## Supporting information for Identification of an orally bioavailable, potent and selective inhibitor of GlyT1

**General Methods**. Unless otherwise indicated, melting points were obtained on a Leica Galen III hot stage and are uncorrected. Proton NMR spectra were obtained using either a Bruker DPX-250, a Bruker, DPX-360 or a Bruker DPX-400 spectrometer. Mass spectra were recorded on a Quattro operating in an electrospray (ES) mode (Note that only the strongest peaks from the mass spectra are reported below). Analytical thin-layer chromatography (TLC) was conducted on precoated silica gel 60 $F_{254}$ plates (Merck). Visualisation of the plates was accomplished by using UV light and/or iodine and/or aqueous potassium permanganate solution. Chromatography was conducted on silica gel 60, 220-440 mesh (Fluka) under low pressure. Solutions were evaporated on a Büchi rotary evaporator under reduced pressure. All starting materials were obtained from commercial sources and used as received unless otherwise indicated.

**2,4-Dichloro-*N*-(4-cyclopropylmethanesulfonyl-l-cyclopropylmethylcyclohexyl-methyl)benzamide (compound 16, DCCCyB):**

*8-(Cyclopropylmethyl)-1,4-dioxaspiro[4.5]decane-8-carbonitrile:*

To a stirred solution of l,4-dioxaspiro[4.5]decane-8-carbonitrile (20 g; 119.61 mmol) and (bromomethyl)cyclopropane (17.76 g;12.6 ml; 131.57 mmol) in THF (100 ml) at -10 $^0$C was added KHMDS (0.5M solution in toluene; 263.15 ml; 131.57 mmol) dropwise and the solution allowed to warm to ambient temperature with stirring for 18 hours. The reaction was cooled in an ice bath and quenched with saturated ammonium chloride solution and the solvent evaporated. The residue was partitioned between EtOAc (300 ml) and water (100 ml adjusted to pH 4 with 1 N HCl). The organic phase was separated, dried over $MgSO_4$, filtered and evaporated to give an orange oil. (21.0 g).

$^1$H NMR δ(ppm)($CDCl_3$): 3.99-3.89 (4 H, m), 2.08 (2 H, d, J = 13.5 Hz), 1.96-1.72 (9 H, m), 0.96-0.82 (1 H, m), 0.59-0.53 (2 H, m), 0.17 (2 H, q, J = 5.1 Hz).

*{[8-(Cyclopropylmethyl)-1 ,4-dioxaspiro[4.5] dec-8-yl] methyl} amine :*

To a stirred suspension of lithium aluminium hydride (1 M solution in ether; 94.9 ml ; 94.9 mmol) at -78 $^0$C was added a solution of 8-(cyclopropylmethyl)-l,4-dioxaspiro[4.5]decane-8-carbonitrile (14 g; 63.26 mmol) in ether (40 ml) over 30 minutes and the mixture stirred cold for 1 hour, then allowed to warm to ambient temperature and stirred for 3 hours. The resultant mixture was cooled in an ice bath and to the mixture was added in turn water (2 ml), 15% NaOH solution (2 ml) and water (2 ml). The resultant white granular solid was collected on a filter and rinsed twice with diethyl ether. The filtrate was evaporated to give the crude product as a colourless oil (12 g).

$^1$H NMR δ (ppm)($CDCl_3$): 3.93 (4 H, s), 2.68 (2 H, s), 1.63-1.57 (4 H, m), 1.54-1.48 (4 H, m), 1.25 (2 H, d, J = 6.6 Hz), 0.65-0.50 (1 H, m), 0.46-0.40 (2 H, m), 0.02 (2 H, q, J = 4.9 Hz). MS (m/e) = 226.

*2,4-Dichloro-N-[(8-cyclopropylmethyl-l,4-dioxaspiro[4.5]dec-8-yl)methyl]benzamide:*

To a solution of {[8-(cyclopropylmethyl)-l,4-dioxaspiro[4.5]dec-8-yl]methyl}amine (7.0 g; 31.0 mmol) and *N*-ethyldiisopropylamine (6.45ml; 37.2 mmol) in DCM (60 ml) at 0 $^0$C was added 2,4-dichlorobenzoyl chloride (4.78 ml; 34.17 mmol) dropwise and the solution stirred for 4 hours warming to ambient temperature. The reaction was partitioned between DCM (50 ml) and water (20 ml). The aqueous phase was extracted with DCM (20 ml) and the combined organics dried over $MgSO_4$, filtered and evaporated to give a colourless oil which was used in the next step without further purification. (11.5 g).

$^1$H NMR δ (ppm)($CDCl_3$): 7.67 (1 H, d, J = 8.3 Hz), 7.43 (1 H, d, J = 2.0 Hz), 7.32 (1 H, dd, J = 2.0, 8.4 Hz), 6.25 (1 H, s), 3.93 (4 H, s), 3.56 (2 H, d, J = 6.2 Hz), 1.67 (8 H, m)), 1.33 (2 H, d, J = 6.7 Hz), 0.75-0.65 (1 H, m), 0.51-0.47 (2 H, m), 0.05 (2 H, q, J = 5.0 Hz). MS (m/e) = 398.

*2,4-Dichloro-N-{[1-(cyclopropylmethyl)-4-oxocyclohexyl]methyl}benzamide:*

2,4-Dichloro-*N*-[(8-cyclopropylmethyl-l ,4-dioxaspiro[4.5]dec-8-yl)methyl]benzamide (11 g; 27.61 mmol) was dissolved in THF (80 ml) and HCl (2 M; 80 ml) and the solution stirred at ambient temperature for 18 hours. The solution was adjusted to pH 9 with 10N NaOH solution and extracted with DCM (2x 75 ml). The combined organics were dried ($MgSO_4$) filtered and evaporated to give an oil which was crystallised from EtOAc isohexane as a white solid (8.0g).

$^1$H NMR δ (ppm)($CDCl_3$): 7.67 (1 H, d, J = 8.3 Hz), 7.44 (1 H, d, J = 2.0 Hz), 7.34 (1 H, dd, J = 2.0, 8.3 Hz), 6.37 (1 H, s), 3.72 (2 H, d, J = 6.4 Hz), 2.57-2.49 (2 H, m), 2.39-2.31 (2 H, m), 1.92-1.80 (4 H, m), 1.43 (2 H, s), 0.77-0.69 (1 H, m), 0.58-0.54 (2 H, m), 0.11 (2 H, q, J = 5.0 Hz). MS (m/e) = 354.

*2,4-Dichloro-N-{[1-(cyclopropylmethyl)-4-hydroxycyclohexyl]methyl}benzamide:*

To a stirred solution of 2,4-dichloro-*N*-{[l-(cyclopropylmethyl)-4-oxocyclohexyl]methyl}benzamide (1 g; 2.82 mmol) in ethanol (20 ml) was added in 4 portions over 30 minutes sodium borohydride (0.15 g; 3.95mmol) and the solution stirred at ambient temperature for 2 hours. Water (1 ml) was added and the methanol evaporated. The residue was partitioned between DCM (50 ml) and water (20 ml). The aqueous phase was extracted with DCM (20 ml) and the combined organics dried over $MgSO_4$, filtered and evaporated to give an oil. The crude product was chromatographed on silica eluted with 20% EtOAc in DCM to give the two isomeric alcohols in approx 1:1 ratio, 350 mg of each isomer as white foamy solids, plus approx 150 mg of mixed fractions.

Less polar alcohol: $^1$H NMR δ (ppm)($CDCl_3$): 7.67 (1 H, d, J = 8.3 Hz), 7.43 (1 H, d, J = 2.0 Hz), 7.33 (1 H, dd, J = 2.0, 8.3 Hz), 6.27 (1 H, s), 3.71 (1 H, d, J = 4.0 Hz), 3.50 (2 H, d, J = 6.3 Hz), 1.83 (2 H, dd, J = 4.0, 13.1 Hz), 1.72 (2 H, d, J = 13.8 Hz), 1.46-1.32 (6 H, m), 0.72-0.66 (1 H, m), 0.52-0.48 (2 H, m), 0.07 (2 H, q, J = 5.0 Hz). MS (m/e) = 354.

More polar alcohol: $^1$H NMR δ (ppm)($CDCl_3$): 7.66 (1 H, d, J = 8.4 Hz), 7.42 (1 H, d, J = 2.0 Hz), 7.32 (1 H, dd, J = 2.0, 8.3 Hz)$_5$ 6.26 (1 H, s), 3.69-3.65 (1 H, m), 3.59 (2 H, d, J = 6.1 Hz), 1.83-1.77 (2 H, m), 1.72-1.68 (2 H, m), 1.63-1.59 (2 H, m), 1.39-1.33 (2 H, m), 1.25 (2 H, d, J = 6.7 Hz), 0.72-0.64 (1 H, m), 0.51-0.47 (2 H, m), 0.04 (2 H, m). MS (m/e) = 354.

*Thioacetic acid 4-cyclopropylmethyl-4-[(2,4-dichlorobenzoylamino)methyl]-cyclohexyl ester:*

To a stirred solution of triphenylphosphine (0.44 g; 1.68 mmol) in THF (20 ml) at 0 $^0$C was added diisopropylazodicarboxylate (0.34 g; 1.68 mmol) and the solution stirred at 0 $^0$C for 90 minutes. To a stirred solution of 2,4-dichloro-*N*-{[l-(cyclopropylmethyl)-4-hydroxycyclohexyl]methyl}benzamide (0.30 g; 0.84 mmol; less polar alcohol isomer) in THF (10 ml) was added thioacetic acid (0.12 ml ; 1.68 mmol). The resulting solution was added dropwise to the triphenylphosphine solution at 0 $^0$C. On complete addition, the solution was stirred at 0 $^0$C for 1 hour then allowed to warm to ambient temperature and stirred for 18 hours. The reaction mixture was partitioned between EtOAc (200 ml) and water (100 ml). The organic phase was washed with brine (100 ml), dried over anhydrous sodium sulfate, filtered and evaporated to give a yellow oily solid. The crude product was chromatographed on silica eluted with 10 - 30 % EtOAc in isohexane to give the product contaminated with triphenylphosphine oxide. The contaminated product was chromatographed on silica eluted with 1 % EtOAc in dichloromethane to give the desired product as an oil (220 mg).

$^1$H NMR. δ (ppm)($CDC1_3$): 7.66 (1 H, d, J = 8.4Hz), 7.43 (1 H, d, J = 2.1Hz), 7.34 (1 H, dd, J = 2.1, 8.4Hz), 6.26 (1 H, s), 3.58 (2 H, d, J = 6.0Hz), 3.45-3.52 (1 H, m), 2.30 (3 H, s), 1.83-1.88 (2 H, m), 1.65-1.74 (4 H, m), 1.44-1.52 (2 H, m), 1.26 (2 H, d, J = 7.0Hz), 0.64-0.71 (1 H, m), 0.47-0.52 (2 H, m), 0.00-0.06 (2 H, m).

*2,4-Dichloro-N-(1-cyclopropylmethyl-4-cyclopropylmethylsulfanylcyclohexyl-methylbenzamide:*

To a stirred solution of thioacetic acid 4-cyclopropylmethyl-4-[(2,4-dichlorobenzoylamino)methyl] cyclohexyl ester (220 mg ; 0.53 mmol) in degassed IPA (3 ml) was added a solution of lithium hydroxide (51 mg; 2.12 mmol) in water (1 ml) and the mixture stirred at ambient temperature for 2 hours. (Bromomethyl)cyclopropane (0.10 ml; 1.06 mmol) was added and the reaction mixture was stirred at ambient temperature for 18 hours. The reaction mixture was evaporated and the residue diluted with EtOAc (50 ml). The organic phase was washed with water (50 ml) and brine (20 ml), dried over anhydrous sodium sulphate, filtered and evaporated to give a yellow oil. The crude product was purified by preparative-plate chromatography on silica eluting with 15 % EtOAc in isohexane to give the desired product as a yellow oil (65 mg).

$^1$H NMR δ (ppm)($CDC1_3$): 7.68 (1 H, d, J = 8.4Hz), 7.42 (1 H, d, J = 2.1Hz), 7.33 (1 H, dd, J = 2.1, 8.4Hz), 6.21-6.23 (1 H, m), 3.59 (2 H, d, J = 6.0Hz), 2.67-2.79 (1 H, m), 2.48 (2 H, d, J = 7.0Hz), 1.87-1.92 (2 H, m), 1.59-1.76 (4 H, m), 1.32-1.41 (2 H, m), 1.25 (2 H, d, J = 6.8Hz), 0.91-1.02 (1 H, m), 0.64-0.75 (1 $H_5$ m), 0.57 (2 H, dd, J = 1.2, 7.9Hz), 0.46-0.49 (2 H, m), 0.18- 0.28 (2 H, m), 0.03-0.04 (2 H, m).

*2,4-Dichloro-N-(4-cyclopropylmethanesuIfonyl-1-cyclopropylmethylcyclohexyl-methyl)benzamide:*

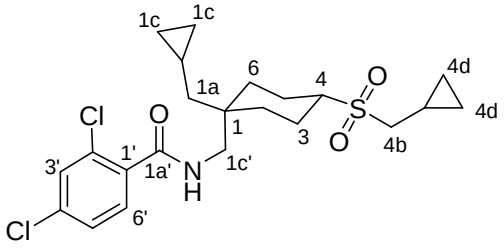
To a stirred solution of 2,4-dichloro-*N*-(1-cyclopropylmethyl-4-cyclopropylmethylsulfanylcyclohexyl methylbenzamide (65 mg ; 0.152 mmol) in acetone (2 ml) was added a solution of oxone (281 mg ; 0.457 mmol) in water (2 ml) and the solution heated at reflux for 1 hour. The reaction was diluted with water (8 ml) and adjusted to pH 7 with saturated $NaHCO_3$ solution. The aqueous mixture was extracted with EtOAc (2x50 ml). The organic layer was dried over anhydrous sodium sufate, filtered and evaporated to give a colourless oil. The oil was purified by preparative plate chromatography eluting with 10 % EtOAc in dichloromethane which give the title compound as a white foam (37 mg).

Detailed analysis of compound **16** is given below:

**$^1$H NMR:**

The proton magnetic resonance spectrum of compound 16 shown below was obtained using a Bruker DRX-500 nuclear magnetic resonance (NMR) spectrometer operating at a frequency of 499.87 MHz. The sample concentration was approximately 3.0% (w/v) in $CDCl_3$. The reference compound was $CHCl_3$ (7.27 ppm). Signal assignments are tabulated following the numbered structural formula of compound 16 below.

Proton Magnetic Resonance Signal Assignments for compound **16**

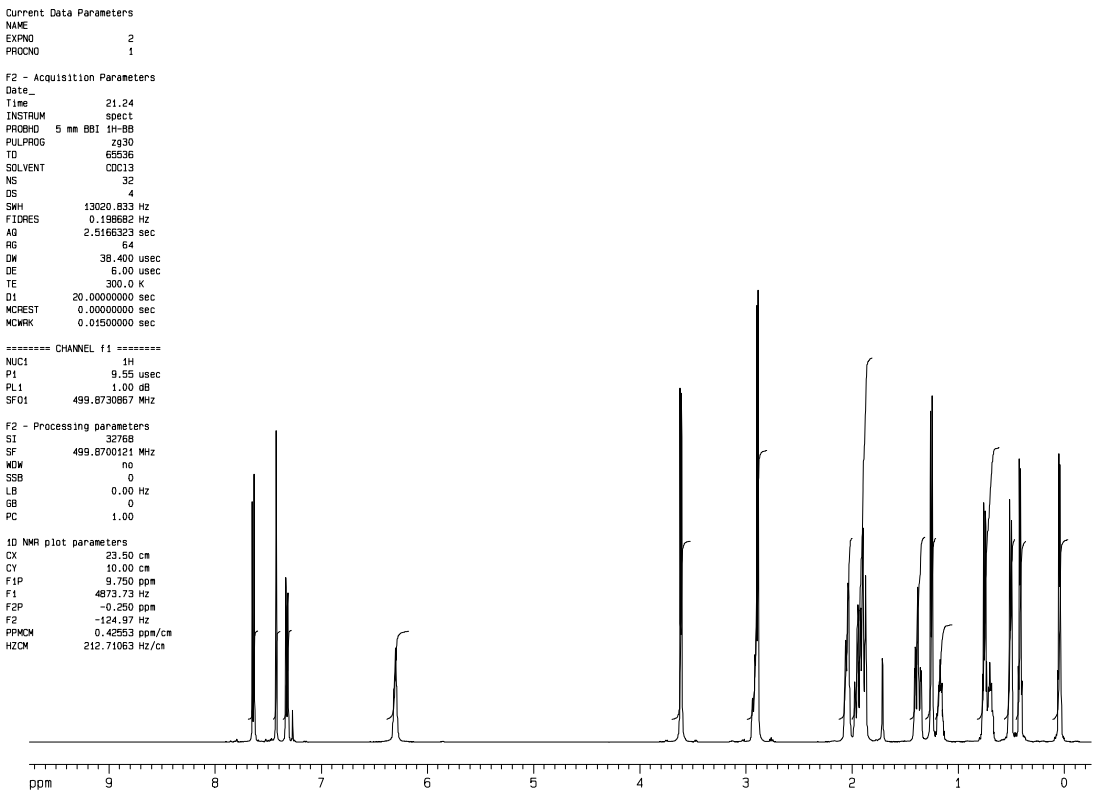



Proton Magnetic Resonance Signal Assignments

| δH (ppm) | Multiplicity[1] | | Assignment[2,3] |
|---|---|---|---|
| 7.64 | d | J=8.3 | $C_{6'}H$ |
| 7.42 | d | J=1.8 | $C_{3'}H$ |
| 7.32 | dd | J=8.3, 1.8 | $C_{5'}H$ |
| 6.30 | t | J=6.3 | $N_{1b'}H$ |
| 3.61 | d | J=6.3 | $C_{1c'}H_2$ |
| 2.94-2.88 | om | | $C_4H$ |
| 2.89 | d | J=7.1 | $C_{4b}H_2$ |
| 2.05 | m | | $C_3H$, $C_5H$ |
| 1.95 | td | J=13.2, 3.2 | $C_3H$, $C_5H$ |
| 1.88 | d | J=13.7 | $C_2H$, $C_6H$ |
| 1.38 | td | J=13.7, 3.7 | $C_2H$, $C_6H$ |
| 1.25 | d | J=6.7 | $C_{1a}H_2$ |
| 1.17 | m | | $C_{4c}H$ |
| 0.75 | m | | $C_{4d}H_2$ |
| 0.70 | m | | $C_{1b}H$ |
| 0.51 | m | | $C_{1c}H_2$ |
| 0.42 | m | | $C_{4d}H_2$ |
| 0.04 | m | | $C_{1c}H_2$ |

1. Multiplicity: s=singlet, d=doublet, t=triplet, m=multiplet, o=overlapped; coupling constants (J) in hertz.
2. Exists as a >100:1 ratio of amide bond rotamers. The resonances at δ 5.86 (m, 1H), 3.13 (m, 2H), and 2.77 (d, J=7.6, 2H) are assigned to the minor amide bond rotamer.
3. The resonance at δ 1.71 (s) is assigned to water.

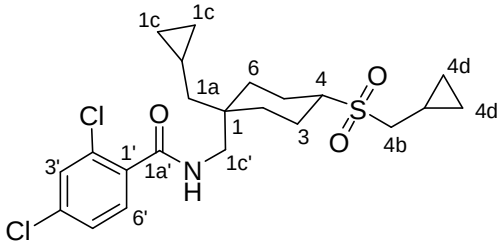
$^{1}$H NMR Spectrum for compound **16**




**$^{13}$C NMR:**

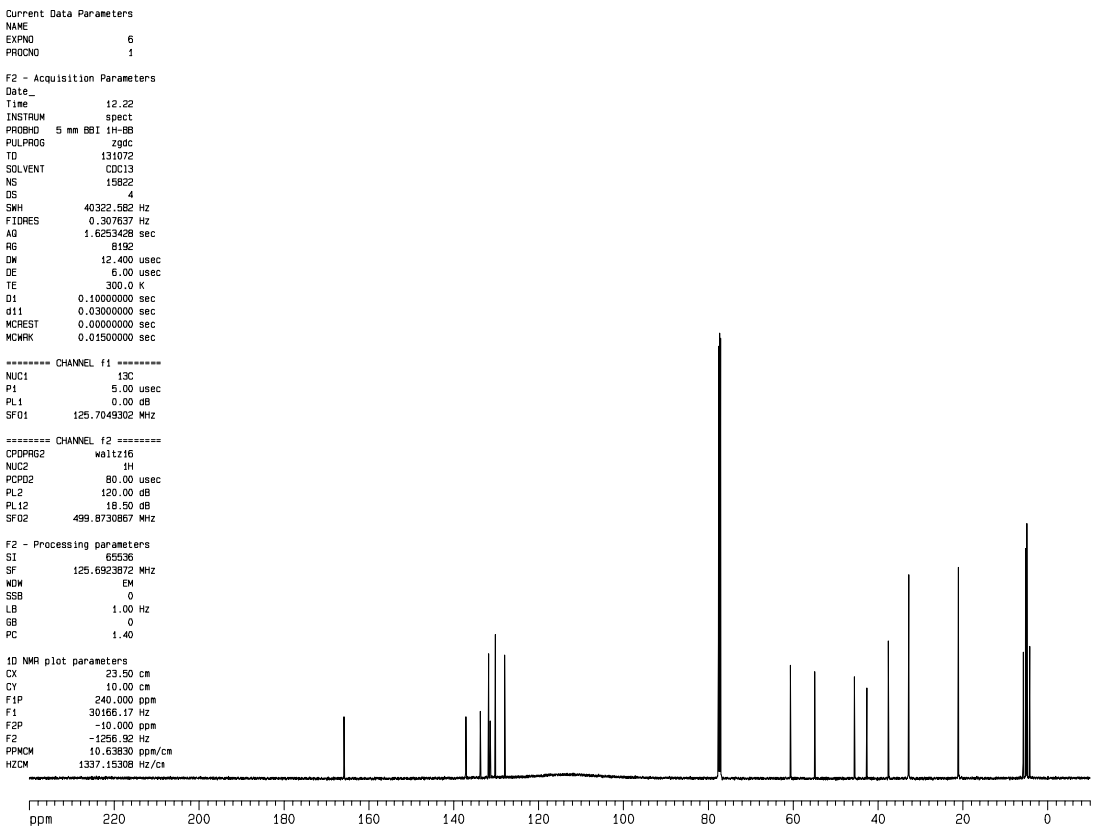
The $^{13}$C spectrum of compound 16 shown below was obtained using a Bruker DRX-500 nuclear magnetic resonance (NMR) spectrometer operating at a frequency of 125.70 MHz. The sample concentration was approximately 3.0% (w/v) in $CDCl_3$. The reference compound was $CDCl_3$ (77.23 ppm). Signal assignments are tabulated following the numbered structural formula of compound 16 below.

$^{13}$C Magnetic Resonance Signal Assignments for compound **16**




| $\delta_C$ (ppm) | Assignment | $\delta_C$ (ppm) | Assignment |
|---|---|---|---|
| 165.78 | $C_{1a'}$ | 45.44 | $C_{1a}$ |
| 137.04 [1] | $C_{4'}$ | 42.67 | $C_{1c'}$ |
| 133.68 [1] | $C_{2'}$ | 37.42 | $C_1$ |
| 131.73 | $C_{6'}$ | 32.74 | $C_2$, $C_6$ |
| 131.38 [1] | $C_{1'}$ | 21.01 | $C_3$, $C_5$ |
| 130.23 | $C_{3'}$ | 5.70 | $C_{1b}$ |
| 127.86 | $C_{5'}$ | 5.15 | $C_{1c}$ (x 2) |
| 60.61 | $C_4$ | 4.85 | $C_{4d}$ (x 2) |
| 54.84 | $C_{4b}$ | 4.19 | $C_{4c}$ |
| 1. Assignments may be interchanged. | | | |

$^{13}$C NMR for compound **16**




**Mass Spectra:**

The mass spectral data was obtained on a Micromass Quadrupole Time-of-Flight (QTof) Ultima API US mass spectrometer. The sample was dissolved in 50:50 water:acetonitrile at a concentration of 10 µg/mL. A 1 µL aliquot of this solution was injected into a HPLC system consisting of a Waters Xterra $C_{18}$ column, 150 x 4.6 mm, 3.5 micron particle size. The flow rate was 1.0 mL/min. The column temperature was 35°C. The mobile phase was water and acetonitrile with 0.05% trifluoroacetic acid added to each. A linear gradient from 60% to 85% acetonitrile in 8 minutes was used followed by an isocratic hold at this composition for 4 minutes. The observed retention time of compound 16 was 4.25 minutes. Ions were generated by electrospray ionization (ESI) in positive ion mode. Polyalanine was used as the reference for calibration. The internal lockmass was leucine enkephalin introduced at 11 µL/min at a concentration of 20 µg/mL. The observed and calculated protonated molecular mass values are tabulated below.

Observed and Calculated Molecular Mass of compound **16**

| Ion Formula | Measured Mass | Calculated Mass |
|---|---|---|
| $[C_{22}H_{29}NO_3Cl_2S + H]^+$ | 458.1324 | 458.1323 |

**Melting point:**

Melting point: 105.3-109.0 °C determined by differential scanning calorimetry.

**HPLC:**

Chromatographic Conditions

| | |
|---|---|
| Column: | Zorbax SB C8 150 x 4.6 mm, 5 µm |
| Column Temperature: | 55ºC |
| Flow Rate: | 1 mL/min |
| Detection: | 210 nm |
| Injection Volume: | 25 µL |
| Run Time: | 35 mins |
| Mobile Phase: | A = 0.1% aq. $H_3PO_4$, B= Acetonitrile, C= Methanol |
| Mobile Phase Program: | The mobile phase composition consists of 10% methanol throughout the analysis, with a gradient between the aqueous phase and acetonitrile that increases from 20% acetonitrile to 75% acetonitrile over 30 minutes, and is then held at 75% acetonitrile for 5 minutes. Bring back to the original conditions and re-equilibrate the column for 7 minutes. |

Relative Retention Times:

| Component | RRT |
|---|---|
| Impurity A | 0.99 |
| Compound **16** | 1.00 (RT = ca. 18 min; 98.5 %) |
| Impurity B | 1.09 |
| Impurity C | 1.11 |

Preparation of Solutions

Diluent: Mix deionized water with an equal volume of acetonitrile.

Sample: Weigh *ca.* 5 mg of sample in 100 mL volumetric flask and dilute to volume with diluent.