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Synthesis of Aryl-Substituted Naphthalene-Linked Pyrrolobenzodiazepine Conjugates as Potential Anticancer Agents with Apoptosis-Inducing Ability

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A library of new aryl-substituted naphthalene C8-linked pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) conjugates with various linker architectures were designed, synthesized, and evaluated for their anticancer activity against a panel of 11 human cancer cell lines. All 32 conjugates show anticancer potential, with some of them exhibiting particularly high activity (0.01–0.19 μM). Thermal denaturation studies showed effective DNA binding capacity relative to DC-81. In assays for biological activity relating to cell-cycle distribution, these PBD conjugates induce G_0/G_1-phase arrest and also cause an increase in the levels of p53 and caspase-9 proteins, followed by apoptotic cell death. One conjugate in particular is the most promising candidate of the series, with the potential to be selected for further studies, either alone or in combination with existing anticancer therapies.

Introduction

Cancer is a major global health problem, representing the second-leading cause of death worldwide.[1] Improvements in treatment and prevention have led to a decrease in cancer deaths, but the number of new diagnoses continues to rise. According to information from the World Health Organization (WHO), it is estimated that there will be 12 million deaths from cancer in 2030. Over the last few years, a number of chemotherapeutic drugs have been developed to treat cancer, and these include DNA binding and alkylating agents. The pyrrolobenzodiazepines (PBDs) are a family of naturally occurring antibiotics such as anthramycin, chicamycin, mazethramycin, porothramycin A, sibiromycin, tomaymycin, oxotomaymycin, prothracarcin, and DC-81 (**1**), all of which have antitumor activity. These compounds are isolated from various *Streptomyces* species,[2] and they bind in the minor groove of DNA, spanning three base pairs and forming a covalent bond between their electrophilic N10–C11 imine moiety and the N2 position of a guanine base.[3,4] PBDs are tricyclic molecules, which in most cases contain a stereogenic center at the C11a position (Figure 1). Over the past few years, various types of conjugates, in which a known antitumor compound or some simple active moiety tethered to a PBD, have been designed, synthesized, and evaluated for biological activity.[5–9] Wang and co-workers recently synthesized indole and enediyne PBD conjugates as potential antitumor agents and explained the correlation between antitumor activity and apoptosis.[10,11]

For the last few years our research group has been involved in the development of new synthetic strategies for the prepa-

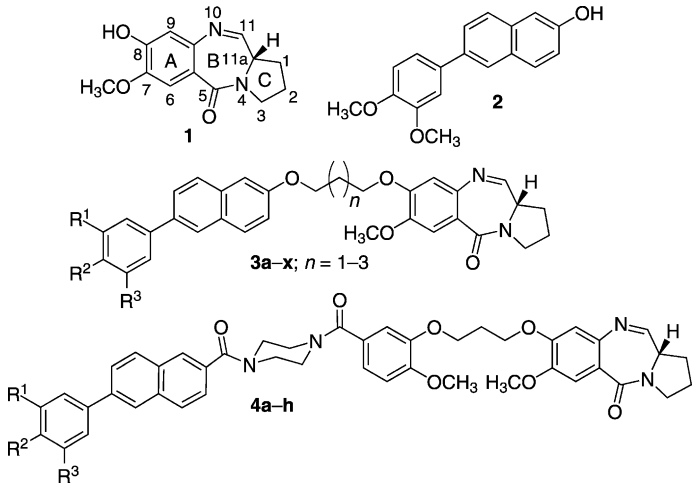

Figure 1. Structures of naturally occurring DC-81 (**1**), 6-(3,4-dimethoxyphenyl)-2-naphthol (**2**), and aryl-substituted naphthalene PBD conjugates **3a–x** and **4a–h**.

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ration of the PBD ring system,[12–14] as well as in the design and synthesis of structurally modified PBDs and their conjugates.[15–20] More recently, we have described some of the PBD conjugates that demonstrate potent apoptotic activity through a mitochondria-mediated pathway.[21–23] Moreover, several piperazine-containing molecules useful as chemotherapeutic agents have been reported over the past few years.[24,25] It is well known that this heterocyclic backbone could act on various pharmacological targets and display anticancer,[26,27] calcium-channel-blocking,[28] and histamine antagonist properties.[29] Furthermore, piperazine-containing PBDs have also shown significant anticancer activity with improved bioavailability.[21,30,31]

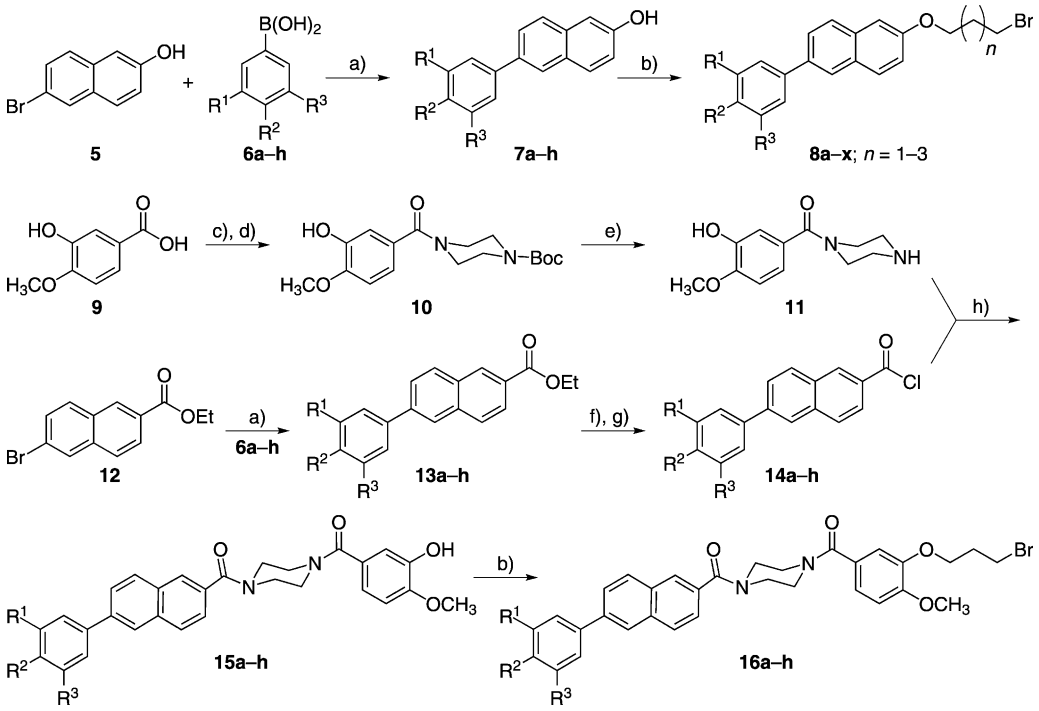
The naphthalene skeleton is an important building block for a large number of clinical drugs[32,33] that possess a variety of biological activities such as anti-inflammatory,[34,35] cardiovascular,[36] antibacterial,[37] and anticancer.[38–40] Naphthalene-based resveratrol analogues are the most effective compounds in causing apoptosis in a human breast cancer cell line and show considerable antiproliferative effects.[41,42] Thus, due to the diverse range of pharmacological activities of naphthalene and its derivatives, we decided to explore various aryl-substituted naphthalene derivatives as pharmacophores in the design of PBD conjugates.

In continuation of these efforts, aryl-substituted naphthalene derivatives were linked to the C8-position of the PBD scaffold (DC-81, **1**) through stable alkane linkers and also by incorporating a piperazine moiety. This led to a new library of PBD conjugates that were evaluated for their anticancer potential. In view of their promising activity, we decided to investigate their role in cytotoxicity, cell-cycle effects, and apoptosis by using the MCF-7 human breast cancer cell line. Representative structures of DC-81 (**1**), 6-(3,4-dimethoxyphenyl)-2-naphthol (**2**, conjugate partner), and their conjugates **3a**–**x** and **4a**–**h** are shown in Figure 1.

Results and Discussion

Chemistry

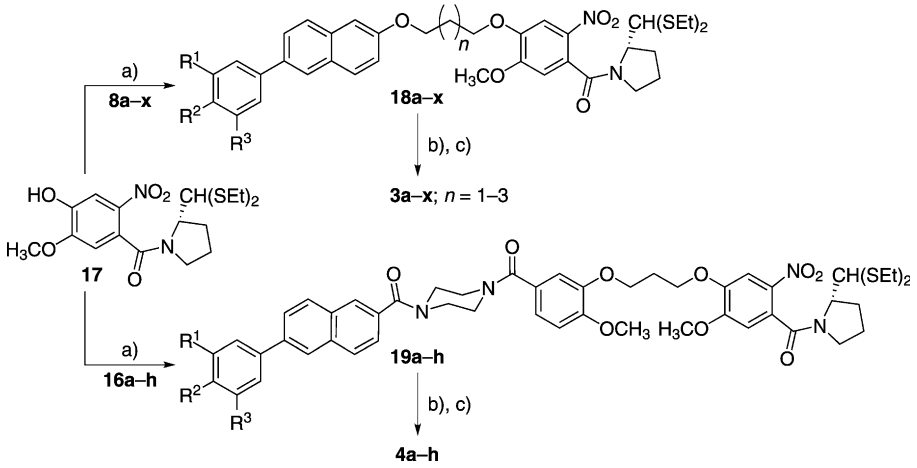
The synthesis of aryl-substituted naphthalene precursors **8a**–**x** and **16a**–**h** is outlined in Scheme 1. Commercially available 6-bromo-2-naphthol (**5**) was treated with phenylboronic acids **6a**–**h** in the presence of aqueous sodium bicarbonate to afford compounds **7a**–**h** via Suzuki palladium-catalyzed cross-coupling reaction; these compounds, upon etherification with various dibromoalkanes using potassium carbonate, produced the corresponding naphthalene precursors **8a**–**x**. For the preparation of the other precursors, isovanillic acid (**9**) was treated with thionyl chloride using catalytic amounts of *N*,*N*-dimethylformamide to produce the acid chloride, which was coupled with *N*-Boc-piperazine in the presence of triethylamine to give the corresponding Boc-protected compound **10**; this, upon deprotection with trifluoroacetic acid, provided compound **11**. Ethyl-6-bromo-2-napthoate (**12**) was allowed to react with boronic acids **6a**–**h** using sodium bicarbonate to afford the aryl-substituted naphthalenes **13a**–**h**. Upon hydrolysis with sodium hydroxide and treatment with thionyl chloride, these provided the corresponding acid chlorides **14a**–**h**, which were coupled with compound **11** using triethylamine to furnish the naphtha-



Scheme 1. *Reagents and conditions*: a) $Pd(PPh_3)_4$, aq. $NaHCO_3$, toluene/EtOH (3:1), 80–90 °C, 2–4 h, (71–90%); b) dibromoalkanes, anhyd. K_2CO_3, dry acetone, reflux, 12–24 h, (78–88%); c) $SOCl_2$, DMF, dry CH_2Cl_2, 0 °C→RT, 2 h, (85%); d) *N*-Boc-piperazine, Et_3N, dry CH_2Cl_2, 0 °C→RT, 4 h, (72%); e) TFA, dry CH_2Cl_2, 0 °C→RT, 2 h, (93%); f) 1 N NaOH, EtOH, 70–80 °C, 1–2 h, (91–95%); g) $SOCl_2$, dry CH_2Cl_2, 0 °C→RT, 2–4 h, (90%); h) Et_3N, dry CH_2Cl_2, RT, 2–4 h, (65–70%).

lene intermediates **15a–h**. Finally, etherification of compounds **15** with 1,3-dibromopropane in the presence of potassium carbonate afforded the aryl-substituted naphthalene precursors **16a–h**.

The synthesis of PBD conjugates **3a–x** and **4a–h** is illustrated in Scheme 2. Compound **17**, (2*S*)-*N*-[4-hydroxy-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethylthioacetal was prepared from vanillin by employing our previously reported procedure.[12–20] Coupling of compound **17** with aryl-



Scheme 2. *Reagents and conditions*: a) anhyd. K_2CO_3, dry acetone, reflux, 12–24 h, (72–83%); b) $SnCl_2{\cdot}2H_2O$, MeOH, reflux, 4–6 h; c) $HgCl_2$, $CaCO_3$, CH_3CN/H_2O (4:1), RT, 8–12 h, (50–73% yield over two steps).

substituted naphthalene precursors **8a–x** and **16a–h** in the presence of potassium carbonate provided the corresponding nitrothioacetols **18a–x** and **19a–h**, which upon reduction with tin(II) chloride dihydrate followed by deprotection and cyclization using mercury(II) chloride/calcium carbonate, afforded the target compounds **3a–x** and **4a–h**; see Table 1 for structures.

Biological activity

DNA interaction: thermal denaturation studies

The DNA binding ability of the new PBD conjugates **3a–x** and **4a–h** was investigated by thermal denaturation studies using calf thymus (CT) duplex DNA at pH 7.0, using protocols reported in our previous work.[22,23] These studies were carried out at a DNA/ligand molar ratio of 1:5. The melting temperature (ΔT_m) for each compound was examined after 0 and 18 h incubation at 37 °C. All conjugates **3a–x** and **4a–h** caused an increase in CT-DNA ΔT_m values in the respective ranges of 1.5–4.0 and 2.8–4.2 °C relative to the naturally occurring DC-81 (**1**) and conjugate partners (**2** and **15g**). Interestingly, conjugates **3g**, **3h**, **4g**, and **4h** elevate the melting temperature by 4.0, 3.8, 4.2, and 4.1 °C, respectively, after 18 h incubation at 37 °C as shown in Table 1. Conjugates **4** show slightly higher ΔT_m values than conjugates **3**, probably due to the presence of a piperazine moiety in the linker. Most of the conjugates in series **3** containing a linker with an odd number of methylene units ($n = 1$ or 3) show slightly higher DNA binding affinity relative to those with an even number of carbons ($n = 2$) in the linker.

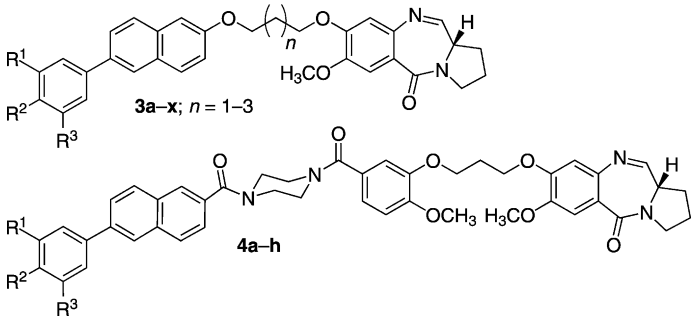
Restriction endonuclease digestion assay

The DNA melting temperature studies indicated that the aryl-substituted PBD conjugates exhibit significant DNA binding affinity. To further confirm their interaction with DNA, a protection assay using restriction endonuclease digestion was performed. The PBD compounds are effective as DNA binding agents particularly at G-rich sequences.[43] Therefore, to determine the binding activity of these PBD conjugates relative to naturally occurring PBD compounds such as DC-81 (**1**), restriction enzyme digestion (RED) assays were carried out for compounds with particular promise, such as **3g** and **4g**, alongside compound **1**. In this assay, a pBR322 vector with a BamHI restriction site (5′-G|GATCC-3′, in which ′|′ denotes the site of cleavage) flanked by G-rich regions was used. The experiment is based on the principle that any compound binding at the BamHI site should impede endonuclease activity toward the DNA substrate by BamHI. Details of the experimental protocol are described in a study reported earlier by our research group.[15] For the assays reported herein, we used various test compound concentrations ranging from 1 to 8 μM. Compounds **3g** and **4g** were observed to effectively bind G-rich regions of DNA along the minor groove at 4 and 8 μM, whereas DC-81 (**1**) did not show binding, as can been seen from the results shown in Figure 2. The inhibitory activity of these PBD conjugates is ranked in the following order: **4g** > **3g** > **1**, and is in agreement with the DNA binding ability as determined by thermal denaturation studies. These assays clearly indicate that the linking of the PBD scaffold with other conjugate partners significantly enhances the binding potential. It has been well established that the PBD scaffold forms a covalent bond with N2 of a guanine base in the DNA, while the other (naphthalene) component of this new conjugate is likely to associate with DNA through noncovalent interactions. Indeed, similar observations were made in previous studies with related PBD conjugates.[44,45]

Anticancer activity

These aryl-substituted naphthalene PBD conjugates **3a–x** and **4a–h** were evaluated for their anticancer activity in selected human cancer cell lines of lung, breast, oral, colon, prostate, ovarian, and cervical tissue by using the sulforhodamine B (SRB) method. Conjugates that exhibit $GI_{50} \leq 10^{-5}$ M are consid-

Table 1. A library of aryl-substituted naphthalene PBD conjugates (**3a–x** and **4a–h**) and their thermal denaturation data with calf thymus (CT)-DNA.



Compd	R^1	R^2	R^3	n	ΔT_m [°C][a] 0 h	18 h
3a	H	F	H	1	2.4	3.0
3b	H	CF_3	H	1	2.0	2.2
3c	H	OCF_3	H	1	2.6	2.6
3d	H	OCH_3	H	1	1.7	1.9
3e	H	$N(CH_3)_2$	H	1	2.2	2.8
3f	H	H	Cl	1	2.8	3.1
3g	H	OCH_3	OCH_3	1	3.2	4.0
3h	OCH_3	OCH_3	OCH_3	1	3.0	3.8
3i	H	F	H	2	2.1	2.5
3j	H	CF_3	H	2	1.9	2.1
3k	H	OCF_3	H	2	1.8	1.8
3l	H	OCH_3	H	2	1.6	1.9
3m	H	$N(CH_3)_2$	H	2	1.8	1.8
3n	H	H	Cl	2	2.1	2.5
3o	H	OCH_3	OCH_3	2	3.5	3.8
3p	OCH_3	OCH_3	OCH_3	2	3.3	3.5
3q	H	F	OCH_3	3	2.1	2.5
3r	H	CF_3	H	3	1.5	1.8
3s	H	OCF_3	H	3	2.1	2.4
3t	H	OCH_3	H	3	1.7	1.7
3u	H	$N(CH_3)_2$	H	3	2.0	2.5
3v	H	H	Cl	3	2.6	2.8
3w	H	OCH_3	OCH_3	3	3.2	3.7
3x	OCH_3	OCH_3	OCH_3	3	3.2	3.5
4a	H	F	H	–	2.9	3.1
4b	H	CF_3	H	–	2.8	2.8
4c	H	OCF_3	H	–	3.0	3.3
4d	H	OCH_3	H	–	2.8	3.5
4e	H	$N(CH_3)_2$	H	–	3.1	3.7
4f	H	H	Cl	–	3.0	3.6
4g	H	OCH_3	OCH_3	–	3.7	4.2
4h	OCH_3	OCH_3	OCH_3	–	3.5	4.1
DC-81 (**1**)	–	–	–	–	0.3	0.7
2	H	OCH_3	OCH_3	–	0.2	0.2
15g	H	OCH_3	OCH_3	–	0.3	0.5

[a] Determined at a PBD/DNA molar ratio of 1:5, for which [CT-DNA] = 100 μM and [ligand] = 20 μM in aqueous sodium phosphate buffer (10 mM sodium phosphate, 1 mM EDTA, pH 7.00 ± 0.01), after incubation at 37 °C for either 0 or 18 h; for CT-DNA alone at pH 7.00 ± 0.01, T_m = 69.6 °C ± 0.01 (mean value from 10 separate determinations); all T_m values are ± 0.05–0.15 °C.

ered to be active on the respective cell lines. All 32 conjugates exhibited significant anticancer activity, with GI_{50} values ranging from 0.01 to 3.41 μM; the positive control compounds, adriamycin and DC-81 (**1**), showed GI_{50} values toward these cell lines in the ranges of 0.1–7.25 and 0.1–2.37 μM, respectively, and the results are listed in Table 2. Interestingly, all the new conjugates showed significant activity in the breast (Zr-75-1, MCF-7) and ovarian (A2780) cancer cell lines. Some interesting trends have been observed for these conjugates in structure–activity relationship (SAR) studies. With respect to substitution on the phenyl moiety of aryl-substituted naphthalene derivatives, all the 3,4-dimethoxy- (**3g**, **3o**, **3w**, and **4g**) and 3,4,5-trimethoxy- (**3h**, **3p**, **3x**, and **4h**) containing aryl-substituted naphthalene PBD conjugates showed significant anticancer activity against all 11 human cancer cell lines relative to other conjugates. The PBD conjugates with an odd number (n = 1 or 3) of methylene units in the linker exhibited greater activity than their counterparts with an even number of units (n = 2) in the linker. PBD conjugates with propyloxy linkers showed slightly better activity than those with pentyloxy linkers. Furthermore, we observed that the incorporation of piperazine along with an isovanillin moiety in the linker between the aryl naphthalene and PBD subunits (series **4**) produced compounds with activity similar to that of the other PBD conjugates. Among all the compounds synthesized, conjugates **3a–h**, **3q**, **3s**, **3w**, **3x**, and **4a–h** exhibited significant anticancer activity, with GI_{50} values ranging from 0.01 to 0.19 μM against the panel of 11 human cancer cell lines.

MTT assays were carried out for eight potent PBD conjugates (**3f–3i**, **3s**, **3w**, **4d**, and **4g**) to identify any cytotoxic effects in MCF-7 cells at 4 μM for 24 h. These PBD conjugates show higher cytotoxicity than DC-81 (**1**) and the conjugate partner **2**; among them, compounds **3g**, **3h**, and **4g** exhibited particularly high activity, as shown in Figure 3.

Effect of PBD conjugates on cell cycle

To investigate the mechanism underlying the cytotoxic effect of these PBD conjugates on cell-cycle progression in the MCF-7 human breast cancer cell line, the DNA content in cell nuclei was measured by flow cytometry. Treatment of MCF-7 cells with PBD conjugates **3g**, **3h**, and **4g** at 4 μM for 24 h caused 87.02, 84.17, and 82.42% accumulation of cells in G_0/G_1 phase, respectively. For comparison, DC-81 (**1**) and conjugate partner

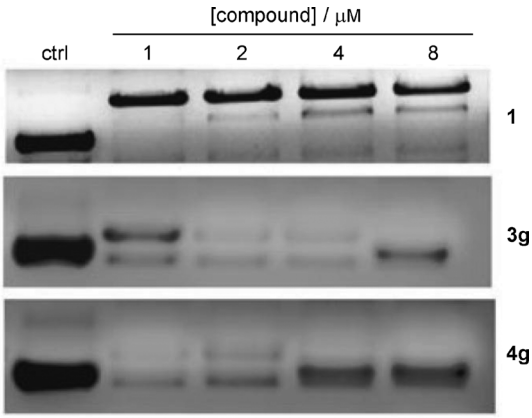


Figure 2. RED assay with DC-81 (**1**) at top; the middle and bottom panels depict RED assay results for compounds **3g** and **4g**. Compound concentrations are indicated; 'ctrl' refers to uncut vector DNA.

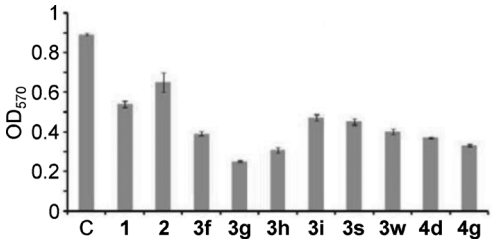


Figure 3. Effect of PBD conjugates on cell viability (in vitro cytotoxicity). MCF-7 cells were treated with conjugates **3f**–**3i**, **3s**, **3w**, **4d**, and **4g** at 4 mM for 24 h in 96-well plates seeded with 10000 cells per well. OD readings were taken at λ 570 nm to determine percent cell viability after treatment with the compounds. Here DC-81 (**1**) and the conjugate partner **2** were used as positive controls; C = negative control (untreated cells). Each experiment was repeated three times, and error bars represent standard deviations, derived from three independent experiments.

2 caused respective G_0/G_1-phase accumulations of 68.14 and 59.80%. Therefore, the increase in the percentage of cells in G_0/G_1 phase clearly shows that these conjugates cause G_1-phase arrest in MCF-7 cells. Conjugate **3g** appears to be the most effective conjugate in effecting cell-cycle arrest, as evident from the data in Table 3. The percentage of apoptotic (G_0 phase) cells in the presence of test compound at 4 μM, as well as at each compound's respective GI_{50} and IC_{50} concentrations, are listed in Table 4.

Table 2. GI_{50} values for PBD conjugates **3a**–**x** and **4a**–**h** in selected human cancer cell lines.

	GI_{50} [μM][a]										
Compd	ZR-75-1[b]	A549[c]	A2780[d]	Hop62[c]	KB[e]	SiHa[f]	Gurav[c]	MCF-7[b]	Colo205[g]	DWD[e]	PC3[h]
3a	0.02	0.13	0.08	0.13	0.15	0.16	0.17	0.07	0.13	0.11	0.09
3b	0.03	0.13	0.05	0.14	0.17	0.17	0.17	0.04	0.14	0.17	0.17
3c	0.13	0.19	0.09	0.14	0.16	0.18	0.14	0.11	0.18	0.14	0.16
3d	0.05	0.19	0.13	0.17	0.16	0.17	0.14	0.08	0.17	0.14	0.16
3e	0.13	0.14	0.07	0.17	0.16	0.18	0.17	0.04	0.18	0.15	0.17
3f	0.09	0.14	0.01	0.16	0.15	0.09	0.16	0.03	0.17	0.02	0.08
3g	0.01	0.11	0.01	0.11	0.01	0.06	0.01	0.01	0.01	0.01	0.07
3h	0.02	0.13	0.01	0.14	0.11	0.08	0.01	0.02	0.02	0.01	0.08
3i	0.14	1.18	0.17	0.19	1.26	1.01	0.17	0.11	2.27	0.18	1.16
3j	0.17	1.47	0.17	2.63	3.27	2.82	2.06	0.17	3.11	2.30	2.48
3k	0.17	1.24	0.19	2.26	3.05	2.52	0.18	0.19	3.13	0.19	2.10
3l	0.18	1.28	0.19	0.19	2.48	3.19	2.92	0.18	2.71	0.19	2.20
3m	0.19	1.49	0.18	2.32	2.22	2.81	2.00	0.17	3.33	2.30	2.48
3n	0.18	1.29	0.19	0.16	3.12	0.19	0.17	0.17	3.41	0.19	1.20
3o	0.17	1.18	0.17	0.19	1.21	2.19	1.42	0.12	2.70	0.17	1.16
3p	0.17	1.21	0.19	0.19	1.27	2.21	1.10	0.14	2.72	0.17	1.32
3q	0.02	0.13	0.08	0.17	0.18	0.16	0.16	0.11	0.18	0.15	0.17
3r	0.13	0.14	0.16	0.18	0.18	1.90	1.80	0.17	2.40	0.18	2.00
3s	0.09	0.12	0.15	0.16	0.16	0.15	0.15	0.09	0.18	0.15	0.17
3t	0.12	0.13	0.15	0.17	0.15	0.16	0.14	0.15	0.15	0.14	2.00
3u	0.08	0.14	0.14	0.17	0.15	0.18	0.17	0.13	2.00	0.15	0.18
3v	0.09	0.14	0.05	0.17	0.17	0.17	0.17	0.14	2.18	0.15	0.18
3w	0.02	0.13	0.01	0.13	0.15	0.14	0.14	0.06	0.16	0.09	0.11
3x	0.06	0.14	0.04	0.14	0.17	0.17	0.14	0.11	0.19	0.09	0.14
4a	0.11	0.12	0.14	0.16	0.17	0.17	0.18	0.11	0.17	0.16	0.17
4b	0.13	0.17	0.17	0.17	0.18	0.19	0.17	0.13	0.15	0.16	0.17
4c	0.14	0.17	0.11	0.16	0.17	0.19	0.17	0.13	0.17	0.18	0.19
4d	0.08	0.02	0.07	0.13	0.16	0.14	0.17	0.05	0.17	0.16	0.17
4e	0.13	0.16	0.17	0.16	0.15	0.17	0.12	0.13	0.14	0.16	0.18
4f	0.11	0.17	0.05	0.17	0.17	0.18	0.17	0.11	0.17	0.18	0.17
4g	0.01	0.01	0.01	0.11	0.14	0.11	0.15	0.02	0.03	0.13	0.05
4h	0.03	0.01	0.05	0.14	0.17	0.18	0.17	0.11	0.16	0.17	0.17
ADR[i]	1.79	7.25	0.16	0.14	0.17	0.17	0.17	0.17	0.14	0.10	1.81
DC-81 (**1**)	2.37	0.16	0.14	0.15	0.17	0.17	0.16	0.17	0.11	1.49	0.20

[a] Values represent the mean of three determinations. [b] Breast cancer. [c] Lung cancer. [d] Ovarian cancer. [e] Oral cancer. [f] Cervical cancer. [g] Colon cancer. [h] Prostate cancer. [i] Adriamycin.

Effect of PBD conjugates on the expression of p53 and caspase-9

The activation of p53 plays an important role in the induction of apoptosis by various agents,[46] and it is well known that *p53* is a tumor-suppressor gene, the product of which exerts its antiproliferative effects through its function as a sequence-specific DNA binding transcription factor. To determine the effect of PBD conjugates on the expression of the p53-dependent apoptotic pathway, MCF-7 cells were treated with conjugates **3g**, **3h**, and **4g** at 4 μM for 24 h, and Western blot analysis was carried out with a p53-specific antibody. The levels of p53 were up-regulated in the presence of all conjugates tested relative to DC-81 (**1**) and conjugate partner **2** (Figure 4a). Similar results were observed in the results of ELISA, as shown in Figure 4b. Interestingly, the increase in the level of p53 protein was more prominent for conju-

Table 3. Cell-cycle distribution of MCF-7 cells with DC-81 (**1**) [4 μM], conjugate partner **2**, and PBD conjugates **3g**, **3h**, and **4g**.[a]

Compd	G_0/G_1	S	G_2/M
Control	52.09 ± 1.38	10.39 ± 0.40	37.51 ± 0.93
DC-81 (**1**)	68.14 ± 0.13	8.48 ± 0.22	23.37 ± 0.33
2	59.80 ± 0.67	10.34 ± 0.40	29.85 ± 0.28
3g	87.02 ± 0.27	4.33 ± 0.32	8.70 ± 0.10
3h	84.17 ± 2.00	5.32 ± 0.35	10.50 ± 1.80
4g	82.42 ± 0.64	5.05 ± 0.13	12.53 ± 0.57

[a] Values represent the mean ± SD of at least three experiments, each performed in triplicate.

Table 4. Percentage of apoptosis (G_0 cells) with DC-81 (**1**), conjugate partner **2**, and PBD conjugates **3g**, **3h**, and **4g**.[a]

Compd	G_0 cells [%] at 4 μM	at GI_{50}	at IC_{50}
Control	3.72 ± 0.25	1.13 ± 0.41	2.76 ± 0.25
DC-81 (**1**)	4.24 ± 0.01	1.67 ± 0.28	3.57 ± 0.36
2	3.33 ± 0.14	1.57 ± 0.40	1.56 ± 0.46
3g	15.26 ± 0.21	4.30 ± 0.64	11.10 ± 1.10
3h	7.25 ± 0.34	2.66 ± 0.51	5.34 ± 0.57
4g	4.55 ± 0.45	1.83 ± 0.26	2.66 ± 0.12

[a] Values represent the mean ± SD of at least three experiments, each performed in triplicate.

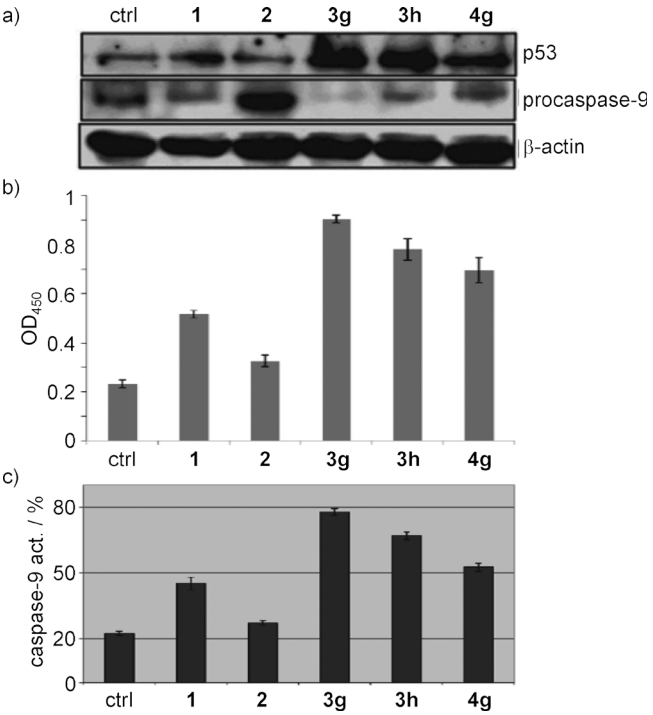


Figure 4. a) Effect of PBD conjugates on the expression of p53 and procaspase-9. MCF-7 cells were treated with DC-81 (**1**), **2**, **3g**, **3h**, and **4g** at 4 mM for 24 h. Cell lysates were collected, and protein expression levels were determined by Western blot analysis using specific antibodies; β-actin was used as loading control. Control (ctrl) lanes: untreated. b) Effect of PBD conjugates on the expression p53. MCF-7 cells were treated with the indicated compounds at 4 mM for 24 h. Cell lysates were collected and observed for p53 protein expression levels using a p53 ELISA kit. c) Effect of PBD conjugates on caspase-9 protein activity. MCF-7 cells were treated with the indicated compounds at 4 mM for 24 h and then subjected to fluorimetry as detailed in the Experimental Section. Here DC-81 (**1**) was used as the positive control with compound **2** as the conjugate partner.

gate **3g**, as evident in Figure 4a and 4b, thus indicating that such PBD conjugates suppress tumor cell proliferation.

Given the observed increase in p53 levels in the presence of these PBD conjugates, we wanted to shed light on the molecular mechanism behind the induction of apoptosis. The MCF-7 cell line lacks endogenous caspase-3,[47] whereas caspase-9 plays an important role in mediating drug-induced apoptosis.[48] Thus the role of caspases was examined in MCF-7 cells treated with conjugates **3g**, **3h**, and **4g** at 4 μM for 24 h. Cell lysates were analyzed for active caspase-9 expression levels by a fluorescence-based caspase-9 assay. Up-regulation of caspase-9 was effected by these conjugates relative to both controls, DC-81 (**1**) and conjugate partner **2**, as shown in Figure 4c. A similar observation was made by Western blot analysis, indicating a degradation of procaspase-9 and production of active caspase-9; such activity is ranked in the order **3g** > **3h** > **4g** as shown in Figure 4a. Apoptosis is more prominent for all three conjugates relative to both controls, and is most prominent in the presence of **3g**, as evident in Figure 4.

Effect of PBD conjugates on DNA

A terminal transferase dUTP nick end labeling (TUNEL) assay was carried out with a view to understand the effects of these PBD conjugates on DNA by treating MCF-7 cells with DC-81 (**1**) and conjugate **3g** at 4 μM for 24 h. Confocal microscopy revealed DNA fragmentation (green FITC staining) in the presence of conjugate **3g** as well as DC-81 (**1**), but not in untreated cells (Figure 5a). Moreover, Hoechst DNA staining also indicated the formation of apoptotic bodies (blebbing) in cells treated with conjugate **3g**, which is more prominent than for cells treated with positive control, DC-81 (**1**), as apparent in Figure 5b. These results further confirm the apoptotic-inducing ability of such PBD conjugates.

Conclusions

In summary, a library of aryl-substituted naphthalene–PBD conjugates were synthesized and evaluated for their anticancer potential. Thermal denaturation studies showed that these conjugates exhibit higher DNA binding affinity than the naturally occurring PBD, DC-81 (**1**). All these PBD conjugates **3a**–**x** and **4a**–**h** showed significant anticancer activity, with GI_{50} values ranging from 0.01 to 3.41 μM, in comparison with some previously reported PBD conjugates such as phosphonate-linked PBD conjugates (GI_{50} values in the 0.17–30.50 μM range),[19] 1,2,3-triazole-linked PBD conjugates (GI_{50} values in the 0.13–30.50 μM range),[20] and triazolobenzothiadiazine-linked PBD conjugates (GI_{50} values in the 0.22–30.30 μM range).[49] Moreover, from the MTT proliferation assay, the three most promising conjugates **3g**, **3h**, and **4g** showed higher cytotoxicity in MCF-7 cells. Flow cytometry (FACS) analysis

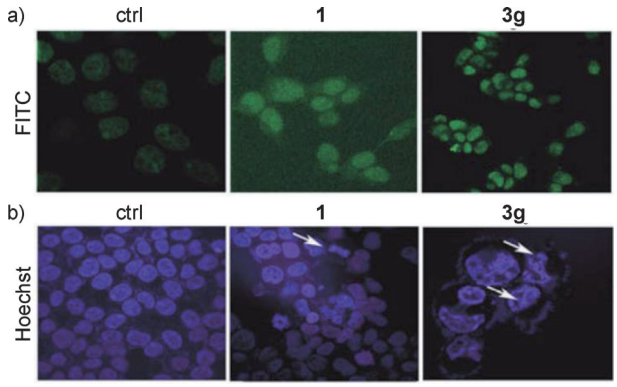


Figure 5. a) Effect of PBD conjugates on DNA fragmentation. MCF-7 cells were treated with DC-81 (**1**) and conjugate **3g** at 4 mM for 24 h. DNA fragmentation was monitored by primary antibody binding to fragmented DNA, followed by detection with FITC-labeled secondary antibody. Apoptotic cells are thus stained green. Here DC-81 was used as positive control; **3g** is the most effective conjugate of this series. Control cells yield only background color. The apoptotic nature of the compounds is apparent by fragmented nuclei in the compound-treated cells. b) Effect of PBD conjugates on apoptosis. MCF-7 cells were treated with DC-81 and conjugate **3g** at 4 mM for 24 h. Hoechst 33258 was used to stain the nuclei of both control and conjugate **3g**-treated cells. Here DC-81 was used as positive control; **3g** is the most effective conjugate in this series. As above, the apoptotic nature of the compounds is apparent by fragmented nuclei in compound-treated cells.

showed a greater cell population in the G_0/G_1 phase, indicating that the PBD conjugates possess the ability to induce apoptosis. To understand the mechanism of cell death, further biological assays such as Western blot analyses of p53, procaspase-9, and caspase-9 were carried out. The results indicated an upregulation of p53 and caspase-9 upon treatment with conjugates **3g**, **3h**, and **4g**. Furthermore, TUNEL assays and Hoechst staining further confirmed apoptosis effected by conjugate **3g**. This investigation reveals that linking of aryl-substituted naphthalene derivatives to the PBD ring system enhances anticancer activity. Based on the results of the studies reported herein, it is evident that conjugate **3g** is a suitable candidate for further detailed studies either alone or in combination with existing therapies.

Experimental Section

Biological assays

Thermal denaturation studies

Compounds **3a**–**x** and **4a**–**h** were subjected to DNA thermal melting (denaturation) studies with duplex calf thymus DNA (CT-DNA) using a modified published procedure.[50] Working solutions were produced by appropriate dilution in aqueous buffer (10 mM Na_2HPO_4/NaH_2PO_4, 1 mM Na_2EDTA, pH 7.00 ± 0.01) containing CT-DNA (100 μM in phosphate) and the PBD (20 μM); these were prepared by the addition of concentrated PBD solutions in MeOH to obtain a fixed PBD/DNA molar ratio of 1:5. The DNA/PBD solutions were incubated at 37 °C for 0 h prior to analysis; samples were monitored at λ 260 nm using a Beckman DU-7400 spectrophotometer fitted with a 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 $d(A_{260})/dT$ derivative plots. Results for each conjugate are shown as the mean ± SD from at least three determinations and are corrected for the effects of MeOH co-solvent using a linear correction term.[51] Ligand-induced alterations 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 PBD-free CT-DNA is 68.5 ± 0.001; the fixed PBD/DNA ratio used did not result in binding saturation of the host DNA duplex for any compound examined.

Cell line maintenance

The MCF-7 (human breast cancer) cell line was obtained from the American Type Culture Collection (ATCC), USA. MCF-7 cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Invitrogen), supplemented with 10% fetal calf serum and 100 U mL^{-1} penicillin and 100 mg mL^{-1} streptomycin sulfate (Sigma). The cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2.

Cell viability (MTT assay)

Cell viability was assessed by MTT assay using the Vybrant MTT cell proliferation assay kit (Invitrogen). The assay is based on the ability of viable cells to reduce MTT to insoluble formazan crystals by mitochondrial dehydrogenase. Briefly, MCF-7 cells were seeded in a 96-well plate (TPP) at a cell density of 10000 cells per well. After incubation overnight, the cells were treated with compounds DC-81 (**1**), **2**, **3f**–**3i**, **3s**, **3w**, **4d**, and **4g** at 4 μM for 24 h. The medium was then discarded and replaced with fresh medium (100 μL) followed by addition of 10 μL MTT dye. Plates were incubated at 37 °C for 2 h. The resulting formazan crystals were solubilized in 100 μL extraction buffer (SDS). The optical density (OD) was read at λ 570 nm using Multimode Varioscan FLASH (Thermo Fischer Scientific).

Cell cycle analysis

MCF-7 cells (5×10^5) were seeded in a 60 mm Petri dish and were allowed to grow for 24 h. Compounds DC-81 (**1**), **2**, **3g**, **3h**, and **4g** (each at 4 μM) were added to the culture medium, and the cells were incubated for an additional 24 h. Cells were harvested with trypsin/EDTA, fixed with ice-cold 70% EtOH at 4 °C for 30 min, washed with PBS and incubated with 1 mg mL^{-1} RNase A solution (Sigma) at 37 °C for 30 min. Cells were collected by centrifugation at 2000 rpm (Heraeus Sorvall swinging bucket rotor (model # 75002000), max speed: 4700 rpm, Heraeus Multifuge 1S-R, Thermo Scientific) for 5 min and further stained with 250 mL DNA staining solution [10 mg propidium iodide (PI), 0.1 mg trisodium citrate, and 0.03 mL Triton X-100 dissolved in 100 mL sterile Milli-Q water at room temperature for 30 min in the dark]. The DNA content of 20000 events was measured by flow cytometry (DAKOCYTOMATION, Beckman Coulter, Brea, CA, USA). Histograms were analyzed using Summit software.

Protein extraction and Western blot analysis

Total cell lysates from cultured MCF-7 cells after compound treatment [DC-81 (**1**), **2**, **3g**, **3h**, and **4g**] as described above were obtained by lysing the cells in ice-cold RIPA buffer (1 × PBS, 1% NP-40, 0.5% sodium deoxycholate, and 0.1% SDS) containing 100 mg mL^{-1} PMSF, 5 mg mL^{-1} aprotinin, 5 mg mL^{-1} leupeptin,

5 mg mL^{-1} pepstatin, and 100 mg mL^{-1} NaF. After centrifugation at 12000 rpm (fixed angle rotor (model # 75003348), max speed: 22000 rpm, Heraeus Multifuge 1S-R, Thermo Scientific) for 10 min, the protein content in the supernatant was quantified by the Bradford method (Bio-Rad) using a Multimode Varioscan instrument (Thermo Fischer Scientific). Protein (50 μg per lane) was applied to a 12% SDS polyacrylamide gel. After electrophoresis, the protein was transferred to a polyvinylidine difluoride (PVDF) membrane (Amersham Biosciences). The membrane was blocked at room temperature for 2 h in TBS containing 0.1% Tween-20 (TBST) containing 5% blocking powder (Santa Cruz Biotechnology). The membrane was washed with TBST for 5 min, and primary antibody was added and incubated at 4 °C overnight. Mouse monoclonal β-actin antibody and rabbit polyclonal procaspase-9 antibody were purchased from Imgenex. Mouse monoclonal p53 antibody was purchased from Santa Cruz Biotechnology. After three TBST washes, the membrane was incubated with corresponding horseradish peroxidase (HRP)-labeled secondary antibody (1:2000; Santa Cruz) at room temperature for 1 h. Membranes were washed with TBST three times for 15 min, and the protein blots were visualized with a Super Signal West Pico chemiluminescence reagent (Thermo Fischer Scientific). Blot images were collected by X-ray autoradiography.

p53 ELISA

Enzyme-linked immunorsorbent assays (ELISA) for p53 were conducted with the p53 ELISA kit from Alexis Biochemical. MCF-7 cells were treated with compounds DC-81 (**1**), **2**, **3g**, **3h**, and **4g** at 4 μM for 24 h. Cell lysates were isolated and added to microplate wells containing p53 antibody. Biotin-conjugated anti-human p53 monoclonal antibody (100 μL) was then added. After the incubation period and washing steps, bound p53 was detected by using streptavidin–HRP secondary antibody (150 μL). The colored product obtained was detected by measuring OD at λ 450 nm; the OD is directly proportional to the amount of p53 protein present in the sample.

Caspase-9 assay

The Apoalert caspase-9/6 fluorescent assay kit (Clonetech, CA, USA) was used according to the manufacturer's recommendations. MCF-7 cells were treated with compounds DC-81 (**1**), **2**, **3g**, **3h**, and **4g** at 4 μM as obtained from FACS analysis. Here the substrate used is LEHD–AMC, which was added to the cell lysates, and incubation was carried out at 37 °C for 1 h. Readings were taken at λ_{ex} 400 nm and λ_{em} 505 nm.

TUNEL assay

Terminal transferase dUTP nick end labeling (TUNEL) assays were conducted with the Apoalert DNA fragmentation assay kit (Clonetech). Apoptosis-induced nuclear DNA fragmentation was determined using this assay, which was conducted according to the manufacturer's recommendations and is based on the principle of terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling. TdT catalyzes the incorporation of fluorescein–dUTP at the free 3′-hydroxy ends of fragmented DNA. Flourescein-labeled DNA can be detected by confocal microscopy.

Chemistry

All chemicals and reagents were purchased from Sigma–Aldrich (St. Louis, MO, USA), Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA) or Spectrochem Pvt. Ltd. (Mumbai, India), and were used without further purification. Reactions were monitored by TLC, performed on glass plates containing silica gel 60 GF_{254}, and visualized by UV light or iodine indicator. Column chromatography was performed with Merck 60–120 mesh silica gel. ^{1}H NMR and ^{13}C NMR spectra were recorded on Gemini Varian VXR Unity (200 MHz) or Bruker UXNMR/XWIN-NMR (300 MHz) instruments. Chemical shifts (δ) are reported in ppm relative to the peak for $(CH_3)_4Si$ as an internal standard, and coupling constants are reported in Hz. High-resolution mass spectra (HRMS) were recorded on a QSTAR XL Hybrid MS–MS mass spectrometer. ESIMS data were recorded on a Micromass Quattro LC instrument using ESI^+ software with a capillary voltage of 3.98 kV and an ESI positive ion trap detector. Melting points were determined with an Electrothermal melting point apparatus and are uncorrected.

General procedure for synthesis of 7a–h (Suzuki coupling)

A catalytic amount (0.02–0.04 mmol) of $Pd(PPh_3)_4$ and aqueous 2 M $NaHCO_3$ (1 mL) were added to a solution of 6-bromo-2-naphthol (**5**, 223 mg, 1 mmol) in toluene (10 mL). A solution of substituted phenylboronic acids (**6a–h**, 1 mmol) in 3 mL EtOH was added and stirred at RT under N_2. After 10 min, the solution was heated at 80–90 °C for 2–4 h. TLC showed the disappearance of starting materials, and the reaction mixture was cooled to RT and extracted with EtOAc (3×30 mL). The organic phases were combined, washed with H_2O and brine, dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure to obtain the crude product. This residue was purified by column chromatography using EtOAc and hexane to afford compounds **7a–h**.

General procedure for synthesis of 8a–x

Anhydrous K_2CO_3 (4 mmol) and various dibromoalkanes (3 mmol) were added to a solution of compounds **7a–h** (1 mmol) in dry acetone (10 mL), and the reaction mixture was held at reflux for 12–24 h. After completion of the reaction, K_2CO_3 was removed by suction filtration, and the solvent was evaporated under reduced pressure to obtain the crude product. This was further purified by column chromatography using EtOAc and hexane to afford compounds **8a–x**.

***tert*-Butyl-4-(3-hydroxy-4-methoxybenzoyl)-1-piperazinecarboxylate (10):** A catalytic amount of DMF (2–4 drops) was added to a stirred solution of 3-hydroxy-4-methoxybenzoic acid (isovanillic acid, 168 mg, 1 mmol) and $SOCl_2$ (0.18 mL, 2.5 mmol) in dry CH_2Cl_2 (5 mL), and the mixture was stirred for 2 h under N_2. The solvent was evaporated under vacuum, the resulting acid chloride was washed with dry CH_2Cl_2 (3×10 mL), and the solvent was evaporated under reduced pressure to obtain the product 3-hydroxy-4-methoxybenzoyl chloride as a light-yellow oil. Owing to potential stability problems, this product was taken directly to the next step. Et_3N (0.14 mL, 2 mmol) was added dropwise to a stirred solution of *N*-Boc-piperazine (186 mg, 1 mmol) in dry CH_2Cl_2 (10 mL) at 0 °C over 10 min; 3-hydroxy-4-methoxybenzoyl chloride (220 mg, 1.2 mmol) dissolved in dry CH_2Cl_2 (5 mL) was then added at the same temperature. The reaction was brought to RT and stirred for another 2 h. After completion of the reaction, the solvent was evaporated and extracted with CH_2Cl_2 (3×20 mL), washed with aqueous $NaHCO_3$ (2 M) followed by brine, separated, and dried

over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure and further purified by column chromatography using with EtOAc and hexane (1:1) as eluent to afford compound **10** as a white solid.

(3-Hydroxy-4-methoxybenzoyl)-1-piperazine (11): Trifluoroacetic acid (TFA; 0.9 mL, 13 mmol) was added slowly to compound **10** (336 mg, 1 mmol) in dry CH_2Cl_2 (25 mL) at 0 °C, and the reaction mixture was stirred at RT for 2 h. After completion, the reaction mixture was concentrated, and the resulting compound was suspended in aqueous $NaHCO_3$ (2 M, 20 mL), the solution was extracted into CH_2Cl_2 (3–30 mL), and the combined organic phases were washed with H_2O and brine (2×30 mL), dried over anhydrous Na_2SO_4, and the solvent was evaporated under reduced pressure to obtain the product. This compound was used without further purification for next step to afford compound **11** as a light-yellow oil.

General procedure for synthesis of 13a–h

Compounds **13a–h** were prepared by following the method described for the preparation of compound **7** (Suzuki cross-coupling reaction), employing compound **12** (279 mg, 1 mmol) and **6a–h** (1 mmol). The crude product was purified by column chromatography using EtOAc and hexane to afford compounds **13a–h**.

General procedure for synthesis of 14a–h

Aqueous NaOH (1 N, 1 mL) was added dropwise to a stirred solution of ethyl-6-(substituted phenyl)-2-naphthoate (**13a-h**, 1 mmol) in EtOH (10 mL) at RT, and the reaction mixture was held at reflux (70–80 °C) for 1–2 h. After completion of the reaction, the mixture was acidified to pH 7 with 12 N HCl and extracted with CH_2Cl_2 (3× 30 mL), dried over Na_2SO_4, and the EtOH solvent was removed under reduced pressure to obtain the acid product as a white solid (91–95% yield). DMF (2–3 drops) was added to a stirred suspension of acid compound (1 mmol) and $SOCl_2$ (0.18 mL, 2.5 mmol) in dry CH_2Cl_2 (5 mL), and stirring was continued at RT for 2–4 h under N_2. The solvent was evaporated under reduced pressure to obtain the product (**14a–h**) as a pale-yellow oil, which, due to potential stability problems, was used directly in the next step.

General procedure for synthesis of 15a–h

Et_3N (0.14 mL, 2 mmol) was added dropwise to a stirred solution of compound **11** (186 mg, 1 mmol) in dry CH_2Cl_2 (10 mL) at 0 °C over 10 min; acid chloride (**14a–h**, 1.2 mmol) dissolved in dry CH_2Cl_2 (5 mL) was then added at the same temperature. The reaction was brought to RT and stirred for another 2–4 h. After completion of the reaction, the solvent was evaporated and extracted with CH_2Cl_2 (3×20 mL). The reaction mixture was washed with aqueous $NaHCO_3$ (2 M) followed by brine, separated, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to obtain crude product, which was further purified by column chromatography with EtOAc and hexane to afford the pure compounds **15a–h**.

General procedure for synthesis of 16a–h

Compounds **16a–h** were prepared according to the method described for the general procedure for the synthesis of **8a–x**, employing compounds **15a–h** (1 mmol) and 1,3-dibromopropane (3 mmol). The crude product was purified by column chromatography using EtOAc and hexane to afford compounds **16a–h**.

General procedure for synthesis of 18a–x and 19a–h

Anhydrous K_2CO_3 (552 mg, 4 mmol) and naphthalene precursors (**8a–x** and **16a–h** 1 mmol) were added to a solution of (2*S*)-*N*-[4-hydroxy-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethylthioacetal (**17**, 400 mg, 1 mmol) in dry acetone (20 mL). The reaction mixture was held at reflux for 12–24 h. After completion of the reaction as indicated by TLC, K_2CO_3 was removed by suction filtration, and the solvent was removed under vacuum. The crude product was further purified by column chromatography using EtOAc and hexane to afforded pure nitro compounds **18a–x** and **19a–h**.

General procedure for synthesis of 3a–x and 4a–h

$SnCl_2{\cdot}2H_2O$ (1 mmol) was added to a solution of nitro compounds (**18a–x** and **19a–h**, 0.5 mmol) in MeOH (20 mL) and held at reflux for 2–4 h. The MeOH was evaporated under vacuum, and the aqueous layer was carefully adjusted to pH 8 with a solution of 10% $NaHCO_3$ and then extracted with EtOAc (3×30 mL). The combined organic phase was dried over anhydrous Na_2SO_4 and evaporated under vacuum to afford the aminodiethyl thioacetal, which due to potential stability problems, was taken directly to the next step. A solution of aminodiethyl thioacetal (0.5 mmol), $HgCl_2$ (1.13 mmol), and $CaCO_3$ (1.23 mmol) in CH_3CN/H_2O (4:1) was stirred slowly at RT until TLC indicated complete loss of starting material (12 h). The reaction mixture was diluted with EtOAc (30 mL) and filtered through a Celite bed. The clear-yellow organic supernatant was washed with 5% $NaHCO_3$ (20 mL) and brine (20 mL), and the combined organic phase was dried over anhydrous Na_2SO_4. The organic layer was evaporated under vacuum and purified by column chromatography using MeOH/$CHCl_3$ to give the final products **3a–x** and **4a–h**.

7-Methoxy-8-{6-(4-fluorophenyl)-2-naphthyl]oxy)propoxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (3a): White solid (170 mg, 65% yield): R_f=0.3 (2% MeOH/$CHCl_3$); mp: 69–71 °C; $[\alpha]_D^{27}$=+198.0 (c=0.1, $CHCl_3$); IR (KBr): ν_{max}=3317 (br), 2929, 2873, 1605, 1507, 1431, 1253, 1202, 1056, 839 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ=7.91 (s, 1H, ArH), 7.73 (d, J=3.8 Hz, 1H, imine-H), 7.59–7.71 (m, 4H, ArH), 7.52 (s, 1H, ArH), 7.28 (s, 1H, ArH), 7.11–7.21 (m, 4H, ArH), 6.89 (s, 1H, ArH), 4.25–4.37 (m, 4H, 2×OCH_2), 3.86 (s, 3H, OCH_3), 3.43–3.83 (m, 3H, NCH, NCH_2), 2.36–2.49 (m, 2H, CH_2), 1.94–2.15 ppm (m, 4H, 2× CH_2); (ESI) MS: m/z 525 $[M+1]^+$; HRMS (ESI m/z) for $C_{32}H_{30}N_2O_4F$, calcd 525.2451, found 525.2440 $[M+1]^+$.

7-Methoxy-8-{6-(4-trifluoromethyl)phenyl)-2-naphthyl]oxy)propoxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (3b): White solid (201 mg, 70% yield): R_f=0.3 (2.5% MeOH/$CHCl_3$); mp: 84–86 °C; $[\alpha]_D^{27}$=+155.0 (c=0.1, $CHCl_3$); IR (KBr): ν_{max}=3325 (br), 2959, 2876, 1609, 1508, 1433, 1326, 1255, 1200, 1119, 1066, 843 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ=7.98 (s, 1H, ArH), 7.76–7.85 (m, 4H, ArH), 7.74 (s, 2H, ArH), 7.72 (d, J= 3.3 Hz, 1H, imine-H), 7.28 (s, 1H, ArH), 7.21 (s, 2H, ArH), 7. 15–7.19 (m, 1H, ArH), 6.44 (s, 1H, ArH), 4.24–4.37 (m, 4H, 2×OCH_2), 3.87 (s, 3H, OCH_3), 3.42–3.82 (m, 3H, NCH, NCH_2), 2.35–2.48 (m, 2H, CH_2) 1.92–2.11 ppm (m, 4H, 2×CH_2); (ESI) MS: m/z 575 $[M+1]^+$; HRMS (ESI m/z) for $C_{33}H_{30}N_2O_4F_3$ calcd 575,1328, found 575.1321 $[M+1]^+$.

7-Methoxy-8-{6-(4-trifluoromethoxy)phenyl)-2-naphthyl]oxy)propoxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (3c): White solid (200 mg, 68% yield): R_f = 0.3 (3% MeOH/$CHCl_3$); mp: 75–77 °C; $[\alpha]_D^{27}$ = +103.0 (c = 0.1, $CHCl_3$); IR (KBr): ν_{max} = 3329 (br), 2934, 2866, 1607, 1507, 1464, 1259, 1206, 1054, 847 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ = 7.93 (s, 1H, ArH), 7.79 (d, 2H, J = 8.5 Hz, ArH), 7.72 (s, 1H, ArH), 7.68 (s, 2H, ArH), 7.64 (d, J = 4.3, Hz, 1H, imine-H), 7.52 (s 1H, ArH), 7.31 (d, J = 8.5 Hz, 2H, ArH), 7.14–7.22 (m, 2H), 6.88 (s, 1H, ArH), 4.23–4.38 (m, 4H, 2× OCH_2), 3.95 (s, 3H, OCH_3), 3.49–3.88 (m, 3H, NCH, NCH_2), 2.32–2.46 (m, 2H, CH_2), 1.89–2.14 ppm (m, 4H, 2×CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.5, 162.3, 156.9, 151.0, 148.3, 144.8, 139.8, 137.0, 134.8, 133.7, 129.5, 128.9, 128.3, 127.3, 125.5, 121.1, 120.0, 119.3, 112.3, 111.6, 110.4, 106.3, 96.5, 65.4, 64.2, 56.0, 53.5, 46.4, 29.4, 24.6, 23.0 ppm; (ESI) MS: m/z 591 $[M]^+$; (ESI) MS: m/z 591 $[M+1]^+$; HRMS (ESI m/z) for $C_{33}H_{30}N_2O_5F_3$ calcd 591.1429, found 591.1423 $[M+1]^+$.

7-Methoxy-8-{6-(4-methoxyphenyl)-2-naphthyl]oxy)propoxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (3d): White solid (166 mg, 62% yield): R_f = 0.3 (3% MeOH/$CHCl_3$); mp: 86–88 °C; $[\alpha]_D^{27}$ = +137.0 (c = 0.1, $CHCl_3$); IR (KBr): ν_{max} = 3375 (br), 2930, 2861, 1604, 1506, 1461, 1247, 1178, 1023, 836 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ = 7.91 (s, 1H, ArH), 7.76–7.80 (m, 1H, ArH), 7.73 (d, J = 8.1, 1H, ArH), 7.68 (d, J = 8.1, 1H, ArH), 7.64 (d, J = 3.6 Hz, 1H, imine-H), 7.62 (s, 2H, ArH), 7.52 (s, 1H, ArH), 7.12–7.19 (m, 2H, ArH), 6.98–7.04 (m, 2H, ArH), 6.88 (s, 1H, ArH), 4.27–4.38 (m, 4H, 2×OCH_2), 3.95 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.74–3.81 (m, 1H, NCH), 3.53–3.68 (m, 2H, NCH_2), 2.35–2.48 (m, 2H, CH_2), 2.20–2.31 (m, 2H, CH_2), 1.98–2.09 ppm (m, 2H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.5, 162.3, 158.9, 156.5, 150.5, 147.6, 140.5, 135.8, 133.5, 133.2, 129.4, 129.1, 128.0, 127.0, 125.7, 124.7, 120.1, 119.1, 114.1, 111.5, 110.5, 106.4, 65.4, 64.1, 56.0, 55.2, 53.5, 46.5, 29.4, 29.0, 24.0 ppm; (ESI) MS: m/z 537 $[M+1]^+$; HRMS (ESI m/z) for $C_{33}H_{33}N_2O_5$ calcd 537.3776, found 537.3768 $[M+1]^+$.

7-Methoxy-8-{6-(4-(*N*,*N*-dimethylamino)phenyl)-2-naphthyl]oxy)-propoxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepine-5-one (3e): White solid (164 mg, 60% yield): R_f = 0.3 (3.0% MeOH/$CHCl_3$); mp: 89–91 °C; $[\alpha]_D^{27}$ = +181.0 (c = 0.1, $CHCl_3$); IR (KBr): ν_{max} = 3312 (br), 2927, 1605, 1503, 1433, 1256, 1200, 1057, 806 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 7.90 (s, 1H, ArH), 7.66–7.77 (m, 3H, ArH), 7.62 (d, J = 3.7 Hz, 1H, imine-H), 7.60 (s, 2H, ArH), 7.52 (s, 1H, ArH), 7.12–7.16 (m, 2H, ArH), 6.89 (s, 2H, ArH), 6.85 (s, 1H, ArH), 4.27–4.36 (m, 4H, 2×OCH_2), 3.94 (s, 3H, OCH_3), 3.75–3.90 (m, 1H, NCH), 3.52–3.66 (m, 2H, NCH_2) 3.01 (s, 6H, 2×$N(CH_3)_2$), 2.40–2.48 (m, 2H, CH_2), 2.21–2.29 (m, 2H, CH_2), 1.99–2.07 ppm (m, 2H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.5, 162.3, 156.3, 150.5, 149.7, 147.6, 140.5, 136.3, 132.9, 129.3, 129.0, 127.8, 127.6, 126.9, 125.6, 123.9, 120.1, 118.9, 112.8, 111.4, 110.4, 106.4, 65.4, 64.1, 56.0, 53.5, 46.5, 40.5, 29.4, 29.0, 24.0 ppm; (ESI) MS: m/z 550 $[M+1]^+$; HRMS (ESI m/z) for $C_{34}H_{36}N_3O_4$ calcd 550.2967, found 550.2961 $[M+1]^+$.

7-Methoxy-8-{6-(3-chlorophenyl)-2-naphthyl]oxy)propoxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (3f): White solid (191 mg, 70% yield): R_f = 0.3 (2% MeOH/$CHCl_3$); mp: 80–82 °C; $[\alpha]_D^{27}$ = +210.0 (c = 0.1, $CHCl_3$); IR (KBr): ν_{max} = 3356 (br), 2928, 2873, 1597, 1507, 1431, 1254, 1200, 1169, 851 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 7.94 (s, 1H, ArH), 7.76–7.80 (m, 2H, ArH), 7.66–7.69 (m, 2H, ArH), 7.63 (d, J = 4.5 Hz, 1H, imine-H), 7.57 (d, J = 7.5 Hz, 1H, ArH), 7.52 (s 1H, ArH), 7.30–7.41 (m, 2H, ArH), 7.16–7.21 (m, 2H, ArH), 6.88 (s 1H, ArH), 4.28–4.38 (m, 4H, 2×OCH_2), 3.95 (s, 3H, OCH_3), 3.77–3.87 (m, 1H, NCH), 3.49–3.69 (m, 2H, NCH_2), 2.39–2.46 (m, Hz, 2H, CH_2), 2.24–2.31 (m, 2H, CH_2), 2.00–2.09 ppm (m, 2H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.4, 162.3, 157.9, 150.5, 147.6, 142.8, 140.4, 134.7, 134.5, 133.8, 129.9, 129.6, 128.9, 127.3, 127.0, 126.8, 125.5, 125.3, 125.1, 120.1, 119.3, 111.4, 110.4, 106.3, 65.3, 64.1, 55.9, 53.5, 46.5, 29.4, 28.9, 24.0 ppm; (ESI) MS: m/z 542 $[M+1]^+$; HRMS (ESI m/z) for $C_{32}H_{30}N_2O_4Cl$ calcd 542.2156, found 542.2160 $[M+1]^+$.

7-Methoxy-8-{6-(3,4-dimethoxyphenyl)-2-naphthyl]oxy)propoxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (3g): White solid (186 mg, 66% yield): R_f = 0.3 (3.5% MeOH/$CHCl_3$); mp: 88–90 °C; $[\alpha]_D^{27}$ = +174.0 (c = 0.1, $CHCl_3$); IR (KBr): ν_{max} = 3377 (br), 2932, 2869, 1602, 1510, 1460, 1257, 1219, 1168, 1023, 853 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 7.91 (s, 1H, ArH), 7.73–7.84 (m, 2H, ArH), 7.70 (s, 1H, ArH), 7.64 (d, J = 4.4 Hz, 1H, imine-H), 7.52(s, 1H, ArH), 7.13–7.24 (m, 3H, ArH), 6.98 (dd, J = 2.9 Hz, 5.8 Hz, 1H, ArH), 6.88 (s, 1H, ArH), 6.76 (s, 1H, ArH), 4.26–4.39 (m, 4H, 2×OCH_2), 3.98 (s, 3H, OCH_3), 3.95 (s, 6H, 2×OCH_3), 3.75–3.85 (m, 1H, NCH), 3.49–3.64 (m, 2H, NCH_2) 2.20–2.48 (m, 4H, 2×CH_2), 1.97–1.20 ppm (m, 2H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.4, 162.3, 156.6, 150.7, 149.1, 148.3, 147.6, 140.5, 136.0, 134.0, 133.3, 129.4, 129.0, 128.1, 127.0, 125.8, 125.4, 124.8, 120.1, 119.2, 114.6, 111.4, 110.3, 106.4, 65.4, 64.1, 56.0, 55.8, 53.7, 46.5, 29.4, 28.9, 24.0 ppm; (ESI) MS: m/z 567 $[M+1]^+$; HRMS (ESI m/z) for $C_{34}H_{35}N_2O_6$ calcd 567.2314, found 567.2335 $[M+1]^+$.

7-Methoxy-8-{6-(3,4,5-trimethoxyphenyl)-2-naphthyl]oxy)propoxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (3h): White solid (202 mg, 68% yield): R_f = 0.3 (4% MeOH/$CHCl_3$); mp: 92–94 °C; $[\alpha]_D^{27}$ = +178.0 (c = 0.1, $CHCl_3$); IR (KBr): ν_{max} = 3359 (br), 2929, 2876, 1602, 1509, 1432, 1266, 1209, 1157, 853 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 7.91 (s, 1H, ArH), 7.80 (d, J = 6.0 Hz, 1H, ArH), 7.77 (d, J = 4.5 Hz, 1H, ArH), 7.67 (s, 1H, ArH), 7.64 (d, J = 3.7 Hz, 1H, imine-H), 7.52 (s, 1H, ArH) 7.15–7.21 (m, 2H, ArH), 6.86–6.90 (m, 3H, ArH), 4.27–4.38 (m, 4H, 2×OCH_2), 3.97 (s, 6H, 2×OCH_3), 3.95 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.54–3.82 (m, 3H, NCH, NCH_2), 2.40–2.47 (m, 2H, CH_2), 2.23–2.37 (m, 2H, CH_2), 1.99–2.11 ppm (m, 2H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.5, 162.3, 156.8, 153.4, 151.2, 150.5, 147.7, 140.4, 137.1, 136.4, 133.6, 129.4, 129.0, 127.1, 125.8, 125.3, 120.2, 119.3, 111.5, 110.5, 106.4, 104.3, 65.4, 64.2, 60.9, 56.1, 53.6, 46.6, 29.6, 24.1, 22.6 ppm; (ESI) MS: m/z 597 $[M+1]^+$; HRMS (ESI m/z) for $C_{35}H_{37}N_2O_7$ calcd 597.2862, found 597.2848 $[M+1]^+$.

7-Methoxy-8-{6-(4-fluorophenyl)-2-naphthyl]oxy)butoxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (3i): White solid (188 mg, 70% yield): R_f = 0.3 (2.0% MeOH/$CHCl_3$); mp: 78.5–80.5 °C; $[\alpha]_D^{27}$ = +163.0 (c = 0.1, $CHCl_3$); IR (KBr): ν_{max} = 3335 (br), 2928, 2873, 1603, 1507, 1433, 1253, 1202, 1166, 838 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 7.91 (d, 1H, ArH), 7.78 (d, J = 8.3 Hz, 2H ArH), 7.67 (d, J = 3.0 Hz, 1H, imine-H), 7.62–7.65 (m, 3H, ArH), 7.52 (s, 1H, ArH), 7.13–7.19(m, 4H, ArH), 6.84 (s, 1H, ArH), 4.13–4.24 (m, 4H, 2×OCH_2), 3.93 (s, 3H, OCH_3), 3.77–3.81 (m, 1H, NCH) 3.56–3.75 (m, 2H, NCH_2), 2.28–2.36 (m, 2H, CH_2), 2.03–2.24 (m, 4H, 2×CH_2), 1.60–1.72 ppm (m, 2H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.5, 163.8, 162.3, 160.5, 156.9, 150.6, 147.6, 140.4, 137.2, 135.1, 133.5, 129.4, 128.6, 127.1, 125.6, 125.3, 120.0, 119.3, 115.6, 111.4, 110.3, 106.2, 68.5, 67.3, 56.0, 53.6, 46.5, 29.4, 25.8, 25.6, 24.0 ppm; (ESI) MS: m/z 539 $[M+1]^+$; HRMS (ESI m/z) for $C_{33}H_{32}N_2O_4F$ calcd 539.2862, found 539.2848 $[M+1]^+$.

7-Methoxy-8-{6-(4-trifluoromethyl)phenyl)-2-naphthyl]oxy)butoxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (3j): White solid (199 mg, 68% yield): R_f = 0.3 (2.5% MeOH/$CHCl_3$); mp: 91–93 °C; $[\alpha]_D^{27}$ = +140.5 (c = 0.1, $CHCl_3$);

IR (KBr): ν_{max} = 3326 (br), 2960, 2866, 1606, 1509, 1432, 1326, 1254, 1202, 1120, 1056, 841 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 7.93 (s, 1H, ArH), 7.78 (d, J = 8.3 Hz, 1H, ArH), 7.71 (s, 1H, ArH), 7.68 (d, J = 3.7 Hz, 1H, imine-H), 7.52 (s, 1H, ArH), 7.27–7.38 (m, 3H, ArH), 7.15 (d, J = 7.5 Hz, 1H, ArH), 6.98 (s, 1H, ArH), 6.82–6.85 (m, 3H, ArH), 4.12–4.23 (m, 4H, 2×OCH_2), 3.95 (s, 3H, OCH_3), 3.76–3.87 (m, 1H, NCH), 3.57–3.74 (m, 2H, NCH_2), 2.27–2.34 (m, 2H, CH_2), 2.05–2.25 (m, 4H, 2×CH_2), 1.61–1.73 ppm (m, 2H, CH_2); (ESI) MS: m/z 589 $[M+1]^+$; HRMS (ESI m/z) for $C_{34}H_{32}N_2O_4F_3$ calcd 589.4628, found 589.4619 $[M+1]^+$.

7-Methoxy-8-{6-(4-trifluoromethoxy)phenyl)-2-naphthyl]oxy)butoxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (3k): White solid (211 mg, 70% yield): R_f = 0.3 (3.0% MeOH/$CHCl_3$); mp: 86–88 °C; $[\alpha]_D^{27}$ = +168.0 (c = 0.1, $CHCl_3$); IR (KBr): ν_{max} = 3331 (br), 2956, 2856, 1604, 1508, 1421, 1320, 1265, 1197, 1120, 1045, 840 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 7.94 (s, 1H, ArH), 7.79 (d, J = 8.3 Hz, 1H, ArH), 7.72 (s, 1H, ArH), 7.68 (d, J = 3.7, 1H, imine-H), 7.52 (s, 1H, ArH), 7.29–7.37 (m, 3H, ArH), 7.16 (d, J = 7.5 Hz, 1H, ArH), 6.99 (s, 1H, ArH), 6.81–6.86 (m, 3H, ArH), 4.11–4.22 (m, 4H, 2×OCH_2), 3.94 (s, 3H, OCH_3), 3.77–3.88 (m, 1H, NCH), 3.48–3.55 (m, 2H, NCH_2), 2.24–2.33 (m, 2H, CH_2), 2.07–2.28 (m, 4H, 2×CH_2), 1.64–1.75 ppm (m, 2H, CH_2); (ESI) MS: m/z 605 $[M+1]^+$; HRMS (ESI m/z) for $C_{34}H_{32}N_2O_5F_3$ calcd 605.6155, found 605.6147 $[M+1]^+$.

7-Methoxy-8-{6-(4-methoxyphenyl)-2-naphthyl]oxy)butoxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (3l) White solid (170 mg, 62% yield): R_f = 0.3 (3.0% MeOH/$CHCl_3$); mp: 82–84 °C; $[\alpha]_D^{27}$ = +135.0 (c = 0.1, $CHCl_3$); IR (KBr): ν_{max} = 3336 (br), 2932, 2863, 1597, 1521, 1421, 1326, 1255, 1202, 1132, 1025, 820 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 7.91 (s, 1H, ArH), 7.74–7.78 (m, 2H, ArH), 7.69 (d, J = 3.6, 1H, imine-H), 7.61–7.67 (m, 2H, ArH) 7.52 (s, 1H, ArH), 7.14–7.16 (m, 2H, ArH), 7.02 (s, 1H, ArH), 7.00 (s, 1H, ArH), 6.84 (s, 1H, ArH) 4.12–4.25 (m, 4H, 2×OCH_2), 3.93 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.74–3.81 (m, 1H, NCH), 3.50–3.62 (m, 2H, NCH_2), 2.31–2.41 (m, 2H, CH_2), 2.04–2.27 (m, 4H, 2×CH_2), 1.61–1.74 ppm (m, 2H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 162.3, 158.9, 156.8, 155.1, 152.9, 149.6, 136.5, 133.7, 130.9, 129.4, 129.0, 128.1, 127.1, 125.9, 125.8, 124.8, 119.2, 114.2, 111.5, 110.4, 107.5, 106.3, 68.5, 67.3, 56.1, 55.3, 53.6, 46.6, 29.5, 26.0, 25.6, 24.1 ppm; (ESI) MS: m/z 551 $[M+1]^+$; HRMS (ESI m/z) for $C_{34}H_{35}N_2O_5$ calcd 551.2808, found 551.2801 $[M+1]^+$.

7-Methoxy-8-{6-(4-*N*,*N*-dimethylamino)phenyl)-2-naphthyl]oxy)-butoxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (3m): White solid (168 mg, 60% yield): R_f = 0.3 (3% MeOH/$CHCl_3$); mp: 86–88 °C; $[\alpha]_D^{27}$ = +178.0 (c = 0.1, $CHCl_3$); IR (KBr): ν_{max} = 3332 (br), 2960, 2874, 1607, 1508, 1420, 1337, 1251, 1202, 1120, 1056, 830 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 7.91 (s, 1H, ArH), 7.69–7.76 (m, 4H, ArH), 7.66 (d, J = 3.7 Hz, 1H, imine-H), 7.60 (s, 1H, ArH), 7.52 (s, 1H, ArH), 7.12–7.16 (m, 2H, ArH), 6.85–6.89 (m, 3H, ArH), 4.27–4.36 (m, 4H, 2×OCH_2), 3.93 (s, 3H, OCH_3), 3.75–3.88 (m, 1H, NCH), 3.52–3.66 (m, 2H, NCH_2), 3.02 (s, 6H, $N(CH_3)_2$), 2.40–2.44 (m, 2H, CH_2), 2.21–2.30 (m, 4H, 2×CH_2), 1.98–2.08 ppm (m, 2H, CH_2); (ESI) MS: m/z 564 $[M+1]^+$; HRMS (ESI m/z) for $C_{35}H_{38}N_3O_4$ calcd 564.6860, found 564.6851 $[M+1]^+$.

7-Methoxy-8-{6-(3-chlorophenyl)-2-naphthyl]oxy)butoxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (3n): White solid (194 mg, 70% yield): R_f = 0.3 (2.0% MeOH/$CHCl_3$); mp: 74–76 °C; $[\alpha]_D^{27}$ = +124.0 (c = 0.1, $CHCl_3$); IR (KBr): ν_{max} = 3323 (br), 3058, 2931, 2871, 1597, 1507, 1498, 1253, 1202, 1125, 850 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 7.92 (s, 1H, ArH), 7.79 (d, J = 8.3 Hz, 2H, ArH), 7.68 (d, J = 4.1 Hz, 1H, imine-H), 7.63–7.66 (m, 2H, ArH), 7.52(s, 1H, ArH), 7.13–7.19 (m, 5H, ArH), 6.85 (s, 1H, ArH), 4.12–4.25 (m, 4H, 2×OCH_2), 3.93 (s, 3H, OCH_3), 3.78–3.82 (m, 1H, NCH) 3.58–3.74 (m, 2H, NCH_2), 2.27–2.35 (m, 2H, CH_2), 2.02–2.25 (m, 4H, 2×CH_2), 1.22–1.33 ppm (m, 2H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.5, 162.3, 157.1, 150.6, 147.6, 142.8, 140.4, 134.6, 134.4, 133.9, 129.9, 129.6, 128.8, 127.2, 127.0, 126.8, 125.6, 125.4, 125.1, 120.0, 119.4, 111.4, 110.3, 106.2, 68.4, 67.3, 56.0, 53.5, 46.5, 29.4, 25.8, 25.6, 24.0 ppm; (ESI) MS: m/z 556 $[M+1]^+$; HRMS (ESI m/z) for $C_{33}H_{32}N_2O_4Cl$ calcd 556.2312, found 556.2321 $[M+1]^+$.

7-Methoxy-8-{6-(3,4-dimethoxyphenyl)-2-naphthyl]oxy)butoxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (3o): White solid (182 mg, 63% yield): R_f = 0.3 (3.5% MeOH/$CHCl_3$); mp: 70–72 °C; $[\alpha]_D^{27}$ = +168.0 (c = 0.1, $CHCl_3$); IR (KBr): ν_{max} = 3372 (br), 2931, 2867, 1606, 1509, 1459, 1257, 1220, 1170, 1023, 851 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 7.94 (s, 1H, ArH), 7.91(s, 1H, ArH), 7.79 (d, J = 7.3 Hz, 1H, ArH), 7.76 (s, 1H, ArH), 7.70 (d, J = 7.5 Hz, 1H, ArH), 7.68 (d, J = 4.3 Hz, 1H, imine-H), 7.52(s, 1H, ArH), 7.19–7.24(m, 2H, ArH), 7.15 (s, 1H, ArH), 6.99 (t, J = 7.5 Hz, 1H, ArH), 6.82 (s, 1H, ArH), 4.11–4.26 (m, 4H, 2×OCH_2), 3.99 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 3.78–3.88 (m, 1H, NCH), 3.56–3.75 (m, 2H, NCH_2), 2.27–2.35 (m, 2H, CH_2), 1.88–2.21 (m, 4H, 2×CH_2), 1.20–1.34 ppm (m, 2H, CH_2); (ESI) MS: m/z 581 $(M+23)^+$; HRMS (ESI m/z) for $C_{35}H_{37}N_2O_6$ calcd 581.2573, found 581.2566 $[M+1]^+$.

7-Methoxy-8-{6-(3,4,5-trimethoxyphenyl)-2-naphthyl]oxy)butoxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (3p): White solid (201 mg, 66% yield): R_f = 0.3 (4.0% MeOH/$CHCl_3$); mp: 90–92 °C; $[\alpha]_D^{27}$ = +150.0, (c = 0.1, $CHCl_3$); IR (KBr): ν_{max} = 3354 (br), 2929, 2869, 1607, 1507, 1460, 1429, 1270, 1124, 1010, 851 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 7.92 (s, 1H, ArH), 7.77–7.83 (m, 2H, ArH), 7.77 (d, J = 8.3 Hz, 1H, ArH), 7.67 (d, J = 8.3 Hz, 1H, imine-H), 7.52 (s, 1H, ArH), 7.16 (s, 2H, ArH), 6.87 (s, 2H, ArH), 6.84 (s, 1H, ArH), 4.16–4.30 (m, 4H, 2×OCH_2), 3.96 (s, 6H, 2×OCH_3), 3.93 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.74–3.86 (m, 1H, NCH), 3.53–3.73 (m, 2H, NCH_2) 2.27–2.36 (m, 2H, CH_2), 2.01–2.18 (m, 4H, 2×CH_2), 1.64–1.70 ppm (m, 2H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.5, 162.3, 157.0, 153.4, 150.7, 147.7, 140.5, 137.2, 136.3, 133.7, 129.5, 128.9, 127.1, 125.9, 125.6, 125.3, 120.1, 119.4, 111.4, 110.3, 106.3, 104.4, 68.5, 67.4, 60.9, 56.1, 53.6, 46.6, 29.5, 25.9, 25,6, 24.1 ppm; (ESI) MS: m/z 611 $[M+1]^+$; HRMS (ESI m/z) for $C_{36}H_{39}N_2O_7$ calcd 611.6961, found 611.6953 $[M+1]^+$.

7-Methoxy-8-{6-(4-fluorophenyl)-2-naphthyl]oxy)pentoxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (3q): White solid (187 mg, 68% yield): R_f = 0.3 (2.0% MeOH/$CHCl_3$); mp: 70–72 °C; $[\alpha]_D^{27}$ = +141.0 (c = 0.1, $CHCl_3$); IR (KBr): ν_{max} = 3329 (br), 2932, 2869, 1605, 1505, 1466, 1251, 1202, 1165, 839 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 7.87 (s, 1H, ArH), 7.74 (d, J = 8.3 Hz, 2H, ArH), 7.63–7.65 (m, 2H, ArH), 7.62 (d, J = 4.2 Hz, 1H, imine-H), 7.60 (s, 1H, ArH), 7.49(s, 1H, ArH), 7.09–7.17(m, 4H, ArH), 6.78 (s, 1H, ArH), 4.05–4.17 (m, 4H, 2×OCH_2), 3.94 (s, 3H, OCH_3), 3.77–3.86 (m, 1H, NCH), 3.55–3.72 (m, 2H, NCH_2), 2.26–2.32 (m, 2H, CH_2), 1.90–2.10 (m, 6H, 3×CH_2), 1.68–1.79 ppm (m, 2H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.5, 163.8, 162.2, 160.5, 157.0, 150.7, 147.6, 140.4, 137.1, 135.1, 133.5, 129.4, 128.8, 127.1, 125.6, 125.2, 120.0, 119.9, 115.4, 111.4, 110.3, 106.2, 68.6, 67.5, 56.0, 53.5, 46.5, 29.4, 28.7, 28.5, 24.0, 22.5 ppm; (ESI) MS: m/z 553 $[M+1]^+$; HRMS (ESI m/z) for $C_{34}H_{34}N_2O_4F$ calcd 553.2764, found 553.2775 $[M+1]^+$.

7-Methoxy-8-{6-(4-trifluoromethyl)phenyl)-2-naphthyl]oxy)pentoxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (3r): White solid (183 mg, 61% yield): R_f = 0.3

(2.5% MeOH/$CHCl_3$); mp: 88–90 °C; $[\alpha]_D^{27} = +131.0$ ($c = 0.1$, $CHCl_3$); IR (KBr): $\nu_{max} = 3383$ (br), 2936, 2872, 1606, 1506, 1466, 1328, 1260, 1120, 843 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.98$ (s, 1H, ArH), 7.77–7.84 (m, 4H, ArH), 7.69–7.75 (m, 3H, ArH), 7.67 (d, $J = 3.8$ Hz, 1H, imine-H), 7.52 (s, 1H, ArH), 7.15–7.21 (m, 2H, ArH), 6.82 (s, 1H, ArH), 4.05–4.21 (m, 4H, $2\times OCH_2$), 3.95 (s, 3H, OCH_3), 3.78–3.87 (m, 1H, NCH), 3.55–3.74 (m, 2H, NCH_2), 2.28–2.36 (m, 2H, CH_2), 1.91–2.10 (m, 6H, $3\times CH_2$), 1.61–1.78 ppm (m, 2H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 164.5$, 162.3, 157.4, 150.7, 148.1, 147.6, 144.5, 140.4, 134.5, 134.1, 129.6, 128.8, 127.4, 127.2, 125.9, 125.5, 125.3, 120.0, 119.6, 111.4, 110.3, 106.2, 68.6, 67.6, 56.0, 53.6, 46.5, 29.4, 28.8, 28.5, 24.0, 22.5 ppm; (ESI) MS: m/z 603 $[M+1]^+$; HRMS (ESI m/z) for $C_{35}H_{34}N_2O_4F_3$ calcd 603.2732, found 603.2743 $[M+1]^+$.

7-Methoxy-8-{6-(4-trifluoromethoxy)phenyl)-2-naphthyl]oxy)pentoxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (3s): White solid (225 mg, 73% yield): $R_f = 0.3$ (3% MeOH/$CHCl_3$); mp: 72–74 °C; $[\alpha]_D^{27} = +182.0$ ($c = 0.1$, $CHCl_3$); IR (KBr): $\nu_{max} = 3324$ (br), 2936, 2871, 1603, 1507, 1432, 1260, 1205, 1164, 845 cm^{-1}; H NMR (300 MHz, $CDCl_3$): $\delta = 7.93$ (s, 1H, ArH), 7.79 (dd, $J = 2.2$ Hz, 6.7 2H, Hz, ArH), 7.72 (s, 1H), 7.69 (s, 1H), 7.67 (d, $J = 4.3$ Hz, 1H, imine-H), 7.64 (s, 1H, ArH), 7.53 (s, 1H, ArH), 7.31 (d, $J = 8.3$ Hz, 2H, ArH), 7.18 (dd, $J = 2.2$ Hz, 9.0 Hz, 2H, ArH), 6.82 (s, 1H, ArH), 4.04–4.20 (m, 4H, $2\times OCH_2$), 3.95 (s, 3H, OCH_3), 3.78–3.88 (m, 1H, NCH), 3.56–3.74 (m, 2H, NCH_2), 2.27–2.36 (m, 2H, CH_2), 1.91–2.08 (m, 6H, $3\times CH_2$), 1.69–1.77 ppm (m, 2H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 164.5$, 162.3, 157.1, 150.7, 148.2, 147.6, 140.4, 139.8, 134.6, 133.7, 129.5, 128.8, 128.2, 127.2, 125.5, 121.1, 119.9, 119.4, 112.2, 111.3, 110.2, 106.1, 96.5, 68.5, 67.5, 56.0, 53.5, 46.5, 29.4, 28.7, 28.5, 24.0, 22.5 ppm; (ESI) MS: m/z 619 $[M+1]^+$; HRMS (ESI m/z) for $C_{35}H_{34}N_2O_5F_3$ calcd 619.2681, found 619.2661 $[M+1]^+$.

7-Methoxy-8-{6-(4-methoxyphenyl)-2-naphthyl]oxy)pentoxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (3t): White solid (191 mg, 68% yield): $R_f = 0.3$ (3% MeOH/$CHCl_3$); mp: 78–80 °C; $[\alpha]_D^{27} = +178.0$ ($c = 0.1$, $CHCl_3$); IR (KBr): $\nu_{max} = 3328$ (br), 2926, 2865, 1604, 1506, 1460, 1247, 1179, 1124, 1022, 834 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.91$ (s, 1H, ArH), 7.77 (d, $J = 8.0$ Hz, 2H, ArH), 7.68 (d, $J = 4.1$ Hz, 1H, imine-H), 7.60–7.66 (m, 3H, ArH), 7.52 (s, 1H, ArH), 7.11–7.19 (m, 2H, ArH), 7.02 (d, $J = 8.0$ Hz, 2H, ArH), 6.84 (s, 1H), 4.04–4.20 (m, 4H, $2\times OCH_2$), 3.95 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.69–3.78 (m, 1H, NCH), 3.59–3.66 (m, 2H, NCH_2), 2.25–2.35 (m, 2H, CH_2), 1.86–2.10 (m, 6H, $3\times CH_2$), 1.64–1.81 ppm (m, 2H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 164.5$, 162.2, 158.8, 156.7, 150.7, 147.6, 140.4, 135.7, 133.5, 133.2, 129.3, 129.0, 128.0, 127.0, 125.6, 124.6, 119.9, 119.1, 114.1, 111.3, 110.2, 106.2, 68.6, 67.5, 56.0, 55.2, 53.5, 46.5, 29.5, 28.8, 28.5, 24.0, 22.5 ppm; (ESI) MS: m/z 565 $[M+1]^+$; HRMS (ESI m/z) for $C_{35}H_{37}N_2O_5$ calcd 565.2964, found 565.2950 $[M+1]^+$.

7-Methoxy-8-{6-(4-*N*,*N*-dimethylamino)phenyl)-2-naphthyl]oxy)-pentoxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*]-[1,4]benzodiazepine-5-one (3u): White solid (184 mg, 64% yield): $R_f = 0.3$ (3% MeOH/$CHCl_3$); mp: 82–84 °C; $[\alpha]_D^{27} = +192.0$ ($c = 0.1$, $CHCl_3$); IR (KBr): $\nu_{max} = 3366$ (br), 2929, 2871, 1607, 1509, 1432, 1250, 1205, 1154, 850 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.90$ (s, 1H, ArH), 7.75 (d, $J = 9.0$ Hz, 2H, ArH), 7.68 (d, $J = 3.7$ Hz, 1H, imine-H), 7.62 (d, $J = 8.8$ Hz, 2H, ArH), 7.52 (s, 1H, ArH), 7.10–7.16 (m, 3H), 6.90 (d, $J = 8.8$ Hz, 2H, ArH), 6.81 (s 1H, ArH) 4.06–4.18 (m, 4H, $2\times OCH_2$), 3.95 (s, 3H, OCH_3), 3.76–3.88 (m, 1H, NCH), 3.53–3.73 (m, 2H, NCH_2), 3.02 (s, 6H, $N(CH_3)_2$) 2.28 (m, 2H, CH_2), 1.87–2.10 (m, 6H, $3\times CH_2$), 1.62–1.80 ppm (m, 2H, CH_2); (ESI) MS: m/z 578 $[M+1]^+$; HRMS (ESI m/z) for $C_{36}H_{40}N_3O_4$ calcd 578.3280, found 578.3287 $[M+1]^+$.

7-Methoxy-8-{6-(3-chlorophenyl)-2-naphthyl]oxy)pentoxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (3v): White solid (190 mg, 67% yield): $R_f = 0.3$ (2.0% MeOH/$CHCl_3$); mp: 88–90 °C; $[\alpha]_D^{27} = +167.0$ ($c = 0.1$, $CHCl_3$); IR (KBr): $\nu_{max} = 3333$ (br), 3058, 2933, 2868, 1598, 1498, 1429, 1252, 1202, 1170, 850 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.89$ (s, 1H, ArH), 7.74 (d, $J = 8.3$ Hz, 2H, ArH), 7.68 (d, $J = 4.2$ Hz, 1H, imine-H), 7.62–7.66 (m, 2H, ArH), 7.60 (s, 1H, ArH), 7.51 (s, 1H, ArH), 7.10–7.16 (m, 4H, ArH), 6.78 (s, 1H, ArH), 4.06–4.16 (m, 4H, $2\times OCH_2$), 3.94 (s, 3H, OCH_3), 3.77–3.87 (m, 1H, NCH), 3.53–3.72 (m, 2H, NCH_2), 2.27–2.33 (m, 2H, CH_2), 1.91–2.09 (m, 6H, $3\times CH_2$), 1.71–1.78 ppm (m, 2H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 164.5$, 162.3, 157.2, 150.7, 147.6, 142.9, 140.4, 134.6, 134.5, 133.9, 129.9, 129.5, 128.8, 127.3, 127.0, 126.8, 125.6, 125.4, 125.1, 120.2, 119.4, 111.4, 110.3, 106.2, 68.6, 67.6, 56.1, 53.6, 46.5, 29.5, 28.8, 28.5, 24.0, 22.5 ppm; (ESI) MS: m/z 570 $[M+1]^+$; HRMS (ESI m/z) for $C_{34}H_{34}N_2O_4Cl$ calcd 570.3898, found 570.3891 $[M+1]^+$.

7-Methoxy-8-{6-(3,4-dimethoxyphenyl)-2-naphthyl]oxy) pentoxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (3w): White solid (207 mg, 70% yield): $R_f = 0.3$ (3.5% MeOH/$CHCl_3$); mp: 73–75 °C; $[\alpha]_D^{27} = +162.0$ ($c = 0.1$, $CHCl_3$); IR (KBr): $\nu_{max} = 3326$ (br), 2929, 2870, 1602, 1511, 1458, 1256, 1220, 1167, 1022, 850 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.91$ (s, 1H, ArH), 7.75–7.84 (m, 3H, ArH), 7.68 (d, $J = 4.1$ Hz, 1H, imine-H), 7.52 (s, 1H, ArH), 7.18–7.24 (m, 3H, ArH), 7.15 (s, 1H, ArH), 6.98 (t, $J = 8.3$ Hz, 1H, ArH), 6.82 (s, 1H, ArH), 4.10–4.15 (m 4H, $2\times OCH_2$), 3.99 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 3.79–3.87 (m, 1H, NCH), 3.56–3.74 (m, 2H, NCH_2), 2.28–2.33 (m, 2H, CH_2), 1.85–2.10 (m, 6H, $3\times CH_2$), 1.64–1.75 ppm (m, 2H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 164.5$, 162.8, 156.8, 150.7, 149.1, 148.3, 147.7, 140.5, 135.9, 134.1, 133.4, 129.3, 129.0, 128.1, 127.0, 125.8, 125.3, 124.8, 124.2, 119.2, 114.8, 111.5, 110.4, 106.3, 68.7, 67.6, 56.0, 55.8, 53.9, 46.5, 30.6, 29.4,28.8, 24.0, 22.5 ppm; (ESI) MS: m/z 595 $[M+1]^+$; HRMS (ESI m/z) for $C_{36}H_{39}N_2O_6$ calcd 595.3070, found 595.3079 $[M+1]^+$.

7-Methoxy-8-{6-(3,4,5-trimethoxyphenyl)-2-naphthyl]oxy)pentoxy}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (3x): White solid (193 mg, 62% yield): $R_f = 0.3$ (4.0% MeOH/$CHCl_3$); mp: 78–80 °C; $[\alpha]_D^{27} = +144.0$ ($c = 0.1$, $CHCl_3$); IR (KBr): $\nu_{max} = 3352$ (br), 2930, 2870, 1597, 1504, 1459, 1430, 1262, 1124, 1009, 838 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.91$ (s, 1H, ArH), 7.74–7.82 (m, 3H, ArH), 7.69 (d, $J = 4.1$ Hz, 1H, imine-H), 7.52 (s, 1H, ArH), 7.19 (d $J = 8.2$ Hz, 1H, ArH), 7.15 (s, 1H, ArH), 6.88 (s, 2H, ArH), 6.82 (s, 1H, ArH), 4.07–4.17 (m, 4H, $2\times OCH_2$), 3.96 (s, 6H, $2\times OCH_3$), 3.94 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.77–3.86 (m, 1H, NCH), 3.54–3.73 (m, 2H, NCH_2), 2.26–2.34 (m, 2H, CH_2), 1.90–2.09 (m, 6H, $3\times CH_2$), 1.68–1.77 ppm (m, 2H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 164.5$, 162.3, 157.0, 153.9, 150.7, 147.6, 140.4, 137.1, 136.2, 133.6, 129.4, 128.9, 127.1, 125.9, 125.8, 125.2, 120.0, 119.3, 111.4, 110.3, 106.2, 104.3, 68.7, 67.6, 60.8, 56.1, 53.5, 46.5, 29.4, 28.8, 28.5, 24.0, 22.5 ppm; (ESI) MS: m/z 625 $[M+1]^+$; HRMS (ESI m/z) for $C_{37}H_{41}N_2O_7$ calcd 625.3175, found 625.3162 $[M+1]^+$.

7-Methoxy-8-{6-(4-fluorophenyl)-2-naphthyl]{4-[3-(3-oxypropoxy)-4-methoxybenzoyl]piperazino}methanone}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (4a): White solid (211 mg, 55% yield): $R_f = 0.3$ (4.0% MeOH/$CHCl_3$); mp: 130–132 °C; $[\alpha]_D^{27} = +153.0$ ($c = 0.1$, $CHCl_3$); IR (KBr): $\nu_{max} = 3422$ (br), 2927, 1627, 1512, 1430, 1261, 1220, 1013, 817 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 8.01$ (s, 1H, ArH), 7.93–7.98 (m, 3H, ArH), 7.76 (d, $J = 9.0$ Hz, 1H, ArH), 7.70 (d, $J = 4.3$ Hz, 1H, imine-H), 7.64–7.68 (m, 2H, ArH), 7.54 (s, 1H, ArH), 7.52 (s, 1H, ArH), 7.19 (t, $J = 8.3$ Hz, 2H ArH), 7.05 (s 1H, ArH), 7.00 (d, $J = 8.3$ Hz, 1H, ArH), 6.85–

6.89 (m, 2H, ArH), 4.21–4.33 (m, 4H, 2×OCH_2), 3.93 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.80–3.85 (m, 1H, NCH), 3.50–3.78 (m, 10H, $N(CH_2\text{-}CH_2)_2N$, NCH_2), 2.27–2.43 (m, 4H, 2×CH_2) 2.02–2.10 ppm (m, 2H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.5, 170.3, 164.2, 162.3, 157.8, 151.0, 148.2, 147.7, 143.4, 140.4, 138.9, 133.9, 132.3, 131.6, 128.9, 128.8, 128.6, 127.0, 126.8, 126.4, 125.3, 124.6, 120.5, 120.2, 115.9, 115.6, 112.7, 111.4, 110.9, 110.5, 65.5, 65.2, 56.0, 53.6, 46.6, 42.4, 29.5, 28.9, 24.0 ppm; (ESI) MS: *m/z* 771 $[M+1]^+$; HRMS (ESI *m/z*) for $C_{45}H_{44}N_4O_7F$ calcd 771.8439, found 771.8431 $[M+1]^+$.

7-Methoxy-8-{6-(4-trifluoromethylphenyl)-2-naphthyl]{4-[3-(3-oxypropoxy)-4-methoxybenzoyl]piperazino}methanone}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (4b): White solid (205 mg, 50% yield): R_f = 0.3 (4.5% MeOH/$CHCl_3$); mp: 125–127 °C; $[\alpha]_D^{27}$ = +178.0 (*c* = 0.1, $CHCl_3$); IR (KBr): ν_{max} = 3420 (br), 2929, 1629, 1514, 1436, 1251, 1221, 1113, 820 cm^{-1}; H NMR (300 MHz, $CDCl_3$): δ = 8.06 (s, 1H, ArH), 7.95–7.00 (m, 2H, ArH), 7.89 (d, *J* = 7.3 Hz, 1H, ArH), 7.78 (s, 1H, ArH), 7.75 (s, 1H, ArH), 7.72 (s, 1H, ArH), 7.65 (d, *J* = 3.7 Hz, 1H, imine-H), 7.52–7.59 (m, 3H, ArH), 7.36 (s 1H, ArH), 7.34 (s, 1H, ArH), 6.95–7.06 (m, 2H, ArH), 6.86 (s, 1H, ArH), 4.20–4.34 (m, 4H, 2×OCH_2), 3.93 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 3.77–3.86 (m, 1H, NCH), 3.42–3.74 (m, 10H, $N(CH_2\text{-}CH_2)_2N$, NCH_2), 2.24–2.39 (m, 4H, 2×CH_2) 2.00–2.11 ppm (m, 2H, CH_2); (ESI) MS: *m/z* 821 $[M+1]^+$; HRMS (ESI *m/z*) for $C_{46}H_{44}N_4O_7F_3$ calcd 821.8514, found 821.8505 $[M+1]^+$.

7-Methoxy-8-{6-(4-trifluoromethoxyphenyl)-2-naphthyl]{4-[3-(3-oxypropoxy)-4-methoxybenzoyl]piperazino}methanone}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (4c): White solid (217 mg, 52% yield): R_f = 0.3 (5% MeOH/$CHCl_3$); mp: 120–122 °C; $[\alpha]_D^{27}$ = +141.0 (*c* = 0.1, $CHCl_3$); IR (KBr): ν_{max} = 3444 (br), 2932, 1626, 1513, 1430, 1260, 1220, 1172, 1014, 818 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 8.04 (s, 1H, ArH), 7.91–7.99 (m, 2H, ArH), 7.88 (t, *J* = 7.4 Hz, 1H, ArH), 7.79 (s, 1H, ArH), 7.76 (s, 1H, ArH), 7.72 (s, 1H, ArH), 7.66 (d, *J* = 3.7 Hz, 1H, imine-H), 7.51–7.58 (m, 3H, ArH), 7.37 (s 1H, ArH), 7.33 (s, 1H, ArH), 6.96–7.05 (m, 2H, ArH), 6.87 (s, 1H, ArH), 4.19–4.33 (m, 4H, 2×OCH_2), 3.92 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 3.78–3.84 (m, 1H, NCH), 3.46–3.76 (m, 10H, $N(CH_2\text{-}CH_2)_2N$, NCH_2), 2.26–2.47 (m, 4H, 2×CH_2), 1.98–2.12 ppm (m, 2H, CH_2); (ESI) MS: *m/z* 837 $[M+1]^+$; HRMS (ESI *m/z*) for $C_{46}H_{44}N_4O_8F_3$ calcd 837.4508, found 837.4501 $[M+1]^+$.

7-Methoxy-8-{6-(4-methoxyphenyl)-2-naphthyl]{4-[3-(3-oxypropoxy)-4-methoxybenzoyl]piperazino}methanone}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (4d): White solid (215 mg, 55% yield): R_f = 0.3 (5% MeOH/$CHCl_3$); mp: 141–143 °C; $[\alpha]_D^{27}$ = +173.0 (*c* = 0.1, $CHCl_3$); IR (KBr): ν_{max} = 3426 (br), 2927, 1608, 1512, 1432, 1258, 1220, 1178, 1014, 815 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 8.01 (s, 1H, ArH), 7.90–7.96 (m, 3H, ArH), 7.66 (d, *J* = 4.1 Hz, 1H, imine-H), 7.64–7.70 (m, 3H, ArH), 7.52 (s, 1H, ArH), 7.48 (s, 1H, ArH), 6.98–7.06 (m, 4H, ArH), 6.88. (s 1H, ArH), 6.85 (s, 1H, ArH), 4.19–4.35 (m, 4H, 2×OCH_2), 3.92 (s, 3H, OCH_3), 3.88 (s, 6H, 2×OCH_3), 3.80–3.86 (m, 1H, NCH), 3.50–3.79 (m, 10H, $N(CH_2\text{-}CH_2)_2N$, NCH_2), 2.26–2.46 (m, 4H, 2×CH_2), 2.00–2.13 ppm (m, 2H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.6, 170.4, 164.5, 162.3, 159.4, 151.0, 150.5, 148.2, 147.7, 140.4, 139.6, 134.1, 132.8, 131.8, 131.3, 128.8, 128.5, 128.3, 127.1, 126.9, 126.3, 124.7, 124.4, 120.5, 120.2, 114.3, 112.7, 111.4, 110.9, 110.2, 65.5, 65.3, 56.0, 55.9, 55.3, 53.6, 46.6, 29.5, 28.9, 24.1 ppm; (ESI) MS: *m/z* 783 $[M+1]^+$; HRMS (ESI *m/z*) for $C_{47}H_{47}N_4O_8$ calcd 783.3656, found 783.3643 $[M+1]^+$.

7-Methoxy-8-{6-(4-*N*,*N*-dimethylaminophenyl)-2-naphthyl]{4-[3-(3-oxypropoxy)-4-methoxybenzoyl]piperazino}methanone}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (4e): White solid (214 mg, 54% yield): R_f = 0.3 (5% MeOH/$CHCl_3$); mp: 115–117 °C; $[\alpha]_D^{27}$ = +134.0 (*c* = 0.1, $CHCl_3$); IR (KBr): ν_{max} = 3416 (br), 2924, 1608, 1512, 1431, 1261, 1220, 1135, 1014, 814 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 7.99 (s, 1H, ArH), 7.87–7.92 (m, 3H, ArH), 7.80 (d, *J* = 8.3 Hz, 1H, ArH), 7.64–7.67 (m, 2H, Hz, ArH), 7.63 (d, *J* = 3.8 Hz, 1H, imine-H), 7.51 (s, 1H, ArH), 7.47 (d, *J* = 8.3 Hz, 1H, ArH), 7.04 (s, 1H, ArH), 7.00 (d, *J* = 8.3 Hz, 1H, ArH), 6.84–6.87 (m, 4H, ArH), 4.22–4.34 (m, 4H, 2×OCH_2), 3.92 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.77–3.84 (m, 1H, NCH), 3.55–3.75 (m, 10H, $N(CH_2\text{-}CH_2)_2N$, NCH_2), 3.03 (s, 6H, $N(CH_3)_2$), 2.26–2.41 (m, 4H, 2×CH_2), 2.02–2.08 ppm (m, 2H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.8, 164.5, 162.4, 151.0, 148.5, 147.7, 140.4, 140.0, 134.3, 131.0, 131.4, 128.6, 128.4, 127.9, 126.9, 126.2, 124.3, 123.7, 120.5, 112.7, 111.4, 110.9, 110.4, 65.5, 56.0, 53.6, 46.5, 40.4, 29.6, 28.9, 24.0 ppm; (ESI) MS: *m/z* 796 $[M+1]^+$; HRMS (ESI *m/z*) for $C_{47}H_{50}N_5O_7$ calcd 796.3972, found 796.3966 $[M+1]^+$.

7-Methoxy-8-{6-(3-chloromethoxyphenyl)-2-naphthyl]{4-[3-(3-oxypropoxy)-4-methoxybenzoyl]piperazino}methanone}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (4f): White solid (216 mg, 55% yield): R_f = 0.3 (4.0% MeOH/$CHCl_3$); mp: 118–120 °C; $[\alpha]_D^{27}$ = +180.0 (*c* = 0.1, $CHCl_3$); IR (KBr): ν_{max} = 3421 (br), 2929, 1637, 1516, 1430, 1260, 1156, 1014, 817 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 8.02 (s, 1H, ArH), 7.94–7.97 (m, 3H, ArH), 7.77 (s, 1H, ArH), 7.70 (d, *J* = 3.4 Hz, 1H, imine-H), 7.64–7.69 (m, 4H, ArH), 7.55 (s, 1H, ArH), 7.51 (s, 1H, ArH), 7.16–7.21 (t, *J* = 8.3 Hz, 1H, ArH), 7.06 (s 1H, ArH), 7.01 (d, *J* = 7.5 Hz, 1H, ArH), 6.88 (d, *J* = 8.3 Hz, 1H, ArH), 4.22–4.35 (m, 4H, 2×OCH_2), 3.92 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.79–3.82 (m, 1H, NCH), 3.48–3.74 (m, 10H, $N(CH_2\text{-}CH_2)_2N$, NCH_2), 2.01–2.13 (m, 4H, 2×CH_2), 1.95–2.07 ppm (m, 2H, CH_2); (ESI) MS: *m/z* 788 $[M+1]^+$; HRMS (ESI *m/z*) for $C_{46}H_{44}N_4O_7Cl$ calcd 788.2985, found 788.2976 $[M+1]^+$.

7-Methoxy-8-{6-(3,4-dimethoxyphenyl)-2-naphthyl]{4-[3-(3-oxypropoxy)-4-methoxybenzoyl]piperazino}methanone}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (4g): White solid (203 mg, 50% yield): R_f = 0.3 (5% MeOH/$CHCl_3$); mp: 138–140 °C; $[\alpha]_D^{27}$ = +156.0 (*c* = 0.1, $CHCl_3$); IR (KBr): ν_{max} = 3420 (br), 2926, 1627, 1514, 1429, 1257, 1176, 1016, 814 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 8.01 (s, 1H, ArH), 7.92–7.95 (m, 3H, ArH), 7.81 (s, 1H, ArH), 7.76 (s, 1H, ArH), 7.67 (d, *J* = 3.6 Hz, 1H, imine-H), 7.52 (s, 1H, ArH), 7.12–7.24 (m, 4H, ArH), 6.98 (dd, *J* = 2.9 Hz, 5.8 Hz, 1H, ArH), 6.88 (s, 1H, ArH), 6.76 (s, 1H, ArH), 4.20–4.33 (m, 4H, 2×OCH_2), 4.00 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.76–3.84 (m, 1H, NCH), 3.50–3.74 (m, 10H, $N(CH_2\text{-}CH_2)_2N$, NCH_2), 2.06–2.33 (m, 4H, 2×CH_2), 2.00–2.12 ppm (m, 2H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.5, 170.3, 162.3, 159.3, 150.9, 150.5, 149.1, 148.8, 148.1, 139.7, 134.0, 133.2, 131.9, 131.4, 131.2, 128.7, 128.2, 127.0, 126.8, 126.4, 126.2, 124.8, 124.6, 124.3, 120.4, 120.1, 119.6, 114.2, 112.6, 111.4, 110.8, 110.4, 65.4, 65.2, 55.9, 55.2, 53.8, 46.4, 41.6, 29.5, 28.2, 23.9 ppm; (ESI) MS: *m/z* 813 $[M+1]^+$; HRMS (ESI *m/z*) for $C_{47}H_{49}N_4O_9$ calcd 813.3157, found 813.3148 $[M+1]^+$.

7-Methoxy-8-{6-(3,4,5-trimethoxyphenyl)-2-naphthyl]{4-[3-(3-oxypropoxy)-4-methoxybenzoyl]piperazino}methanone}-(11a*S*)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5-one (4h): White solid (227 mg, 54% yield): R_f = 0.3 (6% MeOH/$CHCl_3$); mp: 104–106 °C; $[\alpha]_D^{27}$ = +170.0 (*c* = 0.1, $CHCl_3$); IR (KBr): ν_{max} = 3432 (br), 2929, 1628, 1513, 1429, 1261, 1127, 1013, 819 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 8.01 (s, 1H, ArH), 7.91–7.98 (m, 3H, ArH), 7.77 (dd, *J* = 1.7 Hz, 6.6 Hz, 1H, ArH), 7.66 (d, *J* = 4.3 Hz, 1H, imine-H), 7.51 (s, 1H, ArH), 6.97–7.05 (m, 3H, ArH), 6.91 (s, 2H, ArH), 6.88 (s, 1H, ArH), 6.86 (s, 1H, ArH), 4.20–4.34 (m, 4H, 2×OCH_2), 3.97 (s, 6H, 2×OCH_3), 3.92 (s, 6H, 2×OCH_3), 3.87 (s, 3H, OCH_3), 3.77–3.83 (m, 1H, NCH), 3.50–3.75 (m, 10H, $N(CH_2\text{-}CH_2)_2N$, NCH_2), 2.28–2.43

(m, 4H, $2\times CH_2$) 1.99–2.09 ppm (m, 2H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 170.6$, 170.5, 164.5, 162.4, 160.9, 153.5, 151.1, 148.3, 140.5, 140.1, 136.4, 134.0, 132.2, 131.7, 129.9, 128.9, 128.6, 127.1, 126.9, 126.6, 125.4, 124.6, 124.2, 120.5, 120.2, 112.7, 111.5, 110.9, 110.4, 104.6, 65.5, 65.3, 56.1, 55.9, 53.6, 50.6, 46.6, 29.6, 28.9, 24.1 ppm; (ESI) MS: *m/z* 843 $[M+1]^+$; HRMS (ESI *m/z*) for $C_{48}H_{51}N_4O_{10}$ calcd 843.3527, found 843.2521 $[M+1]^+$.

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