

Electronic supplementary Information

Design, Synthesis and Biological Evaluation of Imidazo[1,5-*a*]pyridine-PBD Conjugates as Potential DNA-directed Alkylating Agents

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General

(A) Chemistry

All the chemicals and reagents were obtained from Aldrich (Sigma-Aldrich, St. Louis, MO, USA), Lancaster (Alfa Aesar, Johnson Matthew Company, Ward Hill, MA, USA) and were used without purification. Reactions were monitored by TLC, performed on silica gel glass plates containing 60 F-254, and visualization on TLC was achieved by UV light or iodine indicator. Column chromatography was performed with Merck 60-120 mesh silicagel. ¹H NMR spectra were recorded on Gemini Varian VXR-unity (400 and 500 MHz) or Bruker UXNMR/XWIN-NMR (300 MHz) instruments. Chemical shifts (δ) are reported in ppm downfield from internal TMS standard. ESI spectra were recorded on Micro mass, Quattro LC using ESI⁺ software with capillary voltage 3.98 KV and ESI mode positive ion trap detector. High-resolution mass spectra (HRMS) were recorded on QSTAR XL Hybrid MS/MS mass spectrometer. Melting points were determined with an Electrothermal melting point apparatus, and are uncorrected. Optical rotations were measured on Horiba, high sensitive polarimeter, SEPA-300. High-performance liquid chromatography analyses for checking purity of synthesized compounds were recorded on a shimadzu SPD-10A (uv-vis detector)LC-10AT instrument: column, Luna 5u C18(2) 250*4.60 mm Mightysil RP-18 GP 250–4.6 (5 lm); mobile phase, 70% A and 30% B in 25 min (mobile phase A = acetonitrile, B = water); flow rate, 1 mL/min; injected sample, 10 μL; column temp, 27 °C; wavelength, 289 nm. The purity of all the compounds was ≥90% based on analytical HPLC.

(B) Biology

(a) Cell culture

MCF-7 (breast carcinoma cells) were incubated by using DMEM media, supplemented with 10% fetal calf serum, 100 μg/mL pencillin-G and 100 μg/mL streptomycin sulfate. The cell line was maintained at 37 °C in a humidified atmosphere containing 5% CO₂ in the incubator.

(b) MTT cell viability assay

Cell viability was assessed by the MTT based assay, a mitochondrial function assay. It is based on the ability of viable cells to reduce the MTT to insoluble formazan crystals by mitochondrial dehydrogenase. In this assay MCF-7 cells were seeded in a 96-well plate at a density of 10,000 cells/well. After overnight incubation cells were treated with **1**, **13a-13l** at 4 μM concentration and incubated for 24 h. Then the medium was discarded and replaced with

10 μ L MTT dye. Plates were incubated at 37 °C for 2 h. The resulting formazan crystals were solubilized in 100 μ L extraction buffer. The optical density (O.D) was recorded at 570 nm with micro plate reader.

(c) Thermal denaturation studies

The compounds **13a–l** were subjected to DNA thermal melting (denaturation) studies using duplex form calf thymus DNA (CT-DNA) using modification reported procedure.⁴⁸ Working solutions were produced by appropriate dilution in aqueous buffer (10 mM $\text{NaH}_2\text{PO}_4/\text{NaH}_2\text{PO}_4$, 1 mM Na_2EDTA , pH 7.00 \pm 0.01) containing CT-DNA, (100 μ M in phosphate) and the PBD (20 μ M) were prepared by addition of concentrated PBD solutions in methanol to obtain a fixed [PBD]/[DNA] molar ratio of 1:5. The DNA-PBD solutions were incubated at 37 °C for 0 h and 18 h prior to analysis. Samples were monitored at 260 nm using a Beckman DU-7400 spectrophotometer fitted with high performance temperature controller. Heating was applied at a rate of 1 °C min^{-1} in the 40–90 °C range. DNA helix-coil transition temperatures (T_m) were determined from the maxima in the $d(A_{260})/dT$ derivative plots. Results for each compound are shown as mean \pm standard deviation from the least three determinations and are corrected for the effects of methanol cosolvent using a linear correction term. Ligand-induced alteration in DNA melting behavior are given by $\Delta T_m = T_m(\text{DNA+PBD}) - T_m(\text{DNA alone})$, where the T_m value for the PBD free CT-DNA is 69.1 \pm 0.001. The fixed [PBD]/[DNA] ratio used did not result in binding saturation of the host DNA duplex for any compound examined.

(d) Cell cycle analysis

5 X 10⁵ MCF-7 cells were seeded in 60 mm dish and allowed to grow for 24 h, **2** and **4** μ M concentration of **1**, **13 a-13l** compounds were added to the culture media, and the cells were incubated for an additional 24 h. Cells were harvested with Trypsin-EDTA, fixed with ice-cold 70% ethanol at 4 °C for 30 min, washed with PBS and incubated with 1mg/mL RNAase solution (Sigma) at 37 °C for 30 min. Cells were collected by centrifugation at 2000 rpm for 5 min and further stained with 250 μ L of DNA staining solution [10 mg of Propidium Iodide (PI), 0.1 mg of trisodium citrate, and 0.03 mL of Triton X-100 were dissolved in 100 mL of sterile MilliQ water at room temperature for 30 min in the dark]. The DNA contents of 20,000 events were measured by flow cytometer (DAKO CYTOMATION, Beckman Coulter, Brea, CA). Histograms were analyzed by using Summit Software.

(e) Immunofluorescence microscopy studies for γ H2AX staining

MCF-7 cells were seeded on glass cover slips, incubated for 24 h in the presence or absence of test compounds **1** and **13g** at 4 μ M concentration. Following the termination of incubation, cells were fixed with 4% paraformaldehyde, 0.02% glutaraldehyde in PBS and permeabilized by dipping the cells in 100% methanol (-20 °C). Later, cover slips were blocked with 1% BSA in phosphate buffered saline for 1 h followed by incubation with a primary antibody (γ H2AX) followed by secondary antibody. At the end, cells were washed and fixed. Photographs were taken by using the confocal microscope (Olympus).

(f) Protein extraction and Western blot analysis

5 X 10⁵ MCF-7 cells were seeded in 60 mm dish and allowed to grow for 24 h, 4 μ M concentration of **1**, **13f** and **13g** compounds were added to the culture media, and the cells were incubated for an additional 24 h. Total cell lysates from cultured MCF-7 cells were obtained by lysing the cells in ice-cold RIPA buffer (1X PBS, 1% NP-40, 0.5% sodium deoxycholate and 0.1% SDS) and containing 100 μ g/mL PMSF, 5 μ g/mL Aprotinin, 5 μ g/mL leupeptin, 5 μ g/mL pepstatin and 100 μ g/mL NaF. After centrifugation at 12,000 rpm for 10 min, the protein in supernatant was quantified by Bradford method (BIO-RAD) using Multimode variaskan instrument (Thermo-Fischer Scientifics). Fifty micrograms of protein per lane was applied in 12% SDS-polyacrylamide gel. After electrophoresis, the protein was transferred to polyvinylidene difluoride (PVDF) membrane (Amersham Biosciences). The membrane was blocked at room temperature for 2 h in 1X TBS + 0.1% Tween20 (TBST) containing 5% blocking powder (Santacruz). The membrane was washed with TBST for 5 min, and primary antibody was added and incubated at 4 °C overnight. P53, p21 antibodies were purchased from Millipore Company. β -actin was purchased from Imgenex Company. Membranes were washed with TBST three times for 15 min and the blots were visualized with chemiluminescence reagent (Thermo Fischer Scientifics Ltd.). The X-ray films were developed with developer and fixed with fixer solution purchased from Kodak Company.

(g) p53 ELISA

Enzyme-linked immunosorbent assays (ELISA) for p53 were conducted by the p53 ELISA kit from Alexis Biochemical. MCF-7 cells were treated with compounds DC-81 (**1**), **13f** and **13g** at 4 μ M concentration for 24 h. Cell lysates were isolated and added to microplate wells containing p53 antibody. Biotin-conjugated anti-human p53 monoclonal antibody (100 μ L) was added. After the incubation period and washing steps, bound p53 was

detected by using streptavidin–HRP secondary antibody (150 μ L). The coloured product obtained was detected by measuring OD at 450 nm. OD is directly proportional to the amount of p53 protein present in the sample.

Supplementary Table 1: IC₅₀ values of compounds 13a-l in MCF7 cell line.

Compd	IC ₅₀ in μ M
DC-81	5 \pm 0.012
13a	7.21 \pm 0.09
13b	7.18 \pm 0.07
13c	4.16 \pm 0.021
13d	3.74 \pm 0.03
13e	5.74 \pm 0.04
13f	3.26 \pm 0.07
13g	3.16 \pm 0.1
13h	4.28 \pm 0.04
13i	6 \pm 0.09
13j	4.17 \pm 0.08
13k	5.67 \pm 0.10
13l	3.68 \pm 0.011

Supplementary Table 2:

Complex	Binding energy
DNA- 13a	-10.67
DNA- 13b	-10.35
DNA- 13c	-12.24
DNA- 13d	-10.74
DNA- 13e	-11.22
DNA- 13f	-10.95
DNA- 13g	-12.66
DNA- 13h	-9.44
DNA- 13i	-10.28
DNA- 13j	-11.06
DNA- 13k	-13.30
DNA- 13l	-12.76

Spectral data and procedures for compound preparations

Ethyl 2-hydroxyimino-2-(2-pyridyl)acetate (**2**)

A solution of 495 mg (3 mmol) of ethyl (2- pyridyl) acetate (**1**) in 10 mL of glacial acetic acid was cooled in an ice bath and 0.207 mg (3.2 mmol) of sodium nitrite in 5 ml of water was added over a period of 20 minutes, keeping the temperature between 15 to 25 °C. The mixture was stirred for an additional 30 minutes, 50 mL of water was added, and the stirring continued for two more hours. The crystals were washed with water, washed three times with 5% sodium bicarbonate solution and again with water. Recrystallization from methanol yielded 0.488 mg (84%) of white needles. mp 147–152 °C ; ¹H NMR (300 MHz, CDCl₃): δ 7.65 (s, 1H), 6.95-6.89 (m, 2H), 6.75 (s, 1H), 3.98 (q, 2H, *J* = 6.7 Hz), 1.54 (t, 3H, *J* = 6.7 Hz); MS (ESI): *m/z* 195 (M⁺+1).

Ethyl 2-amino-2-(2-pyridyl)acetate (**3**)

To a solution of ethyl 2-hydroxyimino-2-(2-pyridyl)acetate (**2**) (0.194 mg, 1 mmol) in 20 mL of methanol was added catalytic amount of 10% palladium on activated carbon. The mixture was stirred overnight under hydrogen, the reaction mixture was filtered, and the filtrate was evaporated. The crude product **3** was used directly in next step without further purification.

Ethyl 2-(benzoylamino)-2-(2-pyridyl)acetate (**5a**)

To a solution containing ethyl 2-amino-2-(2-pyridyl)acetate (**3**) (0.180 mg, 1 mmol), triethylamine (3 mmol) in 20mL of dichloromethane, benzoyl chloride (**4a**) (1 mmol) in 10mL of dichloromethane was added under nitrogen and stirred at room temperature for about 5 h till the completion of the reaction as monitored by TLC. The reaction mixture was washed with water, extracted with dichloromethane, dried over anhydrous sodium sulphate and subjected to column chromatography using ethyl acetate/hexane (7:3) as an eluent to give compound (**5a**). ¹H NMR (300 MHz, CDCl₃): δ 8.30 (d, 1H, *J* = 7.0 Hz), 8.22 (d, 1H, *J* = 8.7 Hz), 7.90 (d, 2H, *J* = 6.7 Hz), 7.22-7.34 (m, 3H), 6.98 (s, 1H), 6.73 (t, 1H, *J* = 6.7 Hz), 5.66 (d, 1H, *J* = 6.5 Hz), 4.39 (q, 2H, *J* = 6.8 Hz), 1.52 (t, 3H, *J* = 6.8 Hz); MS (EI): *m/z* 285 (M⁺+1).

Ethyl 2-(2-pyridyl)-2-[4-(trifluoromethyl)benzoyl]aminoacetate (**5b**)

The compound **5b** was prepared according to the method described for the compound **5a** by employing compound **4b**. ¹H NMR (300 MHz, CDCl₃): δ 8.56 (d, 1H, *J* = 6.7 Hz), 8.11 (d, 1H, *J* = 7.5 Hz), 8.01 (d, 2H, *J* = 8.3 Hz), 7.70 (d, 2H, *J* = 8.3 Hz), 7.58 (d, 1H, *J* = 7.5 Hz), 7.31-7.25 (m, 1H), 5.77 (d, 1H, *J* = 6.7 Hz), 4.21 (q, 2H, *J* = 7.5 Hz), 3.92 (s, 3H), 1.25 (t, 3H, *J* = 7.5 Hz); MS (ESI): *m/z* 353 (M⁺+1).

Ethyl 2-[(4-methoxybenzoyl)amino]-2-(2-pyridyl)acetate (5c)

The compound **5c** was prepared according to the method described for the compound **5a** by employing compound **4c** (1 mmol). ¹H NMR (300 MHz, CDCl₃): δ 8.33 (m, 2H), 7.82 (d, 2H, *J* = 6.7 Hz), 7.16 (dd, 1H, *J* = 9.8, 6.8 Hz), 6.82 (d, 2H, *J* = 6.7 Hz), 6.67 (d, 1H, *J* = 6.2 Hz), 5.47 (d, 1H, *J* = 6.5 Hz), 4.32 (q, 2H, *J* = 6.9 Hz), 3.92 (s, 3H), 1.52 (t, 3H, *J* = 6.9 Hz); MS (EI): *m/z* 315 (M⁺+1).

Ethyl 3-phenylimidazo[1,5-a]pyridine-1-carboxylate (6a)

To ethyl 2-(benzoylamino)-2-(2-pyridyl)acetate **5a** (0.284 mg, 1 mmol), add 4 ml of POCl₃ and reflux for three hours. This was poured into cold water and neutralized with NaHCO₃ solution. This water layer was extracted three times with ethylacetate. The combined organic phases were dried over anhydrous Na₂SO₄ and evaporated under vacuum. The residue, thus obtained was purified by column chromatography using ethylacetate and hexane as eluant to afford compound **6a** (212 mg, 80%). mp 136–140 °C ; ¹H NMR (300 MHz, CDCl₃): δ 8.29 (d, 1H, *J* = 7.1 Hz), 8.26 (d, 1H, *J* = 9.0 Hz), 7.77 (d, 2H, *J* = 6.7 Hz), 7.42-7.54 (m, 3H), 7.09 (dd, 1H, *J* = 6.6, 6.4 Hz), 6.73 (t, 1H, *J* = 6.6 Hz), 4.46 (q, 2H, *J* = 6.9 Hz), 1.47 (t, 3H, *J* = 6.9 Hz); MS (ESI): *m/z* 267 (M⁺+1).

Ethyl 3-[4-(trifluoromethyl)phenyl]imidazo[1,5-a]pyridine-1-carboxylate (6b)

The compound **6b** was prepared according to the method described for the compound **6a** by employing compound **5b** (352 mg, 1 mmol).

Yield: 250 mg, 75%; ¹H NMR (300 MHz, CDCl₃): δ 8.30 (dd, 2H, *J* = 9.6, 6.7 Hz), 7.90 (d, 2H, *J* = 7.7 Hz), 7.70 (d, 2H, *J* = 8.6 Hz), 7.13 (dd, 1H, *J* = 5.7, 6.7 Hz), 6.80 (t, 1H, *J* = 6.7 Hz), 4.47 (q, 2H, *J* = 6.7 Hz), 1.47 (t, 3H, *J* = 6.7 Hz); MS (ESI): *m/z* 335 (M⁺+1).

Ethyl 3-(4-methoxyphenyl)imidazo[1,5-a]pyridine-1-carboxylate (6c)

The compound **6c** was prepared according to the method described for the compound **6a** by employing compound **5c** (314 mg, 1 mmol).

Yield: 216 mg, 73%; ¹H NMR (300 MHz, CDCl₃): δ 8.21-8.24 (m, 2H), 7.69 (d, 2H, *J* = 9.0 Hz), 7.04 (dd, 1H, *J* = 9.8, 6.7 Hz), 6.98 (d, 2H, *J* = 8.3 Hz), 6.70 (t, 1H, *J* = 7.5 Hz), 4.45 (q, 2H, *J* = 6.7 Hz), 3.87 (s, 3H), 1.46 (t, 3H, *J* = 6.7 Hz); MS (ESI): *m/z* 297 (M⁺+1).

3-Phenyl imidazo[1,5-a]pyridine-1-carboxylic acid (7a)

2N sodium hydroxide (1.22 mL) was added to a solution of **6a** (266 mg, 1 mmol) in THF-H₂O-MeOH (4:1:1) and the mixture stirred at room temperature for 12 h. After most of the THF and methanol were evaporated, the aqueous phase was acidified with 12N HCl to pH 7

and re-extracted with CH₂Cl₂ to give compound **7a** in quantitative yield. This compound was directly used in next step.

3-[4-(Trifluoromethyl)phenyl]imidazo[1,5-a]pyridine-1-carboxylic acid (7b)

This compound was prepared according to the method described for the compound **7a** by employing compound **6b** (334 mg, 1 mmol) and it was directly used for the next step.

3-(4-Methoxyphenyl)imidazo[1,5-a]pyridine-1-carboxylic acid (7c)

This compound was prepared according to the method described for the compound **7a** by employing compound **6c** (296 mg, 1 mmol) and it was directly used for the next step.

tert-Butyl 4-[(3-phenylimidazo[1,5-a]pyridin-1-yl)carbonyl]-1-piperazinecarboxylate (8a)

Dichloromethane (10 mL) solution of compound **7a** (1 mmol) was charged with EDC (1 mmol), HOBT (1mmol) at 0 °C. After twenty minutes Boc protected piperazine dissolved in dichloromethane was added. The solution was stirred at room temperature for 16 h under an atmosphere of nitrogen and then diluted with dichloromethane (20 mL), washed with saturated aqueous sodium bicarbonate solution (50 mL), brine (50 mL), dried over anhydrous sodium sulphate, filtered, and concentrated in vacuo. The crude product was purified by silica gel chromatography using ethylacetate and hexane as eluant to afford compound **8a** (345 mg, 85%). mp 166–170 °C ; ¹H NMR (300 MHz, CDCl₃): δ 8.32 (d, 1H, *J* = 9.0 Hz), 8.29 (d, 1H, *J* = 7.1 Hz), 7.75 (d, 2H, *J* = 7.3 Hz), 7.42-7.54 (m, 3H), 6.99 (t, 1H, *J* = 6.9 Hz), 6.70 (t, 1H, *J* = 6.9 Hz), 4.49 (brs, 2H), 3.78 (brs, 2H), 3.53 (s, 4H), 1.47 (s, 9H); MS (ESI): *m/z* 407 (M⁺+1).

tert-Butyl 4-(3-[4-(trifluoromethyl)phenyl]imidazo[1,5-a]pyridin-1-ylcarbonyl)-1-piperazinecarboxylate (8b)

The compound **8b** was prepared according to the method described for the compound **8a** by employing compound **7b** (306 mg, 1 mmol).

Yield: 388 mg, 82%; ¹H NMR (300 MHz, CDCl₃): δ 8.37 (d, 1H, *J* = 9.0 Hz), 8.29 (d, 1H, *J* = 7.5 Hz), 7.90 (d, 2H, *J* = 8.3 Hz), 7.70 (d, 2H, *J* = 9.0 Hz), 7.05 (dd, 1H, *J* = 6.7, 5.2 Hz), 6.79 (t, 1H, *J* = 7.5 Hz), 4.31-4.59 (brs, 2H), 3.70-3.88 (brs, 2H), 3.60-3.49 (m, 4H), 1.47 (s, 9H); MS (ESI): *m/z* 475 (M⁺+1).

tert-Butyl 4-[3-(4-methoxyphenyl)imidazo[1,5-a]pyridin-1-yl]carbonyl-1-piperazinecarboxylate (8c)

The compound **8c** was prepared according to the method described for the compound **8a** by employing compound **7c** (268 mg, 1 mmol).

Yield: 305 mg, 70%; ^1H NMR (500 MHz, CDCl_3): δ 8.31 (d, 1H, J = 8.6 Hz), 8.20 (d, 1H, J = 7.6 Hz), 7.64 (d, 2H, J = 8.6 Hz), 7.01 (d, 2H, J = 8.6 Hz), 6.96 (dd, 1H, J = 6.7, 8.6 Hz), 6.68 (t, 1H, J = 6.7 Hz), 4.33-4.64 (brs, 2H), 3.88 (s, 3H), 3.65-3.84 (brs, 2H), 3.53 (s, 4H), 1.47 (s, 9H); MS (ESI): m/z 437 ($\text{M}^+ + 1$).

(3-Phenylimidazo[1,5-a]pyridin-1-yl)(piperazino)methanone (9a)

To a solution of Boc-protected compound **8a** (406 mg, 1 mmol) in dry dichloromethane was added trifluoroacetic acid (1.2 mL, 15.8 mmol) at 0 °C and stirred under nitrogen for 8 h, the reaction mixture was then concentrated in vacuum to afford the compound **9a** and then it was used directly in the next step.

Piperazino3-[4-(trifluoromethyl)phenyl]imidazo[1,5-a]pyridin-1-ylmethanone (9b)

This compound was prepared according to the method described for the compound **9a** by employing compound **8b** (474 mg, 1 mmol) and it was directly used for the next step.

[3-(4-Methoxyphenyl)imidazo[1,5-a]pyridin-1-yl](piperazino)methanone (9c)

This compound was prepared according to the method described for the compound **19a** by employing compound **8c** (436 mg, 1 mmol) and it was directly used for the next step.

(2S)-N-[4-(3-Bromopropoxy)-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal (11a)

To a solution of compound **10** (400 mg, 1 mmol) in dry acetone (15 ml) was added, anhydrous K_2CO_3 (553 mg, 4 mmol), 1,3-dibromopropane (256 mg, 1.2 mmol) and the mixture was stirred at reflux temperature for 48 h. The reaction was monitored by TLC using EtOAc-hexane (1:1). After completion of the reaction as indicated by TLC, K_2CO_3 was removed by filtration and the solvent was evaporated under reduced pressure, diluted with water and extracted with ethyl acetate. The combined organic phases were dried over Na_2SO_4 and evaporated under vacuum. The residue, thus obtained was purified by column chromatography using ethyl acetate and hexane (2:3) to afford compound **11a** as yellow liquid (500 mg, 96%). ^1H NMR (CDCl_3 , 200 MHz): δ 7.65 (s, 1H), 6.80 (s, 1H), 4.86 (d, 1H, J = 4.3 Hz), 4.72-4.61 (m, 1H), 4.25 (t, 2H, J = 6.0 Hz), 3.95 (s, 3H), 3.65-3.55 (m, 2H), 3.31-3.15 (m, 2H), 2.90-2.60 (m, 4H), 2.40-1.70 (m, 6H), 1.45-1.21 (m, 6H); MS (ESI): m/z 521 ($\text{M} + 1$) $^+$.

(2S)-N-[4-(4-Bromobutyloxy)-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal (11b)

The compound **11b** was prepared according to the method described for compound **11a** by employing **10** (400 mg, 1 mmol) and 1,4-dibromobutane (268 mg, 1.2 mmol) to afford the

compound **11b** (492 mg, 92%). ^1H NMR (CDCl_3 , 200 MHz): δ 7.65 (s, 1H), 6.80 (s, 1H), 4.87 (d, 1H, $J = 4.3$ Hz), 4.71-4.60 (m, 1H), 4.15 (t, 2H, $J = 6.0$ Hz), 3.95 (s, 3H), 3.59-3.42 (m, 2H), 3.30-3.15 (m, 2H), 2.85-2.65 (m, 4H), 2.40-1.60 (m, 8H), 1.40-1.21 (m, 6H); MS (ESI): m/z 536 ($M+1$) $^+$.

(2S)-N-[4-(5-Bromopentyloxy)-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal (11c)

The compound **11c** was prepared according to the method described for compound **11a** by employing **10** (400 mg, 1 mmol) and 1,5-dibromopentane (270 mg, 1.2 mmol) to afford the compound **11c** (522 mg, 94%). ^1H NMR (CDCl_3 , 200 MHz): δ 7.64 (s, 1H), 6.80 (s, 1H), 4.83 (d, 1H, $J = 4.3$ Hz), 4.72-4.62 (m, 1H), 4.15-4.05 (t, 2H, $J = 6.0$ Hz), 3.95 (s, 3H), 3.51-3.43 (m, 2H), 3.30-3.16 (m, 2H), 2.85-2.65 (m, 4H), 2.40-1.61 (m, 10H), 1.41-1.23 (m, 6H); MS (ESI): m/z 550 ($M+1$) $^+$.

4-[(6-Bromohexyl)oxy]-5-methoxy-2-nitrophenyl-2-[di(ethylsulfanyl)methyl]tetrahydro-1H-1-pyrrolylmethanone (11d)

The compound **11d** was prepared according to the method described for compound **11a** by employing **10** (400 mg, 1 mmol) and 1,6-dibromopentane (292 mg, 1.2 mmol) to afford the compound **11d** (507 mg, 90%). ^1H NMR (CDCl_3 , 200 MHz): δ 7.63 (s, 1H), 6.80 (s, 1H), 4.83 (d, 1H, $J = 4.3$ Hz), 4.74-4.60 (m, 1H), 4.14-4.03 (t, 2H, $J = 6.0$ Hz), 3.95 (s, 3H), 3.51-3.43 (m, 2H), 3.30-3.16 (m, 2H), 2.85-2.65 (m, 4H), 2.40-1.61 (m, 12H), 1.41-1.23 (m, 6H); MS (ESI): m/z 564 ($M+1$) $^+$.

(4-3-[4-(2-[Di(ethylsulfanyl)methyl]tetrahydro-1H-1-pyrrolylcarbonyl)-2-methoxy-5-nitrophenoxy]propylpiperazino)(3-phenylimidazo[1,5-a]pyridin-1-yl)methanone (12a)

To a solution of 2S-N-[4-(3-bromopropoxy)-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal (**11a**) (521 mg, 1.0 mmol) in dry DMF (20 mL) was added anhydrous K_2CO_3 (5.0 mmol) and (3-Phenylimidazo[1,5-a]pyridin-1-yl)(piperazino)methanone of formula (**9a**) (306 mg, 1.0 mmol). The reaction mixture was stirred at room temperature for 24 h and the reaction was monitored by TLC using ethyl acetate-hexane (60%) as a solvent system. After the completion of reaction ice was added to the reaction mixture followed by extraction with ethyl acetate (3×20 mL) and finally washed with brine solution. Then the solvent was evaporated under vacuum to afford the crude product. This was further purified by column chromatography using ethyl acetate-hexane (80%) as a solvent system to obtain the pure product **12a** as a yellow solid. Yield 560 mg, 75%; mp 71-76 °C; ^1H NMR (CDCl_3 , 300 MHz): δ 1.35 (q, 6H, $J = 6.4$ Hz), 1.48-2.35 (m,

11H), 2.51-2.94 (m, 9H), 3.15-3.35 (m, 2H), 3.92 (s, 3H), 4.20 (t, 2H, $J = 6.0$ Hz), 4.65-4.75 (m, 1H) 4.86 (d, 1H, $J = 3.7$ Hz), 6.75 (t, 1H, $J = 6.4$ Hz), 6.81 (s, 1H), 7.04 (t, 1H, $J = 6.9$ Hz), 7.44-7.60 (m, 3H), 7.69 (s, 1H), 7.77 (d, 2H, $J = 6.9$ Hz), 8.28-8.34 (m, 2H). ESIMS: m/z 747 ($M^+ + 1$).

(4-4-[4-(2-[Di(ethylsulfanyl)methyl]tetrahydro-1H-1-pyrrolylcarbonyl)-2-methoxy-5-nitrophenoxy]butylpiperazino)(3-phenylimidazo[1,5-a]pyridin-1-yl)methanone (12b)

This compound was prepared according to the method described for compound **12a**, employing compound **11b** (535 mg, 1 mmol) and compound **9a** (1 mmol) to obtain the pure product **12b** as a yellow solid. Yield 570 mg, 75%; mp 75-80 °C; ^1H NMR (CDCl_3 , 300MHz): δ 1.33 (q, 6H, $J = 7.5$ Hz), 1.70-2.33 (m, 11H), 2.46-2.86 (m, 10H), 3.16-3.31 (m, 2H), 3.93 (s, 3H), 4.12 (t, 2H, $J = 6.0$ Hz), 4.61-4.70 (m, 1H), 4.81 (d, 1H, $J = 3.7$ Hz), 6.70 (t, 1H, $J = 6.7$ Hz), 6.76 (s, 1H), 6.99 (dd, 1H, $J = 7.5, 6.7$ Hz), 7.42-7.55 (m, 3H), 7.62 (s, 1H), 7.72 (d, 2H, $J = 7.5$ Hz), 8.27 (d, 1H, $J = 7.5$ Hz), 8.33 (d, 1H, $J = 9.0$ Hz). ESIMS: m/z 761 (M^+).

(4-5-[4-(2-[Di(ethylsulfanyl)methyl]tetrahydro-1H-1-pyrrolylcarbonyl)-2-methoxy-5-nitrophenoxy]pentylpiperazino)(3-phenylimidazo[1,5-a]pyridin-1-yl)methanone (12c)

This compound was prepared according to the method described for compound **12a**, employing compound **11c** (549 mg, 1 mmol) and compound **9a** (1 mmol) to obtain the pure product **12c** as a yellow solid. Yield 581 mg, 75%; mp 64-68 °C; ^1H NMR (CDCl_3 , 300 MHz): δ 1.34 (q, 6H, $J = 7.7$ Hz), 1.44-2.36 (m, 13H), 2.40-2.86 (m, 11H), 3.15-3.32 (m, 2H), 3.93 (s, 3H), 4.08 (t, 2H, $J = 6.3$ Hz), 4.61-4.72 (m, 1H), 4.81 (d, 1H, $J = 3.5$ Hz), 6.70 (t, 1H, $J = 6.3$ Hz), 6.76 (s, 1H), 6.99 (dd, 1H, $J = 6.4, 6.2$ Hz), 7.41-7.57 (m, 3H), 7.61 (s, 1H), 7.73 (d, 2H, $J = 6.9$ Hz), 8.28 (d, 1H, $J = 6.9$ Hz), 8.32 (d, 1H, $J = 9.0$ Hz). ESIMS: m/z 775 ($M^+ + 1$).

(4-6-[4-(2-[Di(ethylsulfanyl)methyl]tetrahydro-1H-1-pyrrolylcarbonyl)-2-methoxy-5-nitrophenoxy]hexylpiperazino)(3-phenylimidazo[1,5-a]pyridin-1-yl)methanone (12d)

This compound was prepared according to the method described for compound **12a**, employing compound **11d** (563 mg, 1 mmol) and compound **9a** (1 mmol) to obtain the pure product **12d** as a yellow solid. Yield 552 mg, 70%; mp 65-70 °C; ^1H NMR (CDCl_3 , 300 MHz): δ 1.20-1.28 (m, 2H), 1.36 (q, 6H, $J = 7.1$ Hz), 1.40-1.68 (m, 3H), 1.73-2.39 (m, 9H), 2.40-2.88 (m, 12H), 3.15-3.31 (m, 2H), 3.93 (s, 3H), 4.01-4.13 (m, 2H), 4.57-4.71 (m, 1H), 4.81 (d, 1H, $J = 3.7$ Hz), 6.70 (t, 1H, $J = 6.8$ Hz), 6.75 (s, 1H), 7.00 (dd, 1H, $J = 6.7, 6.0$ Hz),

7.40-7.56 (m, 3H), 7.61 (s, 1H), 7.70-7.75 (m, 2H), 8.27 (d, 1H, $J = 6.7$ Hz), 8.34 (d, 1H, $J = 9.0$ Hz). ESIMS: m/z 789 (M^+).

(4-3-[4-(2-[Di(ethylsulfanyl)methyl]tetrahydro-1H-1-pyrrolylcarbonyl)-2-methoxy-5-nitrophenoxy]propylpiperazino)3-[4-(trifluoromethyl)phenyl]imidazo[1,5-a]pyridin-1-ylmethanone (12e)

This compound was prepared according to the method described for compound **12a**, employing compound **11a** (521 mg, 1 mmol) and compound **9b** (1 mmol) to obtain the pure product **12e** as a yellow solid. Yield 611 mg, 75%; mp 83-88 °C; ^1H NMR (CDCl_3 , 300 MHz): δ 1.10-1.29 (m, 1H), 1.35 (q, 6H, $J = 7.4$ Hz), 1.73-2.35 (m, 10H), 2.62-2.90 (m, 9H), 3.15-3.31 (m, 2H), 3.93 (s, 3H), 4.20 (t, 2H, $J = 6.8$ Hz), 4.60-4.71 (m, 1H), 4.82 (d, 1H, $J = 3.7$ Hz), 6.76 (s, 1H), 6.80 (dd, 1H, $J = 7.3, 6.9$ Hz), 7.06 (dd, 1H, $J = 6.4, 5.4$ Hz), 7.65 (s, 1H), 7.80 (d, 2H, $J = 8.3$ Hz), 7.91 (d, 2H, $J = 8.1$ Hz), 8.30 (d, 1H, $J = 7.3$ Hz), 8.38 (d, 1H, $J = 9.2$ Hz). ESIMS: m/z 815 (M^+).

(4-4-[4-(2-[Di(ethylsulfanyl)methyl]tetrahydro-1H-1-pyrrolylcarbonyl)-2-methoxy-5-nitrophenoxy]butylpiperazino)3-[4-(trifluoromethyl)phenyl]imidazo[1,5-a]pyridin-1-ylmethanone (12f)

This compound was prepared according to the method described for compound **12a**, employing compound **11b** (535 mg, 1 mmol) and compound **9b** (1 mmol) to obtain the pure product **12f** as a yellow solid. Yield 622 mg, 75%; mp 75-80 °C; ^1H NMR (CDCl_3 , 300 MHz): δ 1.33 (q, 6H, $J = 7.3$ Hz), 1.68-2.36 (m, 12H), 2.48-2.88 (m, 10H), 3.12-3.31 (m, 2H), 3.93 (s, 3H), 4.06-4.19 (m, 2H), 4.61-4.71 (m, 1H), 4.81 (d, 1H, $J = 3.2$ Hz), 6.72-6.82 (m, 2H), 7.04 (t, 1H, $J = 7.8$ Hz), 7.62 (s, 1H), 7.81 (d, 2H, $J = 8.1$ Hz), 7.92 (d, 2H, $J = 7.9$ Hz), 8.30 (d, 1H, $J = 7.1$ Hz), 8.35 (d, 1H, $J = 8.8$ Hz). ESIMS: m/z 844 ($M^+ + 1$).

(4-5-[4-(2-[Di(ethylsulfanyl)methyl]tetrahydro-1H-1-pyrrolylcarbonyl)-2-methoxy-5-nitrophenoxy]pentylpiperazino)3-[4-(trifluoromethyl)phenyl]imidazo[1,5-a]pyridin-1-ylmethanone (12g)

This compound was prepared according to the method described for compound **12a**, employing compound **11c** (549 mg, 1 mmol) and compound **9b** (1 mmol) to obtain the pure product **12g** as a yellow solid. Yield 590 mg, 70%; mp 84-89 °C; ^1H NMR (CDCl_3 , 300 MHz): δ 1.33 (q, 6H, $J = 7.5$ Hz), 1.46-2.36 (m, 13H), 2.46-2.89 (m, 11H), 3.14-3.34 (m, 2H), 3.93 (s, 3H), 4.08 (t, 2H, $J = 6.2$ Hz), 4.60-4.72 (m, 1H), 4.82 (d, 1H, $J = 3.5$ Hz), 6.72-6.82 (m, 2H), 7.06 (dd, 1H, $J = 6.7, 6.4$ Hz), 7.61 (s, 1H), 7.81 (d, 2H, $J = 8.3$ Hz), 7.89 (d, 2H, $J = 8.1$ Hz), 8.31 (d, 1H, $J = 6.9$ Hz), 8.36 (d, 1H, $J = 9.2$ Hz). ESIMS: m/z 829 (M^+).

(4-6-[4-(2-[Di(ethylsulfanyl)methyl]tetrahydro-1H-1-pyrrolylcarbonyl)-2-methoxy-5-nitrophenoxy]hexylpiperazino)3-[4-(trifluoromethyl)phenyl]imidazo[1,5-a]pyridin-1-ylmethanone (12h)

This compound was prepared according to the method described for compound **12a**, employing compound **11d** (563 mg, 1 mmol) and compound **9b** (1 mmol) to obtain the pure product **12h** as a yellow solid. Yield 685 mg, 80%; mp 85-90 °C; ¹H NMR (CDCl₃, 300 MHz): δ 1.17-1.70 (m, 14H), 1.72-2.17 (m, 6H), 2.39-2.89 (m, 12H), 3.13-3.33 (m, 2H), 3.94 (s, 3H), 4.07 (t, 2H, *J* = 6.1 Hz), 4.60-4.72 (m, 1H), 4.81 (d, 1H, *J* = 3.7 Hz), 6.72-6.83 (m, 2H), 7.05 (dd, 1H, *J* = 9.0, 6.4 Hz), 7.61 (s, 1H), 7.81 (d, 2H, *J* = 8.3 Hz), 7.92 (d, 2H, *J* = 8.1 Hz), 8.29 (d, 1H, *J* = 7.1 Hz), 8.37 (d, 1H, *J* = 9.0 Hz). ESIMS: *m/z* 858 (*M*⁺+1).

(4-3-[4-(2-[Di(ethylsulfanyl)methyl]tetrahydro-1H-1-pyrrolylcarbonyl)-2-methoxy-5-nitrophenoxy]propylpiperazino)[3-(4-methoxyphenyl)imidazo[1,5-a]pyridin-1-yl]methanone (12i)

This compound was prepared according to the method described for compound **12a**, employing compound **11a** (521 mg, 1 mmol) and compound **9c** (1 mmol) to obtain the pure product **12i** as a yellow solid. Yield 621 mg, 80%; mp 85-90 °C; ¹H NMR (CDCl₃, 300 MHz): δ 1.19-1.29 (m, 2H), 1.34 (q, 6H, *J* = 7.5 Hz), 1.73-2.35 (m, 8H), 2.62-2.87 (m, 10H), 3.14-3.32 (m, 2H), 3.88 (s, 3H), 3.93 (s, 3H), 4.18 (t, 2H, *J* = 5.8 Hz), 4.61-4.69 (m, 1H), 4.82 (d, 1H, *J* = 3.7 Hz), 6.68 (t, 1H, *J* = 6.6 Hz), 6.76 (s, 1H), 6.97 (dd, 1H, *J* = 6.6 Hz), 7.00 (d, 2H, *J* = 8.6 Hz), 7.25 (s, 1H), 7.63 (d, 2H, *J* = 6.9 Hz), 8.19 (d, 1H, *J* = 7.1 Hz), 8.31 (d, 1H, *J* = 9.0 Hz). ESIMS: *m/z* 777 (*M*⁺+1).

(4-4-[4-(2-[Di(ethylsulfanyl)methyl]tetrahydro-1H-1-pyrrolylcarbonyl)-2-methoxy-5-nitrophenoxy]butylpiperazino)[3-(4-methoxyphenyl)imidazo[1,5-a]pyridin-1-yl]methanone (12j)

This compound was prepared according to the method described for compound **12a**, employing compound **11b** (535 mg, 1 mmol) and compound **9c** (1 mmol) to obtain the pure product **12j** as a yellow solid. Yield 593 mg, 75%; mp 79-84 °C; ¹H NMR (CDCl₃, 300 MHz): δ 1.18-1.29 (m, 2H), 1.36 (q, 6H, *J* = 7.5 Hz), 1.69-2.34 (m, 10H), 2.49-2.88 (m, 10H), 3.13-3.31 (m, 2H), 3.88 (s, 3H), 3.93 (s, 3H), 4.11 (t, 2H, *J* = 6.0 Hz), 4.61-4.70 (m, 1H), 4.82 (d, 1H, *J* = 3.7 Hz), 6.68 (t, 1H, *J* = 7.0 Hz), 6.76 (s, 1H), 6.96 (dd, 1H, *J* = 9.8, 6.7 Hz), 7.01 (d, 2H, *J* = 9.0 Hz), 7.61 (s, 1H), 7.63 (d, 2H, *J* = 9.0 Hz), 8.19 (d, 1H, *J* = 6.7 Hz), 8.32 (d, 1H, *J* = 9.0 Hz). ESIMS: *m/z* 791 (*M*⁺+1).

(4-5-[4-(2-[Di(ethylsulfanyl)methyl]tetrahydro-1H-1-pyrrolylcarbonyl)-2-methoxy-5-nitrophenoxy]pentylpiperazino)[3-(4-methoxyphenyl)imidazo[1,5-a]pyridin-1-yl]methanone (12k)

This compound was prepared according to the method described for compound **12a**, employing compound **11c** (549 mg, 1 mmol) and compound **9c** (1 mmol) to obtain the pure product **12k** as a yellow solid. Yield 563 mg, 70%; mp 80-85 °C; ¹H NMR (CDCl₃, 300 MHz): δ 1.33 (q, 6H, *J* = 7.6 Hz), 1.48-2.35 (m, 12H), 2.47-2.87 (m, 10H), 3.14-3.31 (m, 2H), 3.88 (s, 3H), 3.93 (s, 3H), 4.08 (t, 2H, *J* = 6.0 Hz), 4.61-4.71 (m, 1H), 4.81 (d, 1H, *J* = 3.7 Hz), 6.68 (t, 1H, *J* = 7.1 Hz), 6.76 (s, 1H), 6.97 (dd, 1H, *J* = 6.7, 6.4 Hz), 7.01 (d, 2H, *J* = 8.6 Hz), 7.61-7.65 (m, 3H), 8.22 (d, 1H, *J* = 7.1 Hz), 8.32 (d, 1H, *J* = 9.2 Hz). ESIMS: *m/z* 805 (M⁺+1).

(4-6-[4-(2-[Di(ethylsulfanyl)methyl]tetrahydro-1H-1-pyrrolylcarbonyl)-2-methoxy-5-nitrophenoxy]hexylpiperazino)[3-(4-methoxyphenyl)imidazo[1,5-a]pyridin-1-yl] Methanone (12l)

This compound was prepared according to the method described for compound **12a**, employing compound **11d** (563 mg, 1 mmol) and compound **9c** (1 mmol) to obtain the pure product **12l** as a yellow solid. Yield 614 mg, 75%; mp 87-92 °C; ¹H NMR (CDCl₃, 300 MHz): δ 1.18-1.67 (m, 18H), 1.71-2.36 (m, 8H), 2.63-3.07 (m, 6H), 3.15-3.33 (m, 2H), 3.89 (s, 3H), 3.94 (s, 3H), 4.02-4.13 (m, 2H), 4.59-4.70 (m, 1H), 4.81 (d, 1H, *J* = 3.7 Hz), 6.69-6.79 (m, 2H), 7.02 (d, 3H, *J* = 8.3 Hz), 7.58-7.64 (m, 3H), 8.21 (d, 1H, *J* = 6.7 Hz), 8.28 (d, 1H, *J* = 9.0 Hz). ESIMS: *m/z* 819 (M⁺+1).

(11aS)-7-Methoxy-8-(3-4-[(3-phenylimidazo[1,5-a]pyridin-1-yl)carbonyl]piperazino propoxy)-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5-one (13a)

A solution of the nitro thioacetal **12a** (746 mg, 1mmol) and SnCl₂.2H₂O (1.12 g, 5 mmol) in MeOH (20 mL) was refluxed for 1.5 h or until the TLC (EtOAc/Hexane, 1:1) indicated that reaction was completed. The reaction mixture was cooled and adjusted to pH 8 (sat. aq. NaHCO₃) and then extracted with EtOAc (3 x 30 mL). The combined organic phase was washed with brine (1 x 15 mL), dried (Na₂SO₄) and the solvent removed by rotary evaporation *in vacuo* to afford the amino diethyl thioacetal and due to stability problems directly used in the next step. A solution of amino thioacetal (1 mmol), HgCl₂ (1.19 mg, 4.4 mmol), and CaCO₃ (480 mg, 4.8 mmol) in acetonitrile–water (4:1) was stirred slowly at r.t. for 24 hours until TLC (EtOAc) indicates complete loss of starting material. The reaction mixture was diluted with ethyl acetate (30 mL) and filtered through celite. The clear yellow organic supernatant was extracted with ethyl acetate (2 X 20 mL). The organic layer was washed with sat. aq. NaHCO₃ (2 x 20 mL), brine (2 x 20 mL) and the combined organic phase was dried (Na₂SO₄). The organic layer was evaporated under vacuum and the crude product was purified by column chromatography (2% MeOH–CHCl₃) to afford the

compound **13a** as a white solid; (355 mg, 60% yield). mp 110–115 °C; $[\alpha]_D^{27} +326.6^\circ$ ($c = 0.5$, CHCl₃); ¹H NMR (CDCl₃, 300 MHz): δ 1.54–1.70 (m, 6H), 2.00–2.12 (m, 2H), 2.26–2.34 (m, 2H), 2.53–2.63 (m, 6H), 3.51–3.61 (m, 1H), 3.66–3.73 (m, 1H), 3.76–3.85 (m, 1H), 3.94 (s, 3H), 4.08–4.21 (m, 2H), 6.69 (t, 1H, $J = 7.5$ Hz), 6.79 (s, 1H), 6.97 (dd, 1H, $J = 6.7$, 6.5 Hz), 7.40–7.55 (m, 4H), 7.60 (d, 1H, $J = 4.5$ Hz), 7.74 (dd, 2H, $J = 6.7$, 6.3 Hz), 8.25–8.35 (m, 2H); ¹³C NMR (CDCl₃, 150 MHz): δ 24.1, 26.1, 29.5, 46.5, 53.4, 53.6, 54.8, 56.0, 67.1, 110.4, 111.4, 114.2, 120.9, 121.6, 122.5, 125.2, 128.3, 129.0, 129.1, 140.5, 147.6, 162.4, 164.5; IR (KBr) (Umax/cm⁻¹): ν 3382 (br), 2927, 1599, 1508, 1433, 1378, 1238, 1126, 1075, 1006, 868, 774, 693; ESIMS: m/z 593 ($M^+ + 1$); HRMS (ESI m/z) for C₃₄H₃₇N₆O₄ calcd 593.2876, found 593.2887 ($M + 1$)⁺; purity 97%, C18 column, acetonitrile-water (mobile phase), wavelength 289 nm; white solid.

(11aS)-7-Methoxy-8-(4-4-[(3-phenylimidazo[1,5-a]pyridin-1-yl)carbonyl]piperazino butoxy)-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5-one (13b)

The compound **13b** was prepared according to the method described for the compound **13a**, employing the compound **12b** (761 mg, 1.0 mmol). The crude product was purified by column chromatography (MeOH/CHCl₃, 2%) to afford the compound **13b** as a white solid (363 mg, 60% yield). mp 65–70 °C; $[\alpha]_D^{27} +270.4^\circ$ ($c = 0.5$, CHCl₃); ¹H NMR (CDCl₃, 500MHz): δ 1.68–1.78 (m, 2H), 1.85–1.96 (m, 2H), 1.99–2.10 (m, 4H), 2.25–2.36 (m, 2H), 2.52 (t, 2H, $J = 7.8$ Hz), 2.64 (t, 4H, $J = 4.9$ Hz), 2.72–3.00 (brs, 4H), 3.54–3.62 (m, 1H), 3.7–3.74 (m, 1H), 3.78–3.84 (m, 1H), 3.93 (s, 3H), 4.03–4.16 (m, 2H), 6.71 (t, 1H, $J = 7.8$ Hz), 6.79 (s, 1H), 6.97–7.01 (m, 1H), 7.44–7.54 (m, 4H), 7.64 (d, 1H, $J = 3.9$ Hz), 7.74 (d, 2H, $J = 7.8$ Hz), 8.28 (t, 2H, $J = 7.8$ Hz); ¹³C NMR (CDCl₃, 150 MHz): δ 21.4, 22.5, 24.1, 26.7, 29.5, 46.6, 53.0, 53.6, 56.0, 57.6, 68.5, 110.3, 111.4, 114.3, 120.0, 120.9, 121.7, 122.7, 128.3, 129.0, 129.2, 135.4, 140.3, 147.7, 150.6, 162.4, 163.3; IR (KBr) (Umax/cm⁻¹): ν 3419 (br), 2923, 1631, 1433, 1378, 1165, 1116, 1061, 609; ESIMS: m/z 607 ($M^+ + 1$); HRMS (ESI m/z) for C₃₅H₃₉N₆O₄ calcd 607.3032, found 607.3011 ($M + 1$)⁺; purity 96%, C18 column, acetonitrile-water (mobile phase), wavelength 289 nm; white solid.

(11aS)-7-Methoxy-8-[(5-4-[(3-phenylimidazo[1,5-a]pyridin-1-yl)carbonyl] piperazino pentyl)oxy]-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5-one (13c)

The compound **13c** was prepared according to the method described for the compound **13a**, employing the compound **12c** (774 mg, 1.0 mmol). The crude product was purified by column chromatography (MeOH/CHCl₃, 2%) to afford the compound **13c** as a white solid (372 mg, 60% yield). mp 86–90 °C; $[\alpha]_D^{27} +276.6^\circ$ ($c = 0.5$, CHCl₃); ¹H NMR (CDCl₃, 500

MHz): δ 1.50-1.71 (m, 8H), 1.86-1.94 (m, 2H), 2.00-2.10 (m, 2H), 2.27-2.35 (m, 2H), 2.41 (t, 2H, $J = 7.7$ Hz), 2.52-2.61 (brs, 6H), 3.53-3.59 (m, 1H), 3.67-3.72 (m, 1H), 3.76-3.83 (m, 1H), 3.93 (s, 3H), 3.99-4.11 (m, 2H), 4.13-4.24 (brs, 2H), 6.69 (t, 1H, $J = 6.7$ Hz), 6.73 (s, 1H), 6.97 (t, 1H, $J = 7.7$ Hz), 7.43-7.46 (m, 2H), 7.52 (t, 2H, $J = 7.7$ Hz), 7.60 (d, 1H, $J = 3.8$ Hz), 7.74 (d, 2H, $J = 7.7$ Hz), 8.27 (d, 1H, $J = 7.2$ Hz), 8.32 (d, 1H, $J = 7.4$ Hz); ^{13}C NMR (CDCl_3 , 150 MHz): δ 23.9, 24.2, 26.3, 28.7, 29.6, 46.6, 53.5, 53.7, 56.1, 56.2, 58.5, 68.8, 110.3, 111.5, 114.3, 120.1, 120.9, 121.7, 122.6, 125.2, 125.8, 128.4, 129.1, 129.2, 129.6, 132.7, 140.5, 140.6, 147.7, 150.8, 162.5, 163.4; IR (KBr) ($\text{Umax}/\text{cm}^{-1}$): ν 3331 (br), 2931, 1599, 1507, 1467, 1429, 1367, 1315, 1237, 1126, 1074, 1007, 868, 774, 748, 692; ESIMS: m/z 621 ($\text{M}^+ + 1$); HRMS (ESI m/z) for $\text{C}_{36}\text{H}_{41}\text{N}_6\text{O}_4$ calcd 621.3189, found 621.3187 ($\text{M} + 1$) $^+$; purity 99%, C18 column, acetonitrile-water (mobile phase), wavelength 289 nm; white solid.

(11aS)-7-Methoxy-8-[(6-4-[(3-phenylimidazo[1,5-a]pyridin-1-yl)carbonyl]piperazino-hexyl)oxy]-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5-one (13d)

The compound **13d** was prepared according to the method described for the compound **13a**, employing the compound **12d** (788 mg, 1.0 mmol). The crude product was purified by column chromatography ($\text{MeOH}/\text{CHCl}_3$, 2%) to afford the compound **13d** as a white solid (348 mg, 55% yield). mp 78–83 °C; $[\alpha]_{\text{D}}^{27} +253.7^\circ$ ($c = 0.5$, CHCl_3); ^1H NMR (CDCl_3 , 300 MHz): δ 1.55-1.77 (m, 8H), 1.81-1.95 (m, 4H), 1.99-2.11 (m, 4H), 2.25-2.43 (t, 2H, $J = 7.1$ Hz), 2.49-2.53 (brs, 4H), 3.48-3.56 (m, 1H), 3.64-3.71 (m, 1H), 3.72-3.84 (m, 1H), 3.93 (s, 3H), 3.97-4.13 (m, 4H), 6.67-6.76 (m, 2H), 6.98 (dd, 1H, $J = 6.6, 6.4$ Hz), 7.41-7.57 (m, 4H), 7.61 (d, 1H, $J = 4.1$ Hz), 7.73 (d, 2H, $J = 6.9$ Hz), 8.32 (dd, 2H, $J = 8.1, 6.8$ Hz); ^{13}C NMR (CDCl_3 , 150 MHz): δ 24.1, 25.7, 26.4, 27.1, 28.7, 29.5, 46.5, 53.5, 53.6, 56.0, 58.5, 68.8, 110.2, 111.4, 114.2, 119.9, 120.9, 121.6, 122.5, 125.1, 128.3, 129.0, 129.1, 129.5, 135.2, 140.4, 147.7, 150.7, 162.3, 163.4; IR (KBr) ($\text{Umax}/\text{cm}^{-1}$): ν 3351 (br), 2931, 1599, 1507, 1466, 1429, 1372, 1312, 1235, 1126, 1075, 1005, 868, 772, 693; ESIMS: m/z 635 ($\text{M}^+ + 1$); HRMS (ESI m/z) for $\text{C}_{37}\text{H}_{43}\text{N}_6\text{O}_4$ calcd 635.3345, found 635.3317 ($\text{M} + 1$) $^+$; purity 95%, C18 column, acetonitrile-water (mobile phase), wavelength 289 nm; white solid.

(11aS)-7-Methoxy-8-3-[4-(3-[4-(trifluoromethyl)phenyl]imidazo[1,5-a]pyridin-1-ylcarbonyl)piperazino]propoxy-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5-one (13e)

The compound **13e** was prepared according to the method described for the compound **13a**, employing the compound **12e** (815 mg, 1.0 mmol). The crude product was purified by column chromatography ($\text{MeOH}/\text{CHCl}_3$, 2%) to afford the compound **13e** as a white solid (343 mg, 52% yield). mp 115–120 °C; $[\alpha]_{\text{D}}^{27} +246.9^\circ$ ($c = 0.5$, CHCl_3); ^1H NMR (CDCl_3 , 300

MHz): δ 1.47-1.63 (m, 6H), 2.02-2.11 (m, 4H), 2.26-2.34 (m, 2H), 2.59 (t, 6H, 4.7 Hz), 3.52-3.63 (m, 1H), 3.67-3.73 (m, 1H), 3.75-3.84 (m, 1H), 3.93 (s, 3H), 4.09-4.20 (m, 2H), 6.74-6.82 (m, 2H), 7.02 (dd, 1H, J = 6.7, 6.4 Hz), 7.43 (s, 1H), 7.62 (d, 1H, J = 4.5 Hz), 7.75 (d, 2H, J = 8.3 Hz), 7.92 (d, 2H, J = 7.5 Hz), 8.26 (d, 1H, J = 7.5 Hz), 8.33 (d, 1H, J = 8.0 Hz); ^{13}C NMR (CDCl_3 , 150 MHz): δ 24.1, 26.2, 29.5, 46.6, 53.4, 53.6, 54.8, 56.0, 67.2, 110.5, 111.4, 114.9, 120.1, 121.1, 121.4, 123.0, 125.9, 126.0, 126.1, 128.4, 135.6, 147.7, 150.6, 162.4, 163.1; IR (KBr) ($\text{Umax}/\text{cm}^{-1}$): ν 3384 (br), 2952, 1601, 1534, 1510, 1462, 1432, 1381, 1324, 1240, 1201, 1167, 1125, 1066, 1010, 850, 775, 743, 690, 608; ESIMS: m/z 661 ($\text{M}^+ + 1$); HRMS (ESI m/z) for $\text{C}_{35}\text{H}_{36}\text{F}_3\text{N}_6\text{O}_4$ calcd 661.2750, found 661.2751 ($\text{M} + 1$) $^+$; purity 98%, C18 column, acetonitrile-water (mobile phase), wavelength 289 nm; white solid.

(11aS)-7-Methoxy-8-4-[4-(3-[4-(trifluoromethyl)phenyl]imidazo[1,5-a]pyridin-1-yl carbonyl)piperazino]butoxy-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5-one (13f)

The compound **13f** was prepared according to the method described for the compound **13a**, employing the compound **12f** (829 mg, 1.0 mmol). The crude product was purified by column chromatography ($\text{MeOH}/\text{CHCl}_3$, 2%) to afford the compound **13f** as a white solid (377 mg, 56% yield). mp 119–124 °C; $[\alpha]_{\text{D}}^{27} +253.3^\circ$ (c = 0.5, CHCl_3); ^1H NMR (CDCl_3 , 300 MHz): δ 1.66-1.79 (m, 2H), 1.84-2.18 (brs, 2H), 1.88-1.96 (m, 2H), 2.00-2.10 (m, 2H), 2.25-2.34 (m, 2H), 2.46 (t, 2H, J = 7.5 Hz), 2.56 (t, 4H, J = 4.5 Hz), 3.50-3.62 (m, 1H), 3.65-3.72 (m, 1H), 3.74-3.83 (m, 1H), 3.93 (s, 3H), 4.03-4.16 (m, 2H), 4.24-4.54 (brs, 2H), 6.74-6.81 (m, 2H), 7.03 (dd, 1H, J = 6.7, 6.5 Hz), 7.44 (s, 1H), 7.61 (d, 1H, J = 4.5 Hz), 7.77 (d, 2H, J = 8.3 Hz), 7.90 (d, 2H, J = 7.5 Hz), 8.28 (d, 1H, J = 7.5 Hz), 8.35 (d, 1H, J = 8.0 Hz); ^{13}C NMR (CDCl_3 , 150 MHz): δ 23.1, 24.1, 26.7, 29.5, 46.6, 53.4, 53.6, 56.0, 57.9, 68.6, 110.3, 111.4, 114.9, 120.0, 121.2, 121.4, 122.9, 125.9, 126.0, 128.3, 134.8, 135.7, 140.5, 147.6, 150.7, 162.3, 164.6; IR (KBr) ($\text{Umax}/\text{cm}^{-1}$): ν 3378 (br), 2932, 1602, 1509, 1433, 1375, 1323, 1239, 1168, 1123, 1067, 1009, 851, 775, 741, 689, 607; ESIMS: m/z 675 ($\text{M}^+ + 1$); HRMS (ESI m/z) for $\text{C}_{36}\text{H}_{38}\text{F}_3\text{N}_6\text{O}_4$ calcd 675.2906, found 675.2901 ($\text{M} + 1$) $^+$; purity 97%, C18 column, acetonitrile-water (mobile phase), wavelength 289 nm; white solid.

(11aS)-7-Methoxy-8-(5-[4-(3-[4-(trifluoromethyl)phenyl]imidazo[1,5-a]pyridin-1-yl carbonyl)piperazino]pentyl)-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5-one (13g)

The compound **13g** was prepared according to the method described for the compound **13a**, employing the compound **12g** (843 mg, 1.0 mmol). The crude product was purified by column chromatography ($\text{MeOH}/\text{CHCl}_3$, 2%) to afford the compound **13g** as a white solid

(378 mg, 55% yield). mp 120–125 °C; $[\alpha]_{\text{D}}^{27} +194.0^\circ$ ($c = 0.5$, CHCl_3); ^1H NMR (CDCl_3 , 500 MHz): δ 1.48–1.64 (m, 6H), 1.85–1.95 (m, 2H), 1.98–2.10 (m, 2H), 2.24–2.34 (m, 2H), 2.40 (t, 2H, $J = 7.4$ Hz), 2.47–2.60 (brs, 4H), 3.50–3.59 (m, 1H), 3.65–3.71 (m, 1H), 3.73–3.86 (m, 1H), 3.91 (s, 3H), 3.97–4.11 (m, 2H), 4.36–4.59 (brs, 2H), 6.65–6.83 (m, 2H), 7.01 (s, 1H), 7.43 (s, 1H), 7.59 (s, 1H), 7.77 (d, 2H, $J = 7.4$ Hz), 7.90 (d, 2H, $J = 7.4$ Hz), 8.28 (d, 1H, $J = 6.5$ Hz), 8.34 (d, 1H, $J = 9.3$ Hz); ^{13}C NMR (CDCl_3 , 150 MHz): δ 23.8, 24.1, 26.4, 28.7, 29.5, 46.6, 53.6, 56.1, 58.3, 68.7, 110.3, 111.4, 114.9, 120.0, 121.2, 121.3, 122.9, 125.9, 126.0, 128.3, 133.1, 135.7, 140.5, 147.7, 150.8, 162.4, 164.6; IR (KBr) ($\text{Umax}/\text{cm}^{-1}$): ν 3384 (br), 2928, 1601, 1534, 1510, 1462, 1432, 1366, 1324, 1261, 1239, 1166, 1125, 1066, 1011, 850, 774, 742, 691, 605; ESIMS: m/z 690 ($\text{M}^+ + 1$); HRMS (ESI m/z) for $\text{C}_{37}\text{H}_{40}\text{F}_3\text{N}_6\text{O}_4$ calcd 689.3063, found 689.3076 (M^+); purity 99%, C18 column, acetonitrile-water (mobile phase), wavelength 289 nm; white solid.

(11aS)-7-Methoxy-8-(6-[4-(3-[4-(trifluoromethyl)phenyl]imidazo[1,5-a]pyridin-1-yl carbonyl)piperazino]hexyloxy)-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4] diazepin-5-one (13h)

The compound **13h** was prepared according to the method described for the compound **13a**, employing the compound **12h** (857 mg, 1.0 mmol). The crude product was purified by column chromatography ($\text{MeOH}/\text{CHCl}_3$, 2%) to afford the compound **13h** as a white solid (386 mg, 55% yield). mp 75–80 °C; $[\alpha]_{\text{D}}^{27} +212.5^\circ$ ($c = 0.5$, CHCl_3); ^1H NMR (CDCl_3 , 300 MHz): δ 1.45–1.62 (m, 10H), 1.83–1.93 (m, 2H), 2.01–2.10 (m, 2H), 2.26–2.34 (m, 2H), 2.39 (t, 2H, $J = 6.9$ Hz), 2.55 (t, 4H, $J = 4.7$ Hz), 3.51–3.61 (m, 1H), 3.67–3.72 (m, 1H), 3.75–3.84 (m, 1H), 3.93 (s, 3H), 3.98–4.12 (m, 2H), 4.13–4.28 (brs, 2H), 6.71–6.80 (m, 2H), 7.03 (dd, 1H, $J = 6.6, 6.4$ Hz), 7.43 (s, 1H), 7.60 (d, 1H, $J = 4.3$ Hz), 7.79 (d, 2H, $J = 8.3$ Hz), 7.91 (d, 2H, $J = 8.1$ Hz), 8.29 (d, 1H, $J = 7.1$ Hz), 8.37 (d, 1H, $J = 9.2$ Hz); ^{13}C NMR (CDCl_3 , 150 MHz): δ 24.2, 25.8, 26.6, 27.2, 28.8, 29.6, 46.6, 53.5, 53.7, 56.1, 58.6, 68.9, 110.4, 111.5, 115.0, 120.0, 121.3, 121.4, 123.0, 126.0, 126.1, 128.4, 133.1, 134.9, 135.7, 140.5, 147.8, 150.8, 162.4, 164.6; IR (KBr) ($\text{Umax}/\text{cm}^{-1}$): ν 3343 (br), 2933, 1601, 1534, 1509, 1454, 1431, 1379, 1323, 1237, 1167, 1125, 1066, 1008, 849, 774, 738, 689, 607; ESIMS: m/z 704 ($\text{M}^+ + 1$); HRMS (ESI m/z) for $\text{C}_{38}\text{H}_{42}\text{F}_3\text{N}_6\text{O}_4$ calcd 703.3219, found 703.3218 ($\text{M}^+ + 1$); purity 96%, C18 column, acetonitrile-water (mobile phase), wavelength 289 nm; white solid.

(11aS)-7-Methoxy-8-[3-(4-[3-(4-methoxyphenyl)imidazo[1,5-a]pyridin-1-yl]carbonyl piperazino)propoxy]-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5-one (13i)

The compound **13i** was prepared according to the method described for the compound **13a**, employing the compound **12i** (776 mg, 1.0 mmol). The crude product was purified by column chromatography (MeOH/CHCl₃, 2%) to afford the compound **13i** as a white solid (311 mg, 50% yield). mp 73–78 °C; [α]_D²⁷ +208.6° (*c* = 0.5, CHCl₃); ¹H NMR (CDCl₃, 300 MHz): δ 1.64–1.88 (m, 4H), 1.99–2.13 (m, 4H), 2.26–2.36 (m, 2H), 2.52–2.64 (m, 6H), 3.52–3.62 (m, 1H), 3.66–3.73 (m, 1H), 3.75–3.84 (m, 1H), 3.89 (s, 3H), 3.94 (s, 3H), 4.06–4.28 (m, 4H), 6.66 (t, 1H, *J* = 6.6 Hz), 6.80 (s, 1H), 6.95 (dd, 1H, *J* = 6.9, 6.7 Hz), 7.00 (d, 2H, *J* = 8.4 Hz), 7.44 (s, 1H), 7.62 (d, 1H, *J* = 4.3 Hz), 7.67 (d, 2H, *J* = 8.4 Hz), 8.19 (d, 1H, *J* = 6.9 Hz), 8.28 (d, 1H, *J* = 9.2 Hz); ¹³C NMR (CDCl₃, 150 MHz): δ 24.1, 26.1, 29.5, 46.5, 53.4, 53.6, 54.8, 55.3, 56.0, 67.1, 110.4, 111.5, 114.0, 114.4, 120.0, 120.8, 121.6, 121.8, 122.3, 129.8, 135.0, 140.5, 147.6, 162.3, 163.5; IR (KBr) (U_{max}/cm⁻¹): ν 3393 (br), 2928, 1604, 1508, 1460, 1380, 1250, 1178, 1122, 1008, 837, 749, 689; ESIMS: *m/z* 623 (M⁺+1); HRMS (ESI *m/z*) for C₃₅H₃₉N₆O₅ calcd 623.2981, found 623.2979 (M+1)⁺; purity 91%, C18 column, acetonitrile-water (mobile phase), wavelength 289 nm; white solid.

(11aS)-7-Methoxy-8-[4-(4-[3-(4-methoxyphenyl)imidazo[1,5-a]pyridin-1-yl]carbonyl piperazino)butoxy]-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5-one (13j)

The compound **13j** was prepared according to the method described for the compound **13a**, employing the compound **12j** (790 mg, 1.0 mmol). The crude product was purified by column chromatography (MeOH/CHCl₃, 2%) to afford the compound **13j** as a white solid (318 mg, 50% yield). mp 75–80 °C; [α]_D²⁷ +216.0° (*c* = 0.5, CHCl₃); ¹H NMR (CDCl₃, 300 MHz): δ 1.66–1.83 (6H), 1.88–1.97 (m, 2H), 2.00–2.10 (m, 2H), 2.25–2.33 (m, 2H), 2.46 (t, 2H, *J* = 7.1 Hz), 2.56 (t, 4H, *J* = 4.5 Hz), 3.50–3.61 (m, 1H), 3.66–3.72 (m, 1H), 3.75–3.84 (m, 1H), 3.88 (s, 3H), 3.93 (s, 3H), 4.02–4.23 (m, 4H), 6.66 (t, 1H, *J* = 6.4 Hz), 6.76 (s, 1H), 6.94 (dd, 1H, *J* = 6.4, 6.2 Hz), 7.03 (d, 2H, *J* = 8.8 Hz), 7.44 (s, 1H), 7.61 (d, 1H, *J* = 4.3 Hz), 7.64 (d, 2H, *J* = 8.6 Hz), 8.18 (d, 1H, *J* = 7.1 Hz), 8.28 (d, 1H, *J* = 9.2 Hz); ¹³C NMR (CDCl₃, 150 MHz): δ 24.1, 26.2, 29.6, 46.4, 53.4, 53.6, 54.1, 54.8, 55.2, 56.1, 67.4, 110.3, 111.5, 114.2, 114.6, 120.1, 120.8, 121.6, 121.7, 122.4, 129.8, 135.1, 136.7, 140.5, 144.6, 146.5, 149.8, 162.4, 164.6; IR (KBr) (U_{max}/cm⁻¹): ν 3355 (br), 2932, 1603, 1534, 1508, 1458, 1430, 1380, 1311, 1250, 1124, 1073, 1006, 868, 838, 751, 689; ESIMS: *m/z* 637 (M⁺+1); HRMS (ESI *m/z*) for C₃₆H₄₁N₆O₅ calcd 637.3138, found 637.3113 (M+1)⁺; purity 95%, C18 column, acetonitrile-water (mobile phase), wavelength 289 nm; white solid.

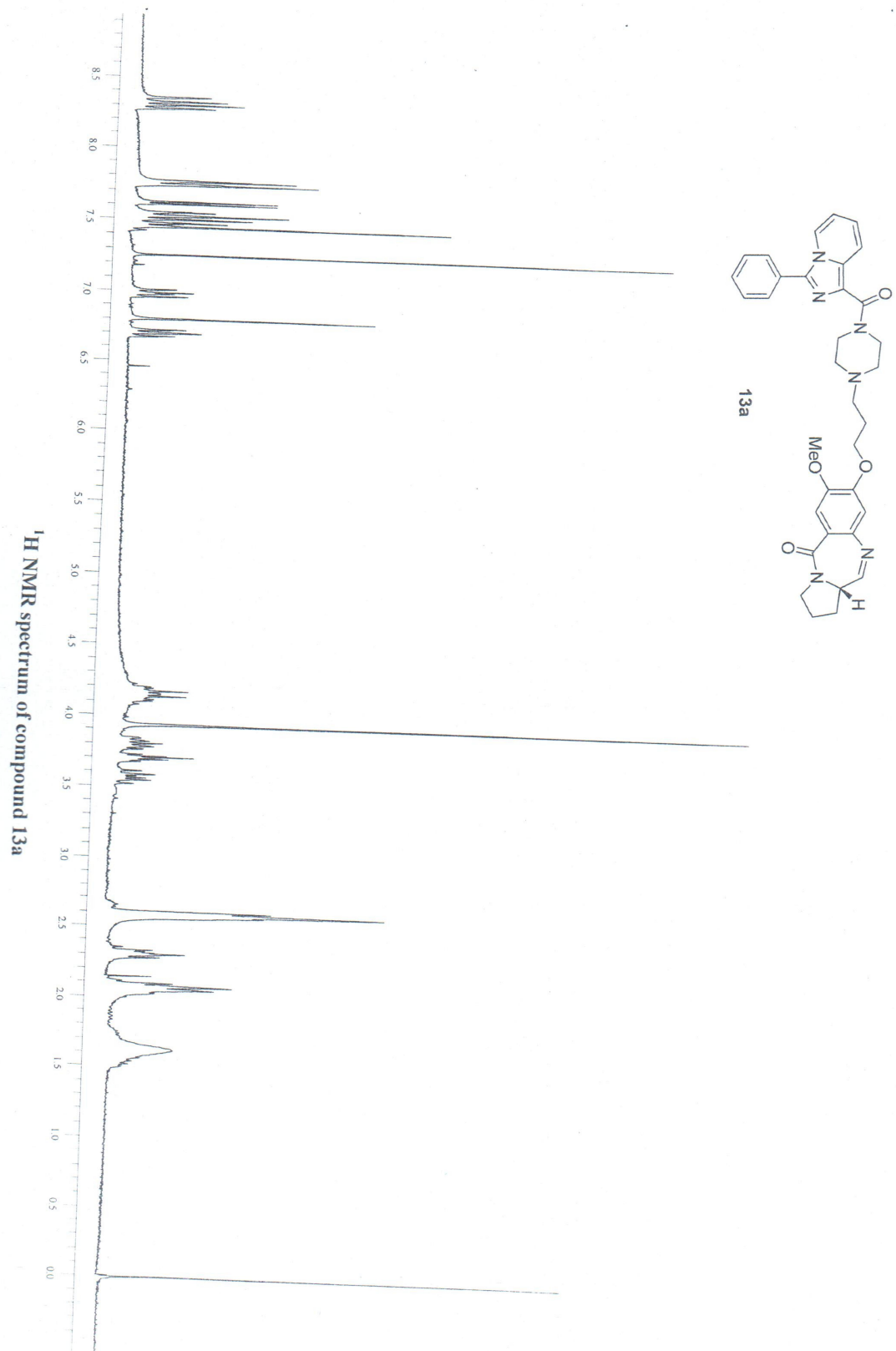
(11aS)-7-Methoxy-8-[5-(4-[3-(4-methoxyphenyl)imidazo[1,5-a]pyridin-1-yl]carbonyl piperazino)pentyl]oxy-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5-one (13k)

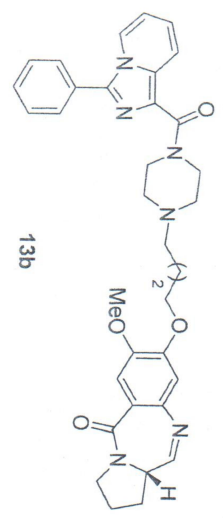
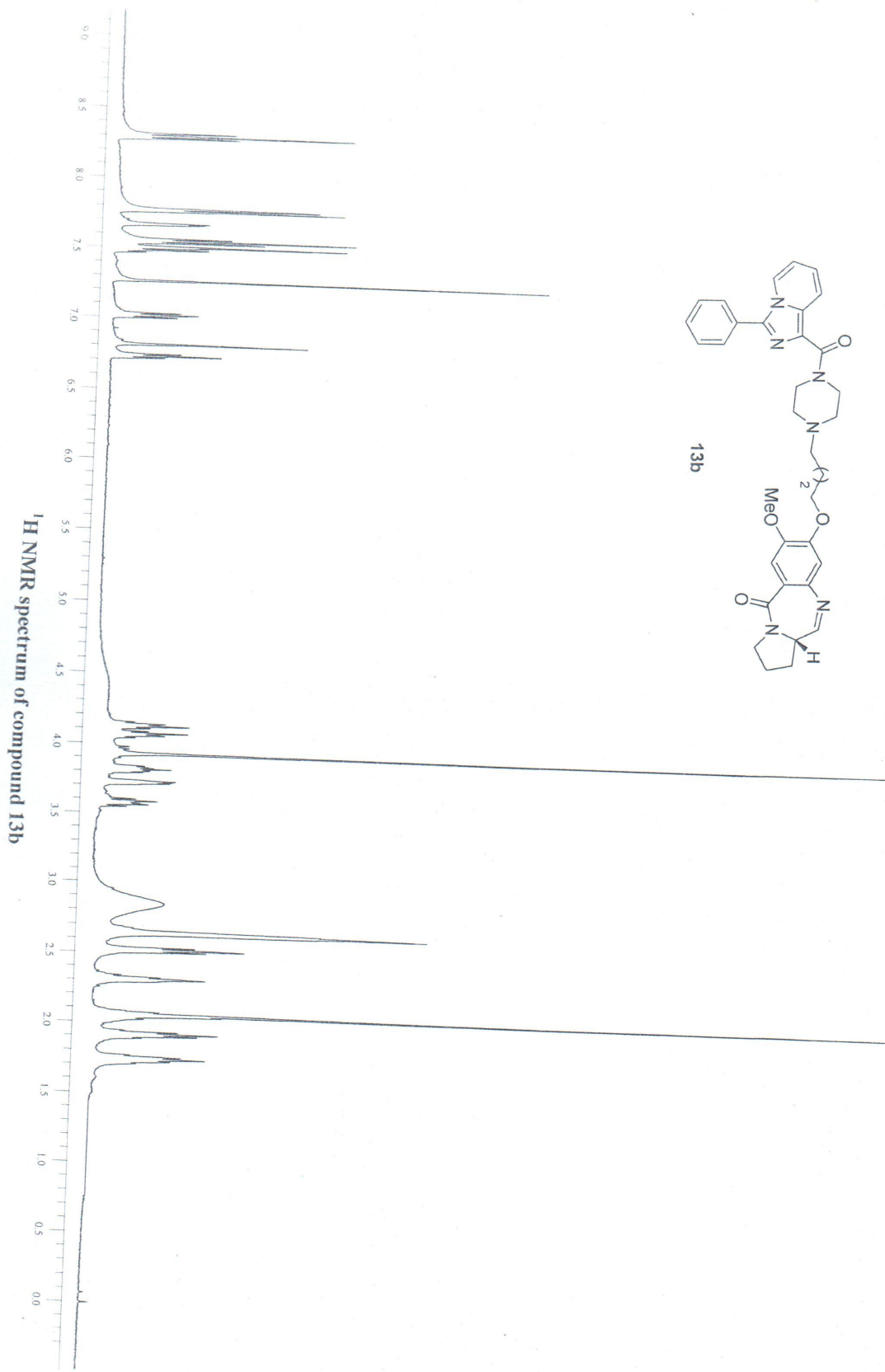
The compound **13k** was prepared according to the method described for the compound **13a**, employing the compound **12k** (804 mg, 1.0 mmol). The crude product was purified by column chromatography (MeOH/CHCl₃, 2%) to afford the compound **13k** as a white solid (377 mg, 58% yield). mp 78–83 °C; [α]_D²⁷ +269.9° (*c* = 0.5, CHCl₃); ¹H NMR (CDCl₃, 300 MHz): δ 1.50-1.72 (m, 8H), 1.84-1.95 (m, 2H), 2.00-2.10 (m, 2H), 2.27-2.35 (m, 2H), 2.41 (t, 2H, *J* = 6.8 Hz), 2.55 (t, 4H, *J* = 4.5 Hz), 3.52-3.61 (m, 1H), 3.66-3.73 (m, 1H), 3.76-3.85 (m, 1H), 3.89 (s, 3H), 3.93 (s, 3H), 4.00-4.12 (m, 2H), 4.36-4.66 (brs, 2H), 6.68 (t, 1H, *J* = 6.2 Hz), 6.76 (s, 1H), 6.97 (dd, 1H, *J* = 6.7, 6.4 Hz), 7.01 (d, 2H, *J* = 8.6 Hz), 7.57 (d, 1H, *J* = 4.2 Hz), 7.61-7.65 (m, 3H), 8.22 (d, 1H, *J* = 7.1 Hz), 8.32 (d, 1H, *J* = 9.2 Hz); ¹³C NMR (CDCl₃, 150 MHz): δ 23.8, 24.1, 26.2, 28.6, 29.5, 46.5, 53.4, 53.6, 55.3, 56.0, 58.3, 68.7, 110.2, 111.4, 114.0, 114.4, 119.9, 120.8, 121.6, 121.8, 122.3, 129.8, 135.0, 136.4, 140.4, 147.7, 150.7, 162.3, 163.5; IR (KBr) (U_{max}/cm⁻¹): ν 3421 (br), 2934, 1603, 1534, 1508, 1460, 1432, 1372, 1312, 1251, 1127, 1072, 1008, 869, 839, 772, 689; ESIMS: *m/z* 651 (M⁺+1); HRMS (ESI *m/z*) for C₃₇H₄₃N₆O₅ calcd 651.3294, found 651.3284 (M+1)⁺; purity 95%, C18 column, acetonitrile-water (mobile phase), wavelength 289 nm; white solid.

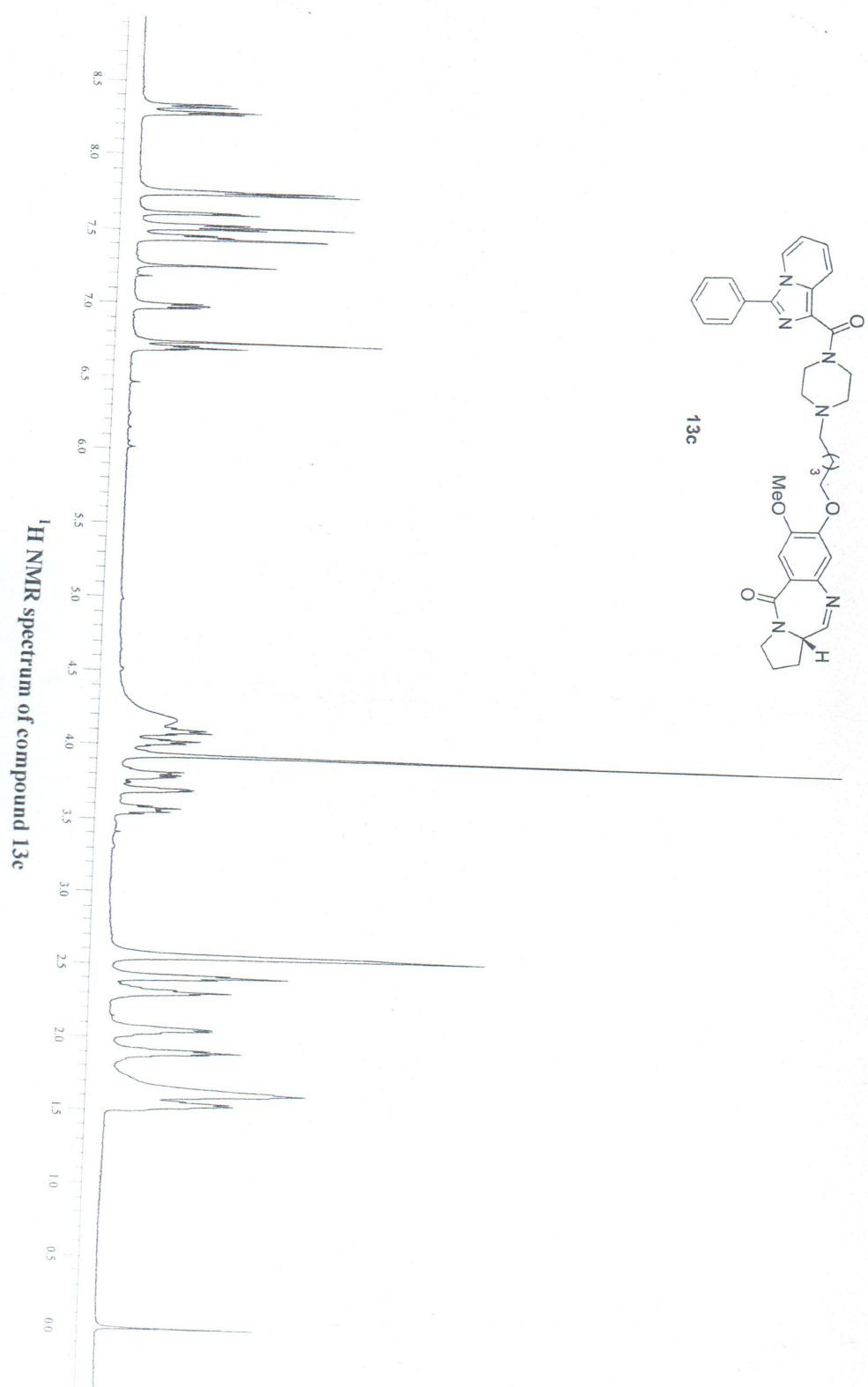
(11aS)-7-Methoxy-8-[6-(4-[3-(4-methoxyphenyl)imidazo[1,5-a]pyridin-1-yl]carbonyl piperazino)hexyl]oxy-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-5-one (13l)

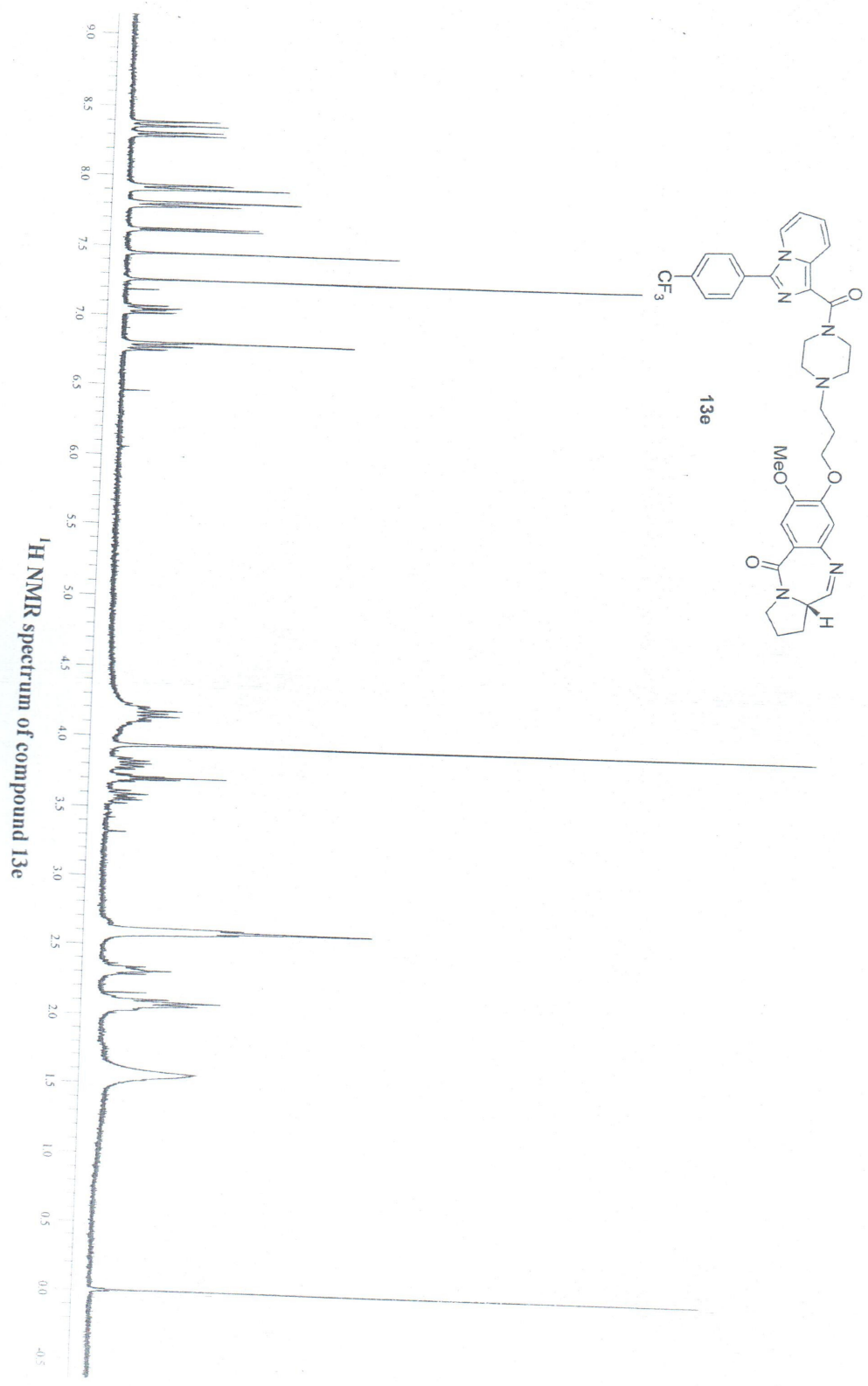
The compound **13l** was prepared according to the method described for the compound **13a**, employing the compound **12l** (818 mg, 1.0 mmol). The crude product was purified by column chromatography (MeOH/CHCl₃, 2%) to afford the compound **13l** as a white solid (365 mg, 55% yield). mp 80–85 °C; [α]_D²⁷ +204.8° (*c* = 0.5, CHCl₃); ¹H NMR (CDCl₃, 300 MHz): δ 1.40-1.59 (m, 8H), 1.72-1.91 (m, 4H), 2.00-2.10 (m, 2H), 2.26-2.33 (m, 2H), 2.37 (t, 2H, *J* = 6.6 Hz), 2.53 (t, 4H, *J* = 4.7 Hz), 3.51-3.63 (m, 1H), 3.66-3.73 (m, 1H), 3.74-3.84 (m, 1H), 3.88 (s, 3H), 3.93 (s, 3H), 3.98-4.12 (m, 2H), 4.13-4.27 (brs, 2H), 6.66 (t, 1H, *J* = 6.2 Hz), 6.73 (s, 1H), 6.94 (dd, 1H, *J* = 6.4, 5.4 Hz), 7.01 (d, 2H, *J* = 9.0 Hz), 7.43 (s, 1H), 7.61 (d, 1H, *J* = 4.3 Hz), 7.65 (d, 2H, *J* = 8.8 Hz), 8.20 (d, 1H, *J* = 7.1 Hz), 8.30 (d, 1H, *J* = 9.2 Hz); ¹³C NMR (CDCl₃, 150 MHz): δ 24.1, 25.7, 26.5, 27.1, 28.7, 29.5, 46.5, 53.4, 53.6, 55.3, 56.0, 58.5, 68.8, 110.2, 111.4, 114.0, 114.4, 120.9, 121.6, 121.9, 122.3, 124.9, 129.8, 135.0, 136.5, 140.5, 147.7, 150.7, 162.3, 163.4; IR (KBr) (U_{max}/cm⁻¹): ν 3414 (br), 2933, 1604, 1534, 1509, 1460, 1431, 1311, 1251, 1178, 1127, 1006, 869, 838, 773, 690; ESIMS: *m/z* 666

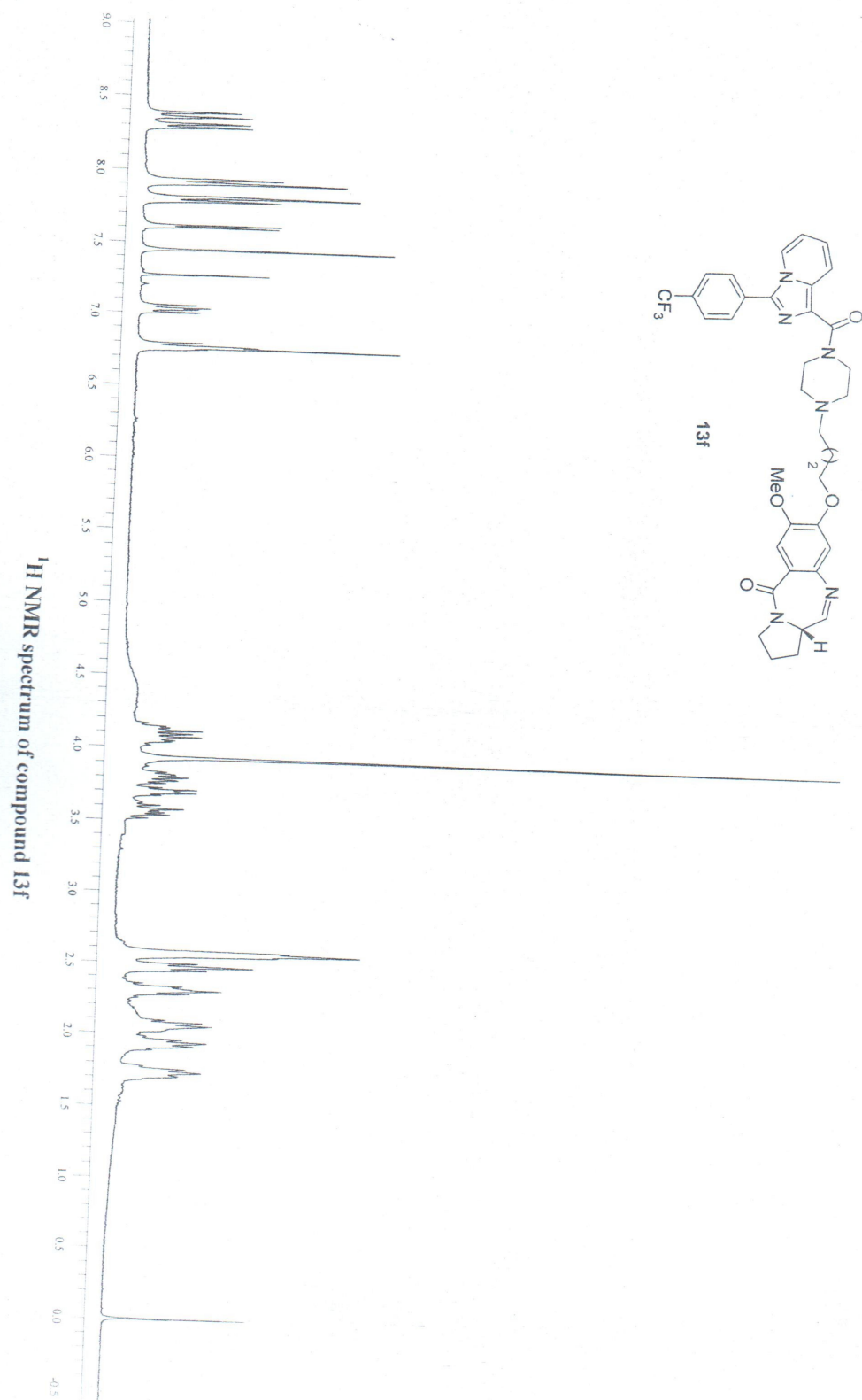
($M^+ + 1$); HRMS (ESI m/z) for $C_{38}H_{45}N_6O_5$ calcd 665.3451, found 665.3483 (M)⁺; purity 96%,
C18 column, acetonitrile-water (mobile phase), wavelength 289 nm; white solid.

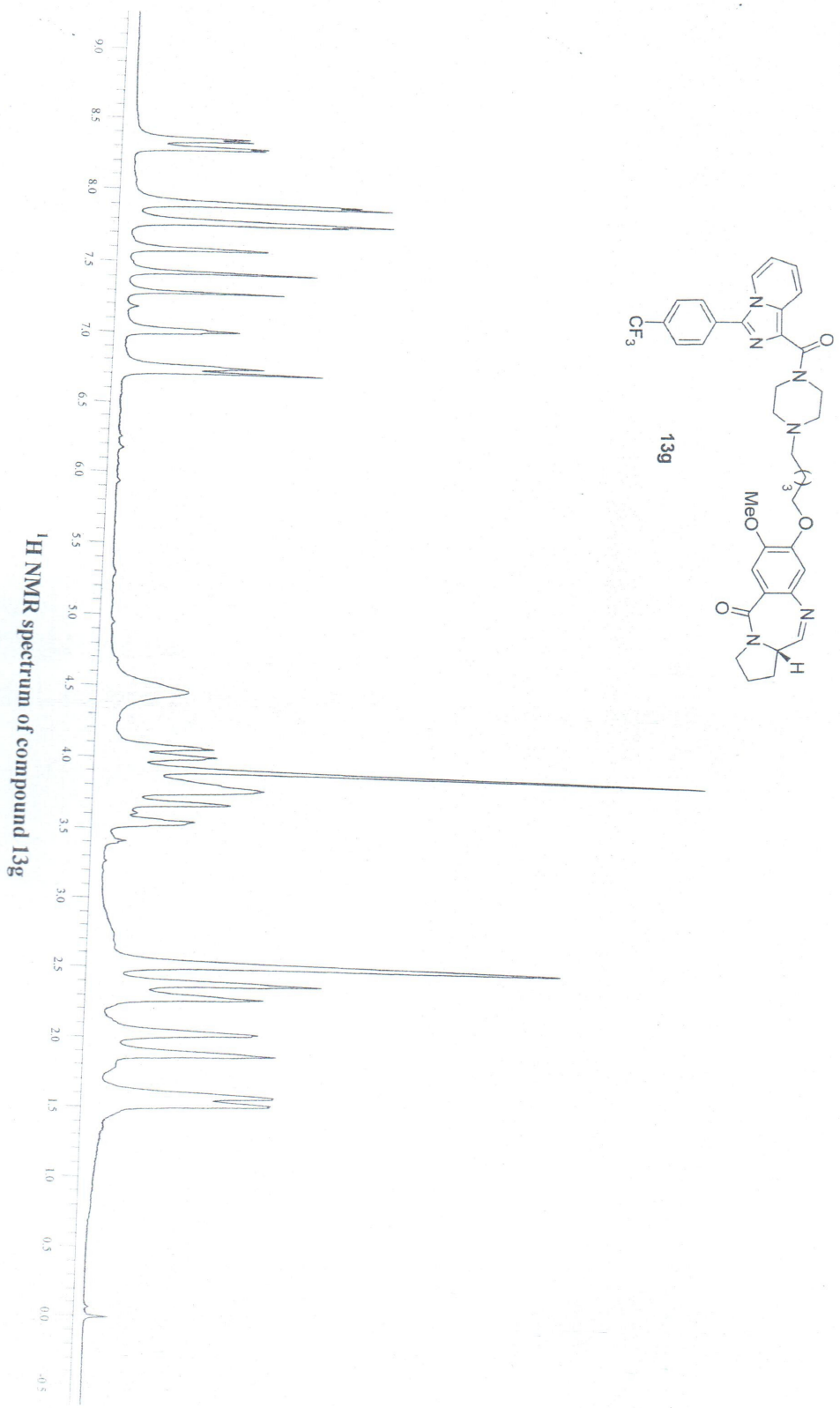


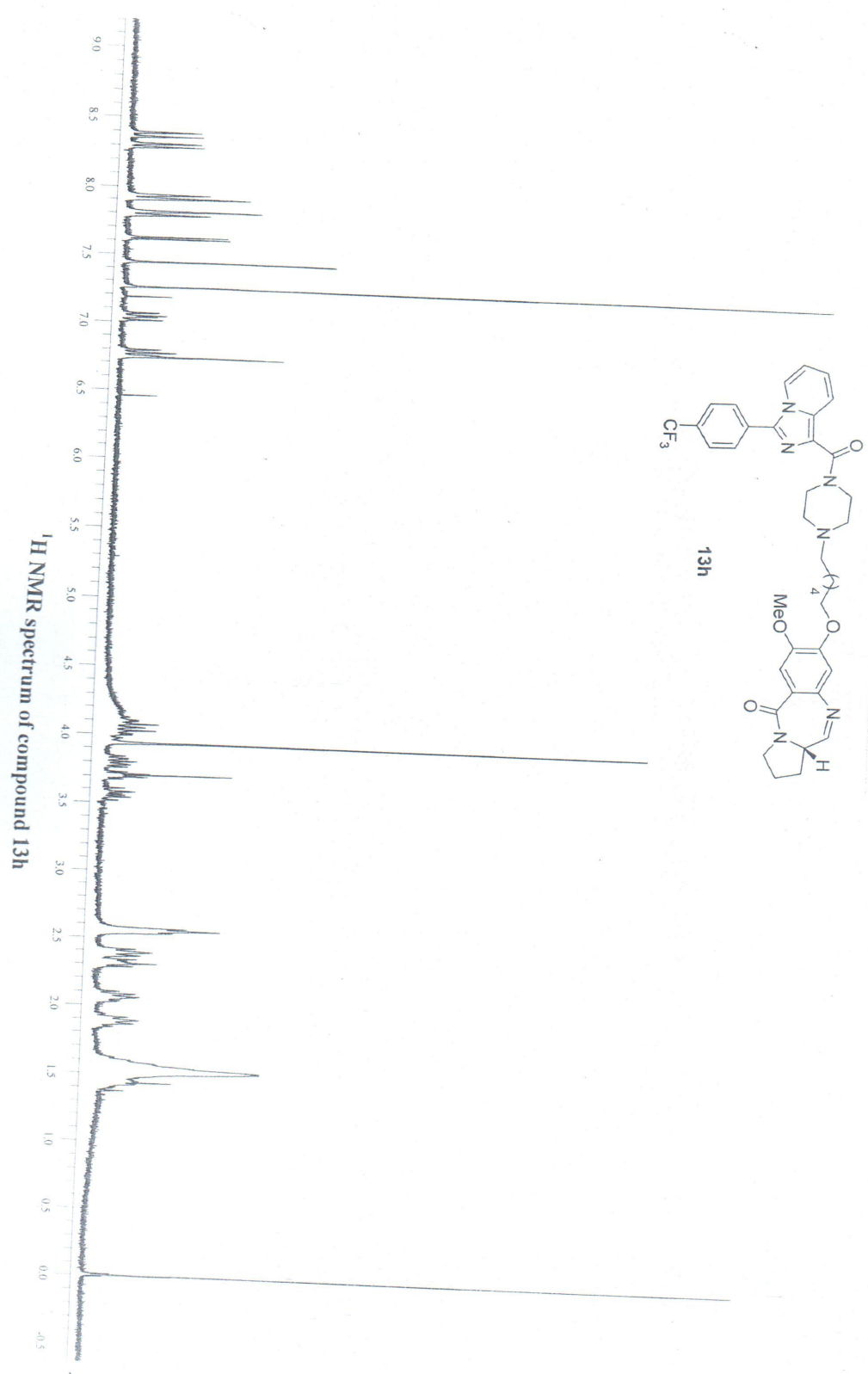


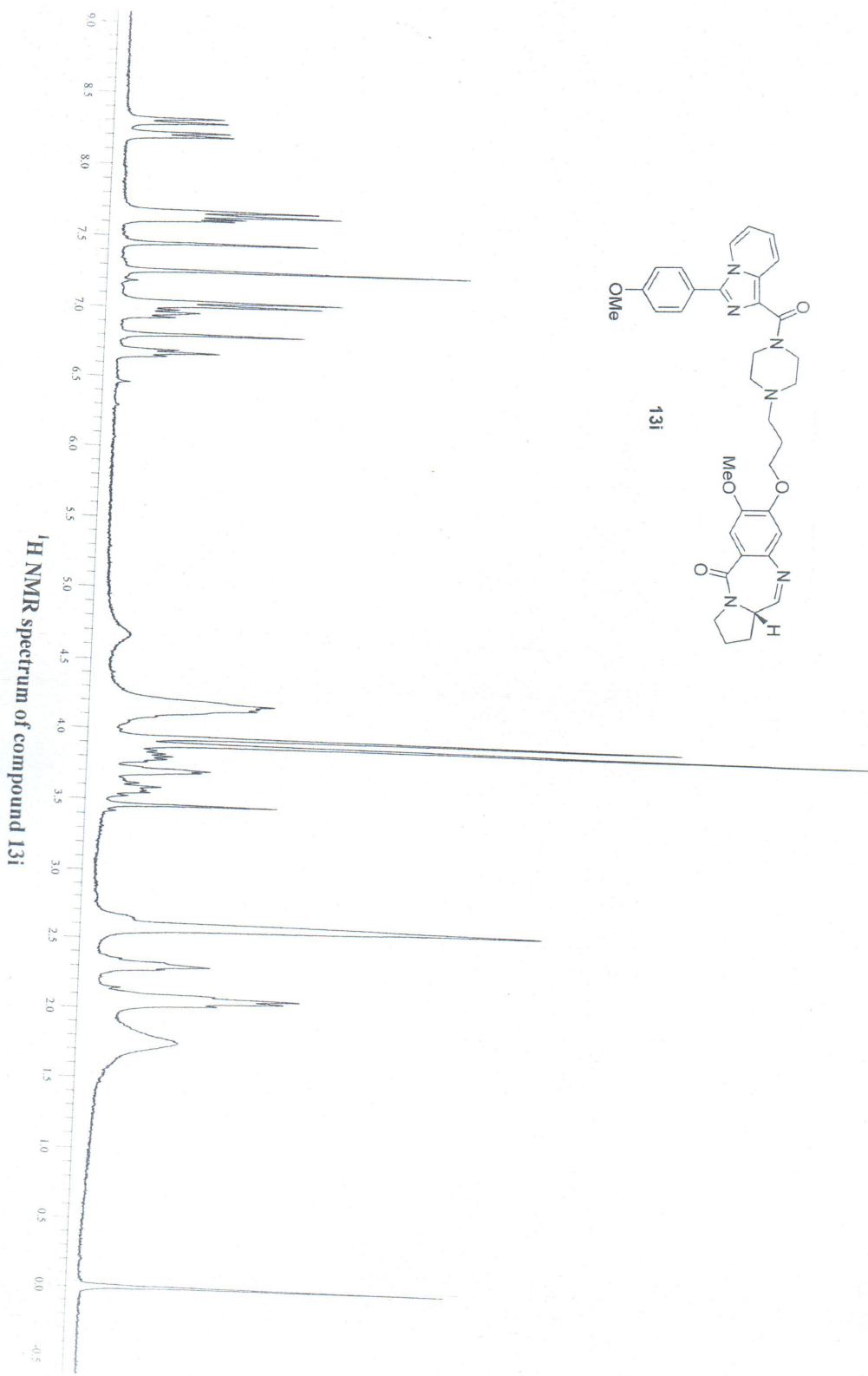


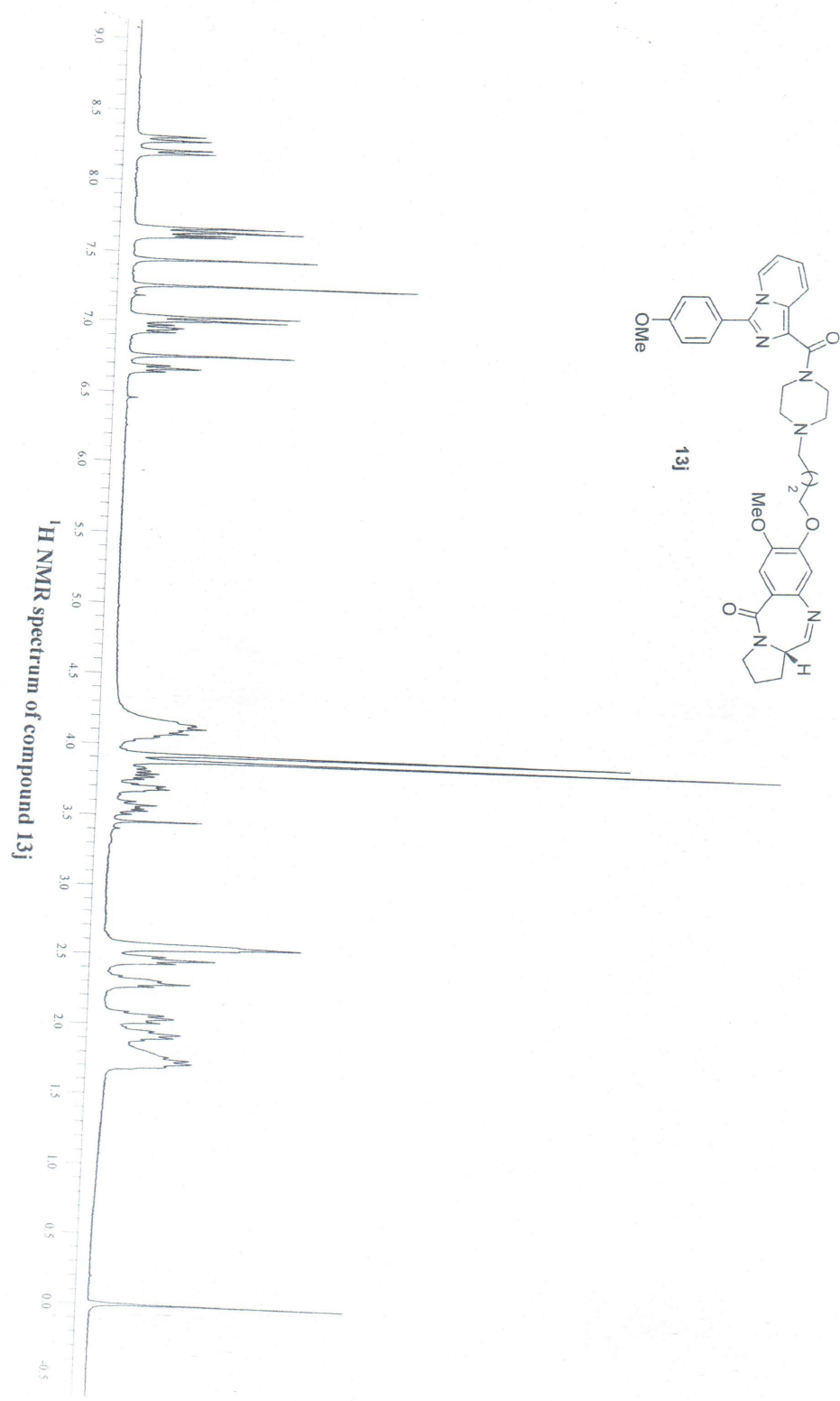


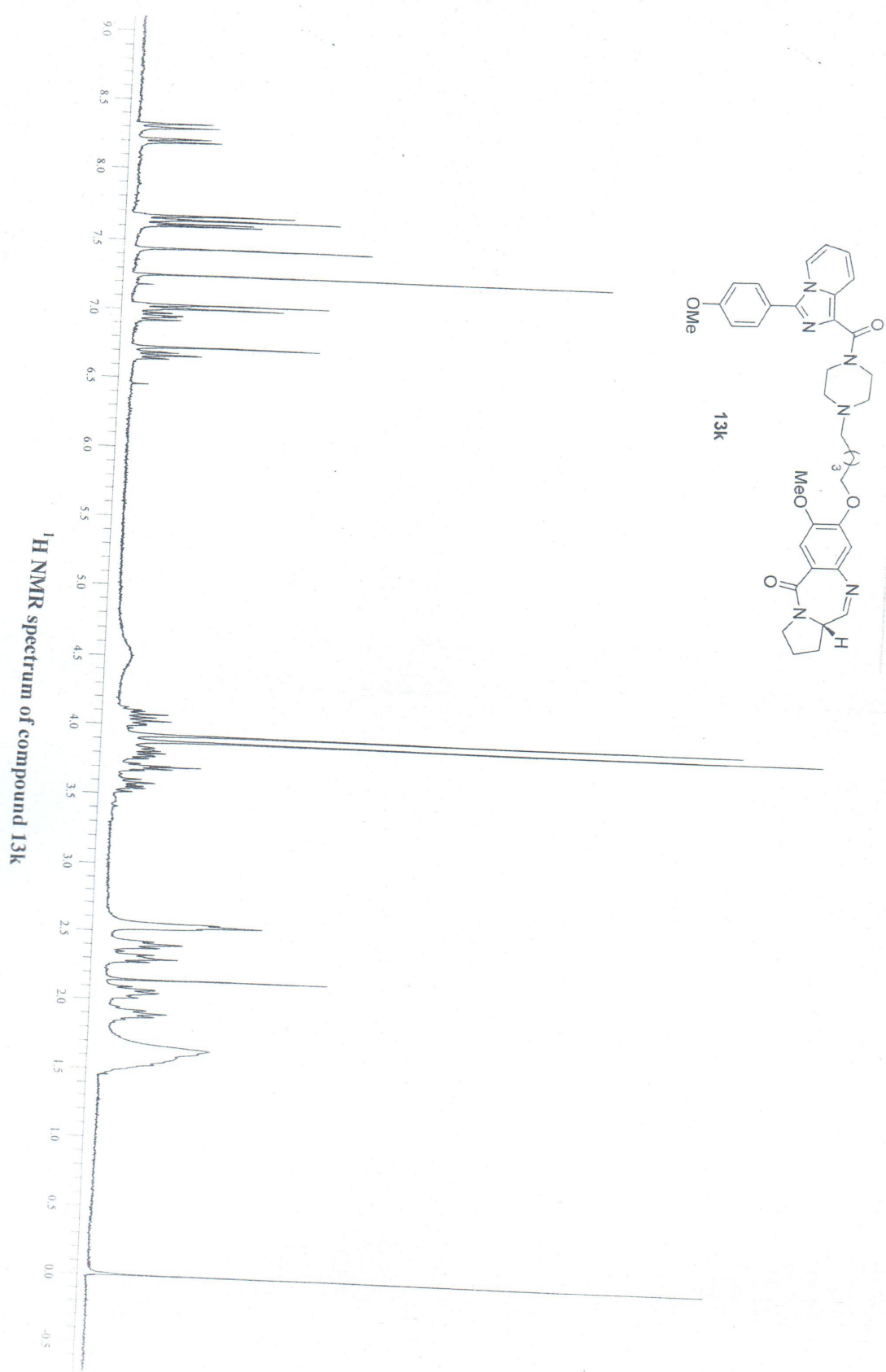


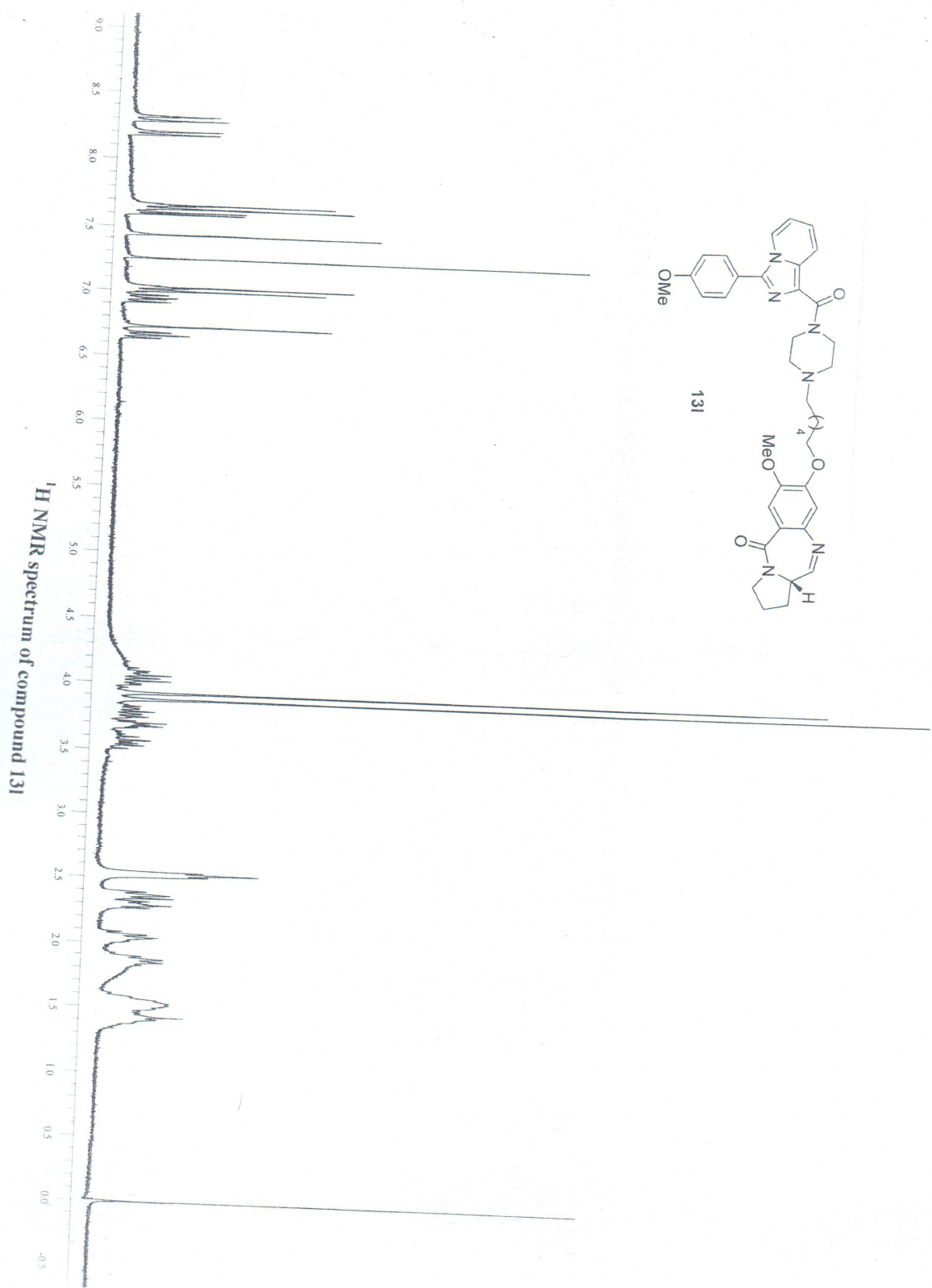












Fri, 16th Jul, 2010 11:58:19

RAMA

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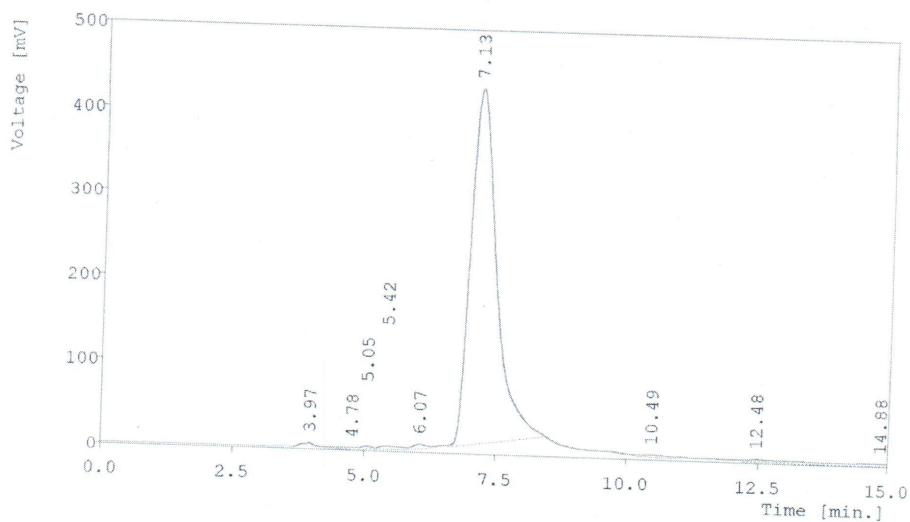
Sample ID : **WS IMP 3imine-1**
Sample : **PBD**
Raw Data : **rama**
Primary : **rama**
Project : **catechol**

Analyst : **ramakrishna**

From : **Tue, 13th Jul, 2010 14:19:09**

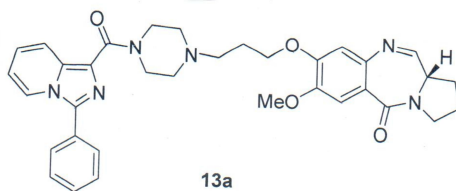
Calibration : **(none)**

Style : **report**



Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	3.970	147.1407	6.887	0.320	1.001	1.571
2	4.780	3.6215	0.459	0.160	0.025	0.105
3	5.050	18.3053	2.031	0.160	0.125	0.463
4	5.420	40.0959	2.142	0.420	0.273	0.489
5	6.070	55.1065	4.001	0.260	0.375	0.913
6	7.130	14331.3179	419.363	0.520	97.534	95.673
7	10.490	19.4165	0.957	0.360	0.132	0.218
8	12.480	39.3802	1.512	0.290	0.268	0.345
9	14.880	39.2752	0.980	0.680	0.267	0.223
-	Total	14693.6597	438.332			



Fri, 16th Jul, 2010 11:59:03

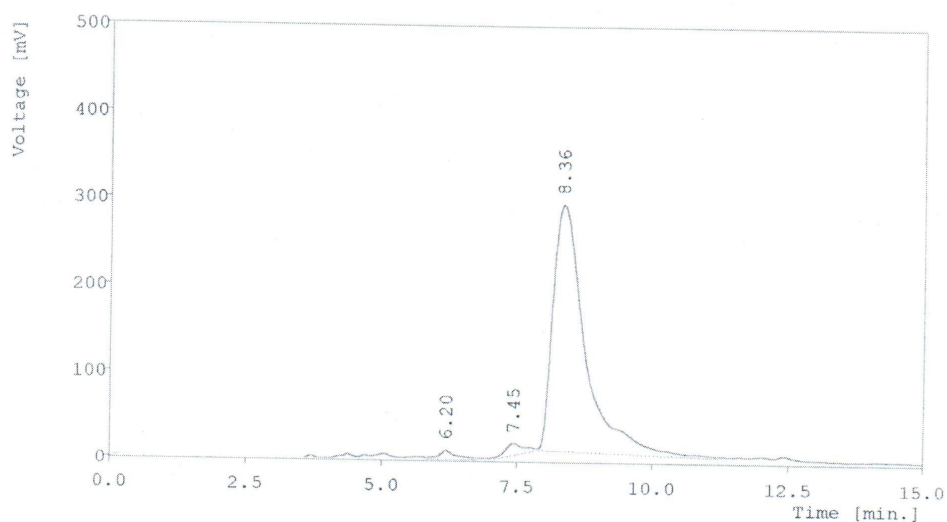
WSIMP4IM

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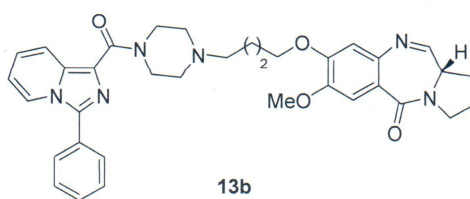
Sample ID : **WS IMP 4imine**
Sample : **PBD**
Raw Data : **wsimp4im**
Primary : **wsimp4im**
Project : **catechol**

Analyst : **ramakrishna**
From : **Tue, 13th Jul, 2010 14:54:26**
Calibration : **(none)**
Style : **report**



Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	6.200	135.4218	8.403	0.210	1.137	2.775
2	7.450	245.8769	12.042	0.550	2.064	3.976
3	8.360	11529.6299	282.397	0.580	96.799	93.249
-	Total	11910.9286	302.841			



Fri, 16th Jul, 2010 12:01:49

WSIMP5IM

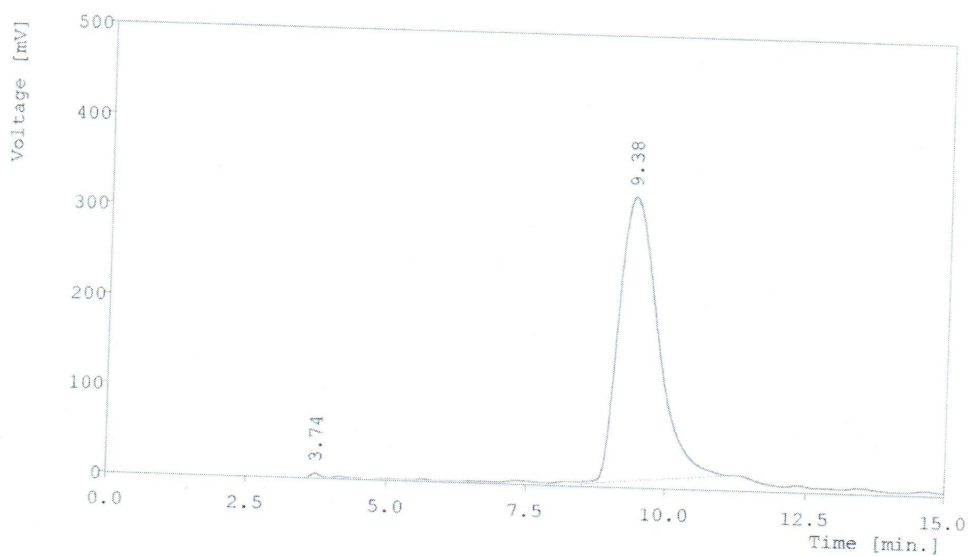
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Sample ID : **WS IMP 5imine**
Sample : **PBD**
Raw Data : **wsimp5im**
Primary : **wsimp5im**
Project : **catechol**

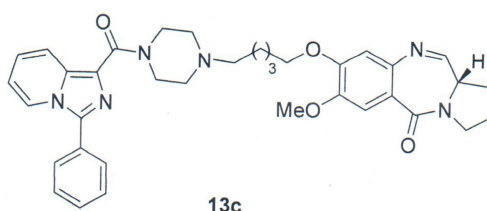
Analyst : **ramakrishna**

From : **Tue, 13th Jul, 2010 15:22:23**
Calibration : **(none)**
Style : **report**



Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	3.740	72.0880	5.565	0.200	0.475	1.745
2	9.380	15096.8995	313.347	0.760	99.525	98.255
-	Total	15168.9875	318.913			



Fri, 16th Jul, 2010 12:03:41

WSIMP6IM

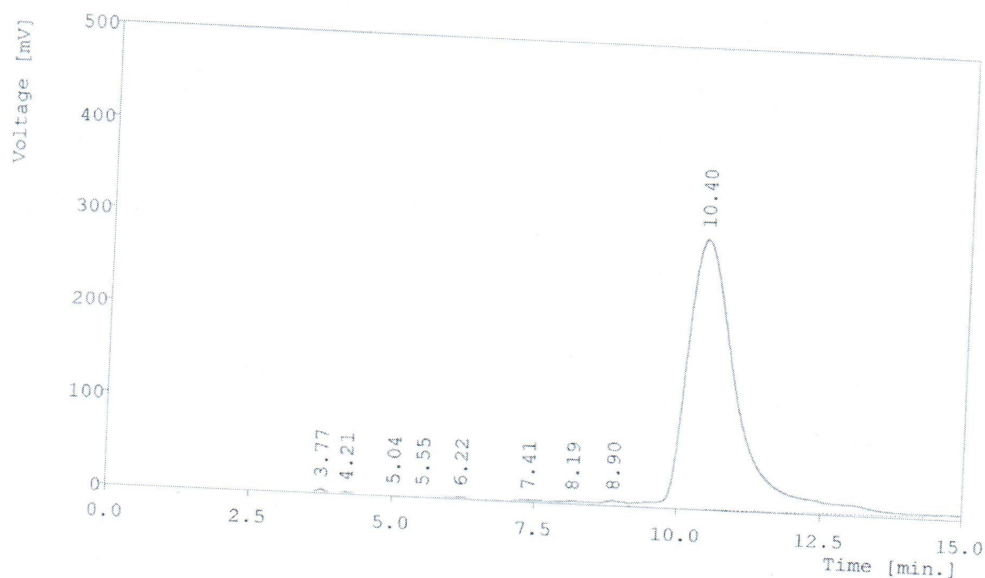
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Sample ID : WS IMP 6imine
Sample : PBD
Raw Data : wsimp6im
Primary : wsimp6im
Project : catechol

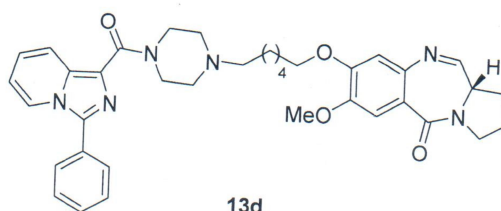
Analyst : ramakrishna

From : Tue, 13th Jul, 2010 16:00:24
Calibration : (none)
Style : report



Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	3.770	118.8693	6.520	0.260	0.656	2.053
2	4.210	105.7509	4.023	0.650	0.584	1.266
3	5.040	72.6023	2.308	0.600	0.401	0.726
4	5.550	58.3057	2.116	0.500	0.322	0.666
5	6.220	95.3941	2.717	0.790	0.527	0.855
6	7.410	101.4566	2.109	0.630	0.560	0.664
7	8.190	89.2177	2.372	0.720	0.492	0.747
8	8.900	108.6444	4.152	0.480	0.600	1.307
9	10.400	17365.5294	291.330	0.850	95.858	91.716
-	Total	18115.7705	317.649			



Fri, 16th Jul, 2010 12:05:04

WIMP3IMN

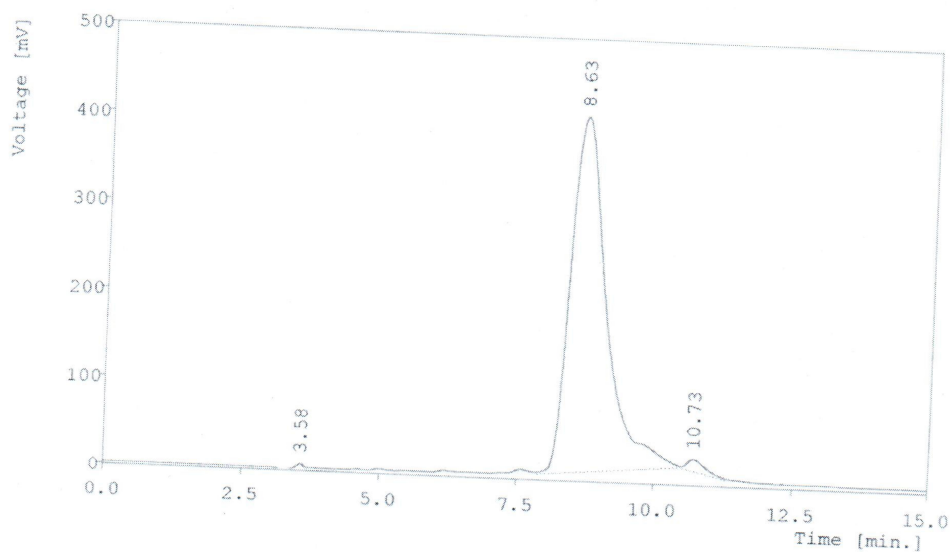
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Sample ID : **W IMP 3imine**
Sample : **PBD**
Raw Data : **wimp3imn**
Primary : **wimp3imn**
Project : **catechol**

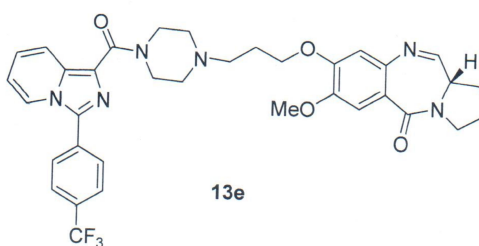
Analyst : **ramakrishna**

From : **Wed, 14th Jul, 2010 9:24:29**
Calibration : **(none)**
Style : **report**



Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	3.580	84.2048	6.264	0.150	0.453	1.492
2	8.630	18246.5087	401.374	0.680	98.173	95.628
3	10.730	255.4550	12.085	0.300	1.374	2.880
-	Total	18586.1685	419.722			



Fri, 16th Jul, 2010 12:12:45

WIMP4IMN

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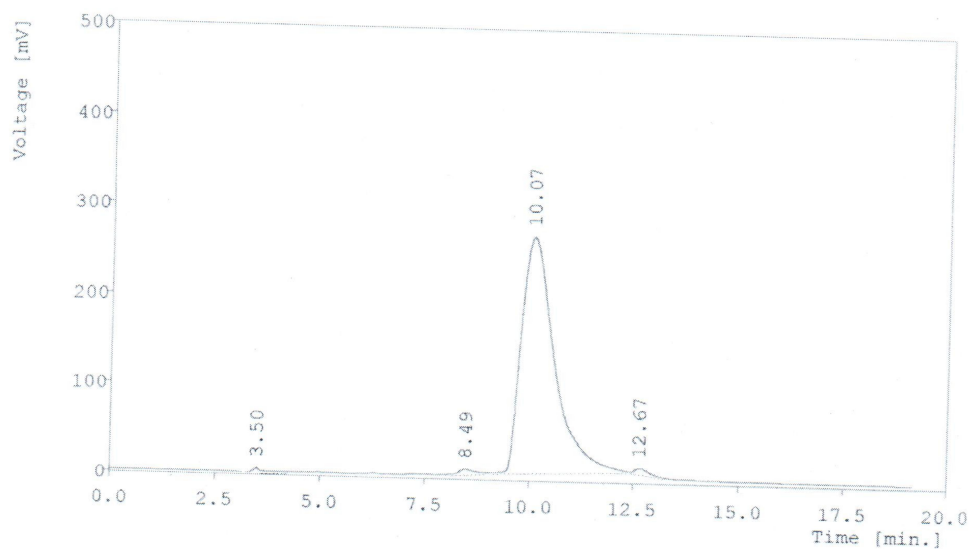
Sample ID : **W IMP 4imine**
Sample : **PBD**
Raw Data : **wimp4imn**
Primary : **wimp4imn**
Project : **catechol**

Analyst : **ramakrishna**

From : **Wed, 14th Jul, 2010 9:51:50**

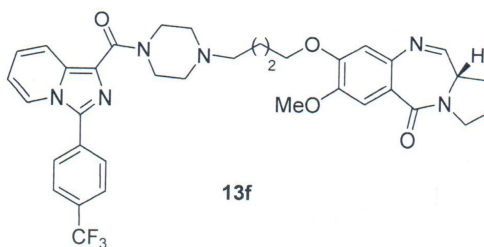
Calibration : **(none)**

Style : **report**



Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	3.500	78.9694	5.742	0.150	0.537	2.065
2	8.490	110.1549	5.147	0.410	0.750	1.851
3	10.070	14375.1521	261.050	0.830	97.814	93.894
4	12.670	132.1225	6.087	0.310	0.899	2.190
-	Total	14696.3989	278.026			



Fri, 16th Jul, 2010 12:11:54

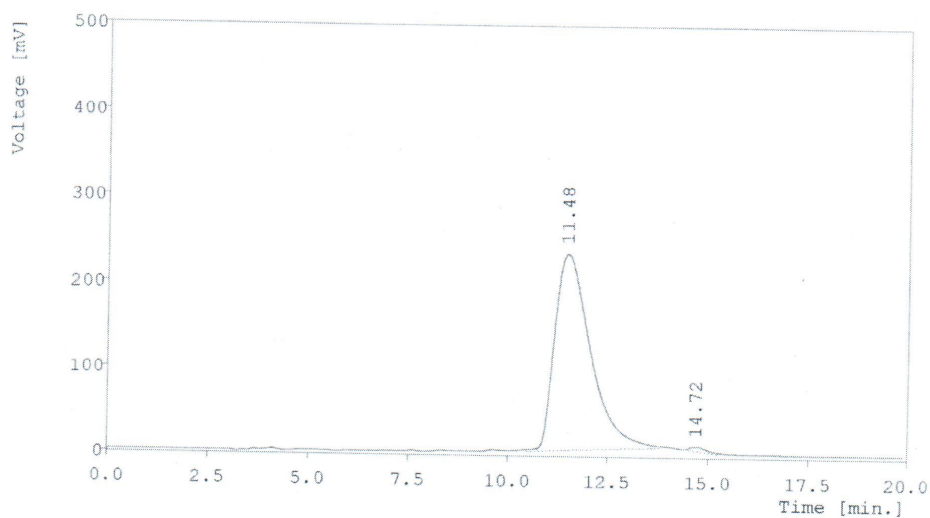
WIMP5IMN

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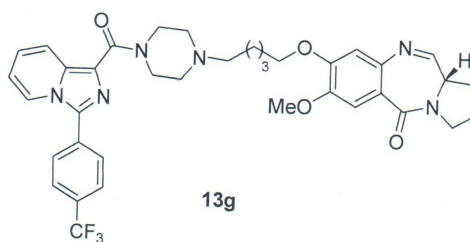
Sample ID : **W IMP 5imine**
Sample : **PBD**
Raw Data : **wimp5imn**
Primary : **wimp5imn**
Project : **catechol**

Analyst : **ramakrishna**
From : **Wed, 14th Jul, 2010 10:16:00**
Calibration : **(none)**
Style : **report**



Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	11.480	14158.4715	227.842	0.960	99.461	98.499
2	14.720	76.7131	3.471	0.320	0.539	1.501
-	Total	14235.1846	231.312			



Fri, 16th Jul, 2010 12:10:35

WIMP6IMN

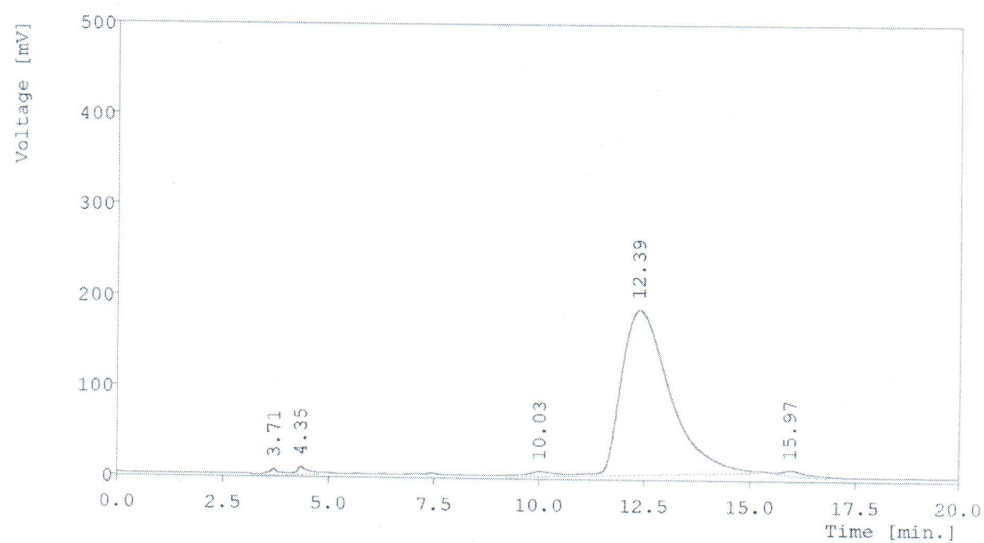
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Sample ID : **W IMP 6imine**
Sample : **PBD**
Raw Data : **wimp6imn**
Primary : **wimp6imn**
Project : **catechol**

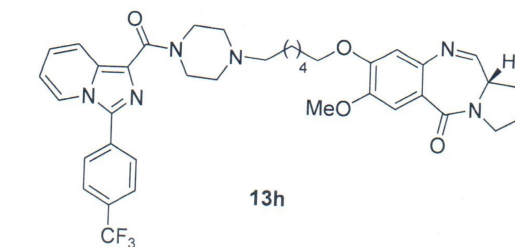
Analyst : **ramakrishna**

From : **Wed, 14th Jul, 2010 10:39:59**
Calibration : **(none)**
Style : **report**



Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	3.710	79.2758	6.508	0.140	0.542	3.198
2	4.350	108.3330	7.818	0.200	0.740	3.841
3	10.030	123.0273	3.960	0.540	0.841	1.946
4	12.390	14130.8024	180.044	1.200	96.587	88.467
5	15.970	116.8814	4.050	0.430	0.799	1.990
6	25.650	71.8386	1.137	0.720	0.491	0.558
-	Total	14630.1585	203.516			



Fri, 16th Jul, 2010 12:14:25

RIMP3IMN

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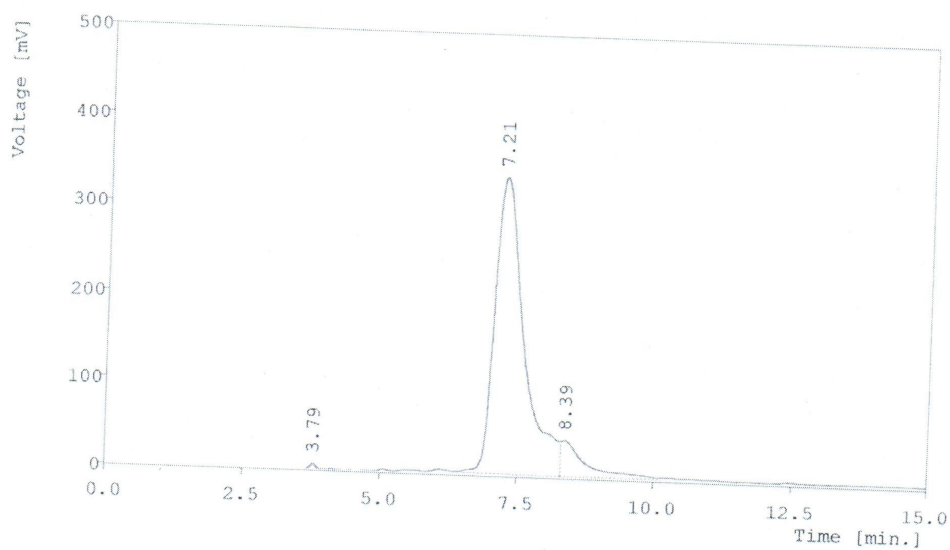
Sample ID : R IMP 3imine
Sample : PBD
Raw Data : rimp3imn
Primary : rimp3imn
Project : catechol

Analyst : ramakrishna

From : Tue, 13th Jul, 2010 16:25:49

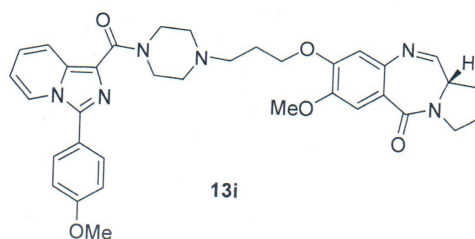
Calibration : (none)

Style : report



Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	3.790	70.7793	7.829	0.120	0.516	2.052
2	7.210	12474.7846	334.621	0.540	91.006	87.724
3	8.390	1162.0710	38.996	0.370	8.478	10.224
-	Total	13707.6349	381.446			



Fri, 16th Jul, 2010 12:15:06

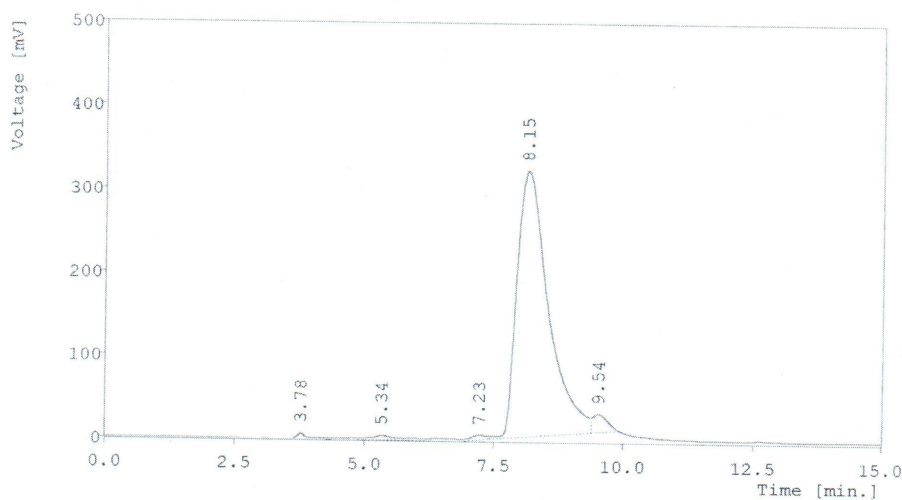
RIMP4IMN

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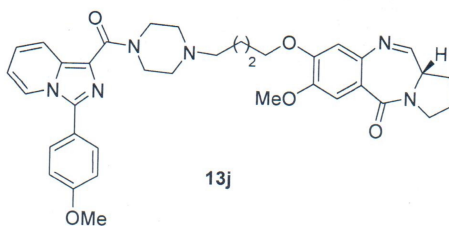
Sample ID : R IMP 4imine
Sample : PBD
Raw Data : rimp4imn
Primary : rimp4imn
Project : catechol

Analyst : ramakrishna
From : Tue, 13th Jul, 2010 16:47:23
Calibration : (none)
Style : report



Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	3.780	93.7756	7.518	0.150	0.676	2.140
2	5.340	79.2437	3.813	0.270	0.571	1.086
3	7.230	64.8253	3.973	0.310	0.467	1.131
4	8.150	13247.2625	316.100	0.630	95.508	89.996
5	9.540	385.1900	19.834	0.350	2.778	5.647
-	Total	13870.2971	351.239			



Fri, 16th Jul, 2010 12:16:04

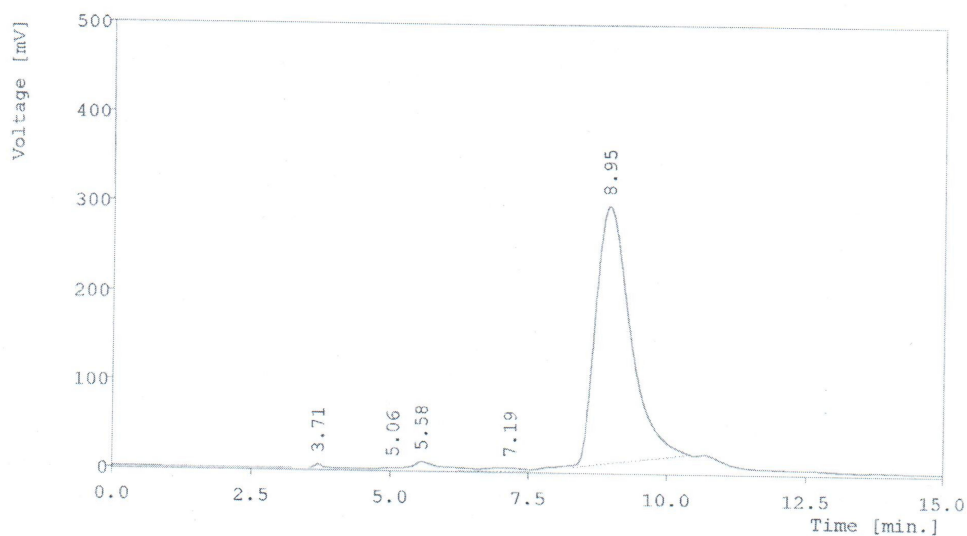
RIMP5IMN

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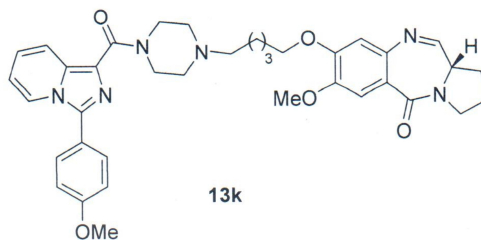
Sample ID : R IMP 5imine
Sample : PBD
Raw Data : rimp5imn
Primary : rimp5imn
Project : catechol

Analyst : ramakrishna
From : Tue, 13th Jul, 2010 17:05:45
Calibration : (none)
Style : report



Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	3.710	107.9762	5.615	0.200	0.838	1.849
2	5.060	51.8403	1.763	0.360	0.403	0.581
3	5.580	286.1717	8.824	0.380	2.222	2.906
4	7.190	69.6578	1.709	0.650	0.541	0.563
5	8.950	12361.9597	285.776	0.670	95.996	94.101
-	Total	12877.6057	303.688			



Fri, 16th Jul, 2010 12:18:01

RIMP6IMN

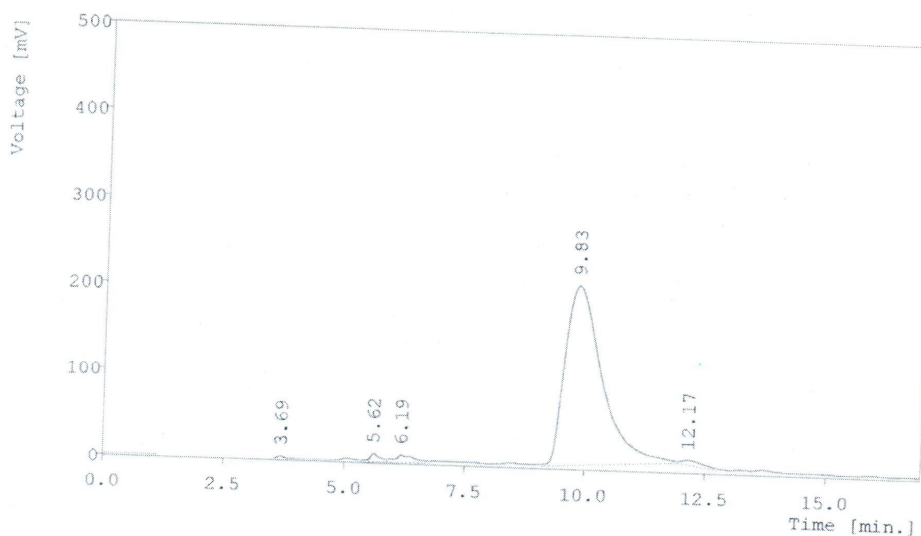
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Sample ID : R IMP 6imine
Sample : PBD
Raw Data : rimp6imn
Primary : rimp6imn
Project : catechol

Analyst : ramakrishna

From : Tue, 13th Jul, 2010 17:28:42
Calibration : (none)
Style : report



Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	3.690	81.9459	4.236	0.210	0.719	1.847
2	5.620	95.7346	8.157	0.190	0.840	3.557
3	6.190	154.3801	7.015	0.350	1.355	3.059
4	9.830	10951.9759	205.940	0.810	96.113	89.798
5	12.170	110.8280	3.991	0.390	0.973	1.739
-	Total	11394.8646	229.339			

