (X = Cl, Br) salts as 5–10 mg/mL solutions in distilled water. The dose required to cause a loss of righting reflex (LRR) for a minimum period of 30 s in 50% of the treated mice was determined. A probit analysis (Minitab) was performed to yield an HD_{50} (hypnotic dose 50, in μmol kg^{-1}) for each compound and 95% confidence limits. The results obtained for compounds **8–18** are given in Table 1, and results for propofol **1** and Org 24447 (racemate of Org 25435 **2**) are included for comparison. All analogues were tested as racemic mixtures.

In contrast to the previous study with compounds with simple alkyl side chains, new compounds where the α-amino substituent was cyclic displayed higher levels of potency than acyclic derivatives. In particular the potency of **8** (HD_{50} = 15 μmol kg^{-1}) compared very favourably with both propofol **1** (HD_{50} = 68 μmol kg^{-1}) and Org 24447 (HD_{50} = 22 μmol kg^{-1}). However incorporation of two acyclic amino substituents led to convulsant side effects for example, **11** and **12**. To further probe these initial SAR a series of analogues incorporating both cyclic and acyclic amines was synthesied that is, compounds **13–18** where $NR_2 \neq NR_2'$.

Once again there was a clear distinction between analogues containing cyclic and acyclic amines; cyclic amino derivatives for example, **13–16** retained good potency while the acyclic analogues **17** and **18** possessed either poor activity or convulsant properties.

To exploit this finding a series of conformationally restricted cyclic analogues of structure **4** were prepared from the bromo acrylate intermediate **7** using the synthetic route outlined in Scheme 2. The anaesthetic

Table 1. Anaesthetic Activity (HD_{50}) of phenolic ester derivatives **8–18**

Compd	NR_2	NR_2'	HD_{50} (μmol kg^{-1})[a]
1 Propofol	N/A	N/A	68
ORG24447	N/A	N/A	22
8	Thiomorpholine	Thiomorpholine	15
9	HHO[b]	HHO[b]	<25
10	Morpholine	Morpholine	38
11	BME[c]	BME[c]	conv
12	BEE[d]	BEE[d]	conv
13	Morpholine	Piperidine	15
14	Morpholine	Thiomorpholine	17
15	Thiomorpholine	Morpholine	19
16	THIQ[e]	Morpholine	21
17	BME[c]	Morpholine	75
18	Morpholine	BEE[d]	conv

[a]Male MF-1 mice (n = 8) were used in all studies (conv = convulsant).
[b]1,4-Hexahydrooxazepine.
[c]Bis(2-methoxyethyl)amine.
[d]Bis(2-ethoxyethyl)amine.
[e]1,2,3,4-Tetrahydroisoquinoline.

activity of compounds **19**–**23** upon iv administration to mice are also shown in Table 2.

A number of these conformationally restricted derivatives exhibited excellent anaesthetic potency for example, **19** ($HD_{50}=24$ μmol kg^{-1}), **20** ($HD_{50}=31$ μmol kg^{-1}) and **21** ($HD_{50}=34$ μmol kg^{-1}).

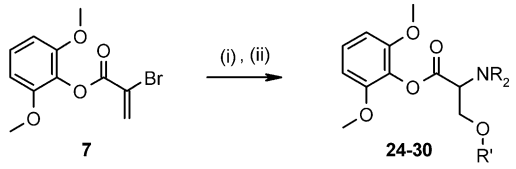
Whilst a number of the bis-amino-α-amino acid analogues displayed comparable or enhanced potency and solubility as compared to Org 24447, all analogues were found to be highly unstable in aqueous solution, precluding further development. Despite this instability the bis-amino-α-amino acid analogues proved that our hypothesis of improving the anaesthetic activity and aqueous solubility by incorporation of heteroatoms into the side chain warranted further investigation.

To improve stability a series of ether side chain analogues **5** and **6** (Fig. 1) were designed and synthesised as outlined in Schemes 3 and 4, respectively. The experimental data for the synthesis of **26**.HCl is given in ref 5.

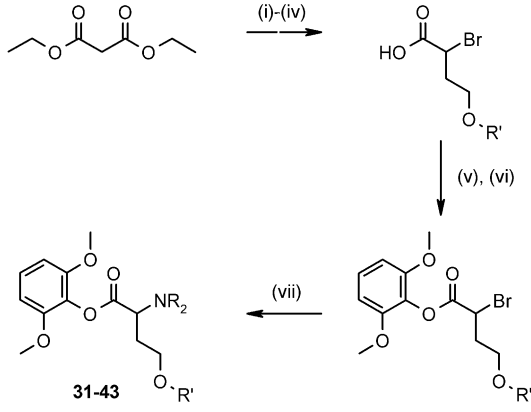
Table 2. Anaesthetic activity (HD_{50}) of phenolic ester derivatives **19**–**23**

Compd	R	HD_{50} (μmol kg^{-1})[a]
1 Propofol	N/A	68
ORG24447	N/A	22
19	Ethyl	24
20	Allyl	31
21	Propionyl	34
22	Methyl	122
23	H	Conv

[a]Male MF-1 mice ($n=8$) were used in all studies (conv = convulsant).



Scheme 3. Synthesis of compounds **24**–**30**: (i) NaOR, ROH; (ii) HNR_2, NEt_3, Toluene, reflux.



Scheme 4. Synthesis of compounds **31**–**43**: (i) Na, 2-bromoethyl methyl ether, EtOH; (ii) KOH, aqEtOH, reflux; (iii) Br_2, HBr, Et_2O; (iv) vacuum distillation; (v) oxalyl chloride, pyridine, DCM; (vi) 2,6-dimethoxyphenol, NEt_3, DCM; (vii) NR_2, NEt_3, toluene, reflux.

A number of the ether side chain analogues exhibited excellent anaesthetic profiles (potency and behavioural pharmacology) compared to propofol **1** and Org 24447, with **26**, **31** and **39** being particularly noteworthy. In contrast to the bis-amino derivatives **8**–**23** these compounds were extremely tolerant to modifications of the α-amino substituent, with both cyclic and acyclic amines retaining good anaesthetic activities. The anaesthetic activity of compounds **24**–**43** upon iv administration to mice are shown in Table 3.

The vast majority of the ether side chain analogues possessed >10 mg mL^{-1} aqueous 'visible' solubility at pH = 4 (data shown in Table 3), whilst a smaller subset possessed excellent solubility for example, **44** = 200 mg mL^{-1} at pH = 2.4, 20 mg mL^{-1} at pH = 5.0. This solubility was allied to acceptable aqueous stability across a pH range suitable for iv administration that is, $pH \geq 4$.

Due to these encouraging results **26**, **31** and **39** were selected for chiral separation and the in vitro and in vivo results for these compounds are shown in Table 4. Semi-preparative chiral HPLC was used to resolve **31** and **39** into their constituent enantiomers, whereas **26** could be resolved by chiral salt formation. This crystallisation method was applicable for providing multigram quantities of pure enantiomer **44**. Enantiomer **44** was assayed (reverse phase and chiral HPLC) across a pH range (1–9) in aqueous medium and was found to be stable with respect to both decomposition and racemisation.

As with other studies involving closely related compounds, there is no direct correlation of [^{35}S]TBPS displacement with in vivo activity for the analogues shown in Table 3.[6] Despite the lack of correlation the in vitro

Table 3. Anaesthetic activity (HD_{50}) of phenolic ester derivatives **24**–**43**

Compd	R′	NR_2	HD_{50} (μmol kg^{-1})[a]
1 Propofol	N/A	N/A	68
ORG24447	N/A	N/A	22
24	Me	Thiomorpholine[b]	21
25	Me	Bisethoxyethylamine[c]	25
26	Me	1,4-Hexahydrooxazepine[d]	30
27	Me	Bismethoxypropylamine[c]	46
28	Me	Morpholine[d]	54
29	Me	Piperidine[c]	146
30	Et	1,4-Hexahydrooxazepine[d]	20
31	Me	1,4-Hexahydrooxazepine[d]	15
32	Me	Hexahydroazepine[c]	17
33	Me	Tetrahydropyridine[c]	22
34	Me	4-Methoxypiperidine[c]	21
35	Me	Piperidine[c]	22
36	Me	Bismethoxyethylamine[d]	27
37	Me	Bisethoxyethylamine[c]	28
38	Me	Morpholine[c]	38
39	Et	1,4-Hexahydrooxazepine[c]	23
40	Et	Hexahydroazepine[c]	25
41	Et	Bismethoxyethylamine[c]	27
42	Et	Morpholine[c]	36
43	Et	Bisethoxyethylamine[b]	47

[a]Male MF-1 mice ($n=8$) were used in all studies.
[b]Solubility of HCl salt = <10mg mL^{-1} @ pH4.
[c]Solubility of HCl salt = 10–20mg mL^{-1} @ pH4.
[d]Solubility of HCl salt = >20mg mL^{-1} @ pH4.

results presented in Table 4 demonstrate modulation of GABAergic function and suggest this mechanism could account at least in part for the anaesthetic activity of these compounds. All in vitro studies were carried out as described by Anderson et al.[4]

Analysis of the results given in Table 4 in conjunction with behavioural pharmacology observations and taking into account synthetic issues led us to select **44** for testing in a more sophisticated anaesthesia model using burst suppression ratio (BSR) detection in the extradural EEG after bolus and continuous infusion.

These experiments, using male Wistar rats, were carried out as described by Vijn and Sneyd.[7] For the bolus study and based on pilot experiments, two doses of each compound, estimated to produce either above or below 60% BSR, were injected iv over 10 s into groups of 5 or 6 rats (60% BSR is regarded as a deep level of anaesthesia). Using linear interpolation between the two fixed doses, an estimate of the dose producing 60% BSR was calculated for each drug, allowing for direct comparisons of their anaesthetic profiles. The mean BSR traces for the higher doses of propofol **1** (47.7 μmol kg^{-1}) and **44** (12.6 μmol kg^{-1}) are shown in Fig. 3.

The data obtained by linear interpolation are shown in Table 5. Analogue **44** is approximately 3.6 times more potent than propofol **1** to induce 60% BSR (11.4 vs 41.0 μmol kg^{-1}). Both compounds produce a rapid rise in burst suppression, with the maximal BSR being observed shortly after injection; propofol **1** (30s) and **44** (32s). However, recovery to righting is considerably faster with **44** than propofol **1** (3.3 min vs 5.6 min).

Following on from the bolus studies, the ability of **44** to maintain a constant level of anaesthesia (BSR = 60%) was studied in a closed loop maintained infusion experiment. Figure 4 shows the BSR traces for propofol **1** and **44** as obtained from these experiments ($n = 4$–5 per compound).

Very stable levels of anaesthesia were obtained with both propofol **1** and **44**. With **44** there was less tendency for the infusion rate to decrease during the 1 h period than with propofol, suggesting less cumulation. On cessation of infusion after 1 h, the BSR response dropped almost immediately with both propofol **1** and **44**, and reached baseline values earlier for **44** than for propofol **1**. Also behaviourally, recovery (gain of righting reflex) after **44** maintained anaesthesia was much faster than after maintenance with propofol **1**.

The relevant parameter values as extracted from the experiments are given in Table 6. It is clear from the data in Table 6 that iv administration of **44** results in a rapid onset and recovery from anaesthesia, which are essential features for a new anaesthetic agent.

In summary a number of water soluble bis-amino-2,6-dimethoxyphenyl ester derivatives were prepared and found to exhibit improved anaesthetic activity in mice relative to propofol **1** and Org 25435 **2**. Of these, compound **44** was further profiled and found to be a superior

Table 4. Anaesthetic activity (HD_{50}) and $GABA_A$ receptor modulatory (TBPS IC_{50}) effects of selected compounds

Compd	HD_{50} (μmol kg^{-1})[a]	TBPS IC_{50} μM
1 Propofol	68	8.1
ORG24447	22	29.3
26 (Racemate)	30	35
44 (ξ of **26**)	16	35.8
45 (ξ of **26**)	40	98.3
31 (Racemate)	20	69.5
46 (ξof **31**)	15	32.5
47 (ξof **31**)	30	>100
39 (Racemate)	15	34.9
48 (ξof **39**)	15	N/D
49 (ξ of **39**)	48	>100

[a]Male MF-1 mice ($n = 8$) were used in all studies (inact = inactive). ξ single isomer of undefined absolute configuration.

Table 5. Parameter values as extracted by linear interpolation of averaged values from individual BSR responses to bolus injections

Parameter	**44** ($n = 14$)	Propofol **1** ($n = 11$)
Dose for 60% BSR (μmol kg^{-1})	11.4±1.5	41.0±2.4
Time to maximum (s)	32±1	30±2
Time to 1st 0.25s BS epoch (s)	12.9±0.6	13.1±0.3
Time to 30% BSR (min)	1.3±0.1	1.5±0.2
Time to righting (min)	3.3±0.1	5.5±0.4

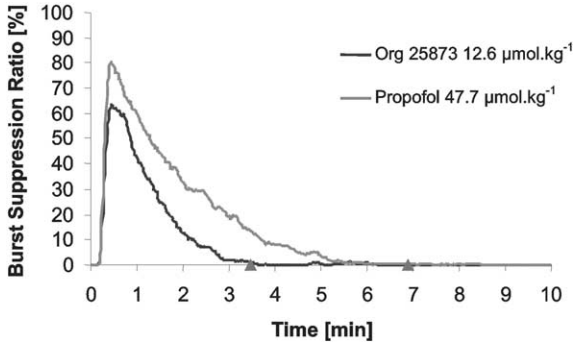


Figure 3. Mean burst suppression ratio (BSR) after the start of a 10 s iv bolus injection of propofol **1**, 47.7 μmol kg^{-1} ($n = 6$); **44**, 12.6 μmol kg^{-1} ($n = 7$). The triangles indicate the moment when the righting reflex returned.

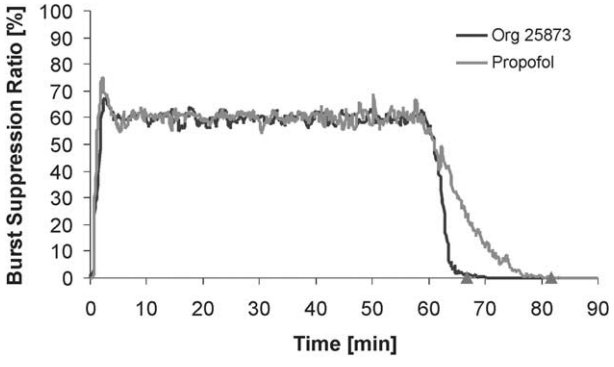


Figure 4. BSR during burst suppression closed-loop infusions at a BSR value of 60% for **44** and propofol **1**. Curves are averages of 4–5 animals. Infusion was stopped after 60 min. The triangles indicate the moment when the righting reflex returned.

Table 6. Parameter values extracted from closed loop infusions to maintain 60% BSR for 1 h periods

Parameter	**44** ($n=4$)	Propofol **1** ($n=5$)
Infusion Rate μmol $kg^{-1}min^{-1}$	5.0±0.4	4.9±0.3
Stability of BSR (%)	2.5±0.3	4.1±0.6
Time to 30% BSR (min)	2.4±0.1	5.4±0.9
Time to righting (min)	6.8±0.4	21.8±1.7

agent to propofol for the induction and maintenance of anaesthesia in rats. Despite the lack of a direct correlation between in vitro and in vivo activity, modulation of GABAergic function may explain at least in part the anaesthetic activity possessed by the analogues disclosed.

Acknowledgements

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5. 2-Bromo-3-methoxypropionic acid, 2,6-dimethoxyphenyl ester.

2-Bromoacrylic acid, 2,6-dimethoxyphenyl ester (12 g, 42 mmol) was dissolved in methanol (300 mL, HPLC grade) and sodium methoxide (0.23 g, 4.2 mmol) added with stirring. The resultant solution was then stirred at room temperature for 30 min prior to the addition of ammonium chloride (0.5 g). The solvent was then removed under reduced pressure and diethyl ether (200 mL) added. The precipitate was removed by filtration and the filtrate evaporated under reduced pressure to give a yellow oil. Chromatography of this oil on silica using toluene as the eluent afforded the title compound (6.3 g, 48%) as a clear oil. ^{1}H NMR ($CDCl_3$): δ 3.48 (s, 3H), 3.82 (s, 6H), 3.85–3.94 (m, 1H), 4.01–4.09 (m, 1H), 4.66 (t, 1H), 6.60 (d, 2H), 7.17 (t, 1H).

2-[*N*-(Hexahydro-4-oxazepinyl)]-3-methoxypropionic acid, 2,6-dimethoxyphenyl ester (**26**).

2-Bromo-3-methoxypropionic acid, 2,6-dimethoxyphenyl ester (2.6 g, 8.2 mmol) was dissolved in toluene (30 mL) with stirring. Triethylamine (2.5 mL, 18.2 mmol) and hexahydro-1,4-oxazepine (1 g, 9.9 mmol) were then added and the reaction mixture heated at 100 °C under nitrogen overnight. The reaction mixture was then cooled to room temperature and diluted with diethyl ether (100 mL). The precipitate was removed by filtration and washed with diethyl ether (100 mL). The combined organic fractions were then extracted with 1N HCl (100 mL). The aqueous phase was subsequently basified to pH14 with 4M NaOH and extracted with diethyl ether (100 mL×2). The combined organic fractions were dried over sodium sulphate, filtered and concentrated under reduced pressure to give a yellow oil. Chromatography of this oil on basic alumina using toluene/ethyl acetate gradient as the eluent afforded the title compound (0.7 g, 26%) as a clear oil which crystallised on standing. Positive Ion ESI $(M+H)^+$ 339.9.

2-[*N*-(Hexahydro-4-oxazepinyl)]-3-methoxypropionic acid, 2,6-dimethoxyphenyl ester hydrochloride (1:1) salt (**26**.HCl).

Hydrogen chloride gas was passed through a solution of 2-[*N*-(hexahydro-4-oxazepinyl)]-3-methoxypropionic acid, 2,6-dimethoxyphenyl ester (0.7 g) in anhydrous dichloromethane for 1–2 min. Most of the dichloromethane was then removed under reduced pressure and the hydrochloride salt precipitated by the addition of dry diethyl ether. The resulting white solid was filtered off and washed with diethyl ether to give the title compound (0.7 g, 90%). ^{1}H NMR ($CDCl_3$ + sodium carbonate): δ 1.85–2.02 (m, 2H), 2.95–3.04 (m, 2H), 3.05–3.17 (m, 2H), 3.43 (s, 3H), 3.67–3.87 (m, 12H), 3.93 (t, 1H), 6.60 (d, 2H), 7.13 (t, 1H).

Positive Ion ESI $(M+H)^+$ 339.9.

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