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Rapid Synthesis of New DNMT Inhibitors Derivatives of Procainamide

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DNA methyltransferases (DNMTs) are responsible for DNA methylation, an epigenetic modification involved in gene regulation. Families of conjugates of procainamide, an inhibitor of DNMT1, were conceived and produced by rapid synthetic pathways. Six compounds resulted in potent inhibitors of the murine catalytic Dnmt3A/3L complex and of human DNMT1, at least 50 times greater than that of the parent compounds. The inhibitors showed selectivity for C5 DNA methyltransferases. The cytotoxicity of the inhibitors was validated on two tumour cell lines (DU145 and HCT116) and correlated with the DNMT inhibitory potency. The inhibition potency of procainamide conjugated to phthalimide through alkyl linkers depended on the length of the linker; the dodecane linker was the best.

Introduction

Epigenetic modifications are known to control gene expression. In mammals, methylation of deoxycytidines was shown to be a major factor in epigenetic regulation and occurs at CpG sites, which are enriched in so-called CpG islands, often located in gene promoters.[1–3] Of note, hypermethylation of the promoters results in gene silencing. The addition of a methyl group on the carbon-5 position of the cytosine is catalysed by C5 DNA methyltransferases (DNMTs).[4–6] Mammalian DNMTs are divided in two major families: DNMT1 and DNMT3 (DNMT3A, 3B and 3L), DNMT2 being a tRNA methyltransferase. With the exception of DNMT3L, which is catalytically inactive, all share a common catalytic mechanism that uses *S*-adenosyl-L-methionine (SAM) as the methyl donor. The epigenetic impairment of information can compromise the normal development of an organism and is involved in various diseases, such as cancer.[7,8] Cancerous cells often present two types of aberrant DNA methylation: global hypomethylation leading to genomic instability and specific hypermethylation of the promoters of certain genes, such as tumour suppressor genes, which silences them. Interestingly, epigenetic modifications are reversible, and it has been shown that inhibitors of DNMTs are able to demethylate the promoters and reactivate tumour suppressor genes.[9–11] Therefore DNMT inhibitors offer great promise for the development of new and more efficient anticancer strategies.

Today, two families of DNMT inhibitors are known:[12] nucleoside and non-nucleoside analogues. Among the latter, procainamide **1**, used over the last 30 years as an antiarrhytmic, was shown to induce significant demethylation of genomic DNA in human cancer cells, together with its analogue, procaine (an anaesthetic).[13,14] These compounds are described to bind to CpG sequences and thereby disturb DNA methylation.[13,15,16] In this study, we used procainamide as a DNA binder to guide an inhibitor of DNMT to CpG-rich regions, in order to increase its local concentration at CpG sites. We have already successfully used this strategy to direct the action of DNA topoisomerase I and II inhibitors to specific DNA sites.[17–21] Here we synthesised several conjugates of procainamide (Scheme 1) by developing a new and rapid synthetic pathway, and found six potent inhibitors of Dnmt3A/3L and DNMT1.

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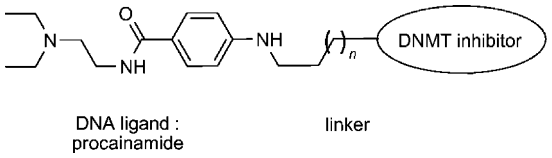


Scheme 1. Schematic representation of the conceived conjugates.

Results and Discussion

Synthesis of RG108–procainamide conjugates

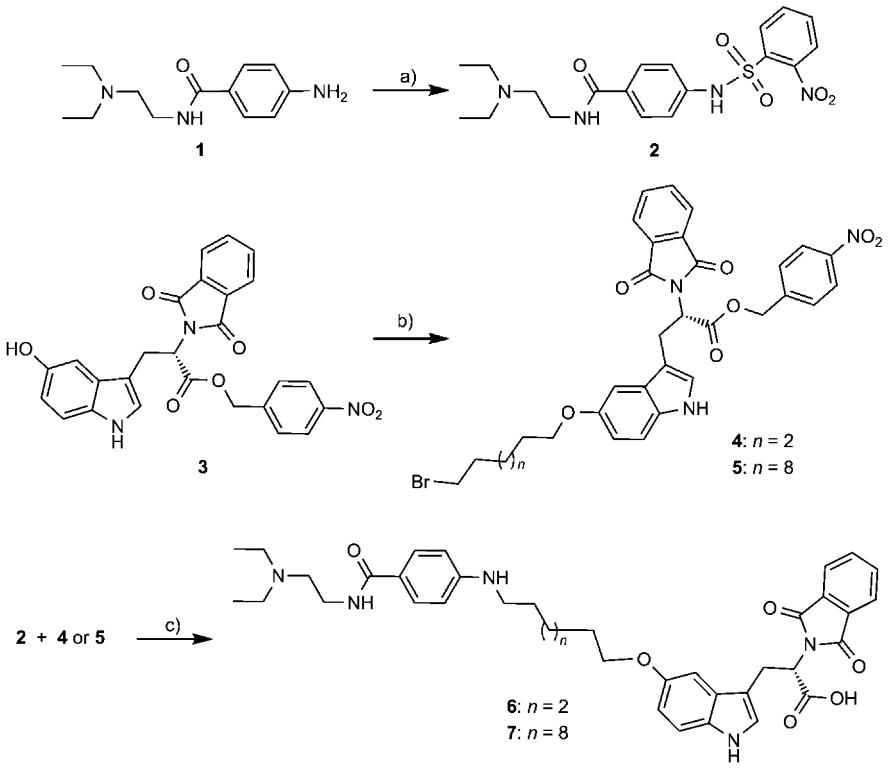
Procainamide **1** was conjugated to the known inhibitor L-RG108 (*N*-phthalimidoyl protected L-tryptophane).[22–24] The conjugation was at the carbon-5 position of the indole as, according to Schirrmacher et al.,[25] this position could be functionalised without any modification of the activity. We developed a rapid pathway requiring few purification steps (Scheme 2). An activation-protection sequence was realised by reacting the primary amine of **1** with a small excess (1.25 equiv) of 2-nitrobenzenesulfonyl chloride (2-nosyl chloride) to give nosyl derivative **2**.[26,27] Compound **3**, an OH-derivatized L-RG108, was functionalised with a bromoalkyl linker of 6 or 12 atoms (**4** and **5**, respectively). An original one-pot reaction was developed: the coupling of **2** to **4** or **5** was performed in Williamson conditions, followed by double deprotection by thiophenol treatment and addition of water to afford **6** and **7**.

The ability of compounds **6** and **7** to inhibit human DNMT1 and the murine catalytic Dnmt3A/3L complex was evaluated in a ^{3}H-methyl group incorporation experiment (Figure 1). DNMT1 was chosen because it has been previously described as a target of procainamide,[13] while, to our knowledge, no specific study has been performed on the Dnmt3A/3L complex. Parent L-RG108 has never been tested on Dnmt3A/3L either, while on human DNMT1 it showed 34% inhibition at 1 mM.[24] Since conjugates **6** and **7** showed a moderate increase in activity compared to parent **1** and L-RG108, a second generation of derivatives coupled at the carboxylic acid of L-RG108 was prepared. In order to explore different lengths of linkers between the L-RG108 and procainamide moieties, the latter was attached to a phthalimide by alkyl linkers of 4 to 14 carbon atoms (Scheme 3). A similar one-pot synthesis was developed: the nosyl derivative of procainamide **2** was reacted with various bromoalkylphthalimides (**B1**–**6**) in the presence of K_2CO_3, and then treated with thiophenol and water to provide the corresponding phthalimidoalkyl derivatives of procainamide (**8**–**13**).

Compounds **9**, **12** and **13** were deprotected by using *N*-methylhydrazine to give the respective primary amines **14**–**16,** and coupled to L-RG108 to give compounds **17, 18** and **19**, respectively (Scheme 4). In addition, the effect of the configuration of the asymmetric carbon atom on RG108 was investigated by coupling primary amine **15** to D-RG108 at the carboxylic acid group to afford **18D**. Analogue **20**, bearing only the linker arm of 12 carbon atoms, was also synthesized by reaction of 1-bromododecane with **2** followed by treatment with thiophenol.

All compounds, L-RG108 amide derivatives (**17**, **18**, and **19**) and intermediate phthalimides (**8** to **13**), were tested on DNMT1 and Dnmt3A/3L activity at 10 μM (Figure 1). Six compounds showed greatly increased activity compared to the parent **1** (Table 1). The inhibition profiles on the two enzymes was comparable.

Interestingly, in the phthalimide family, as the length of the linker increased the inhibition activity increased, up to **12** (bearing the 12 carbon atoms linker). Compounds **12** and **13** proved among the most potent inhibitors together with the L-RG108 conjugates **18** and **19** and D-RG108 conjugate **18D**, all bearing long linker arms (Table 1 and Figure S1 in the Supporting Information). On Dnmt3A/3L, the L configuration was slightly more potent. On DNMT1, **18** and **19** were three- to fourfold less



Scheme 2. a) 2-Nitrobenzenesulfonyl chloride, CH_2Cl_2, 18 h, RT, 55%. b) K_2CO_3, CH_3CN 18 h reflux, 58% for $n=4$, 61% for $n=10$. c) K_2CO_3, KI, CH_3CN, 18 h reflux then thiophenol, K_2CO_3, CH_3CN 3 h reflux, then H_2O 30 min RT, 68% for $n=4$, 58% for $n=10$.

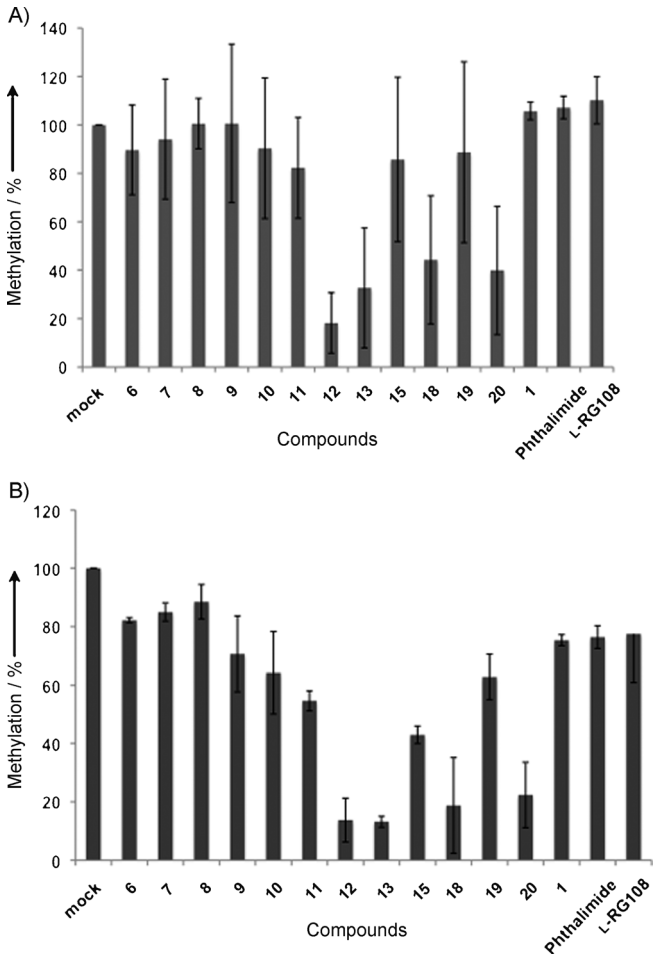
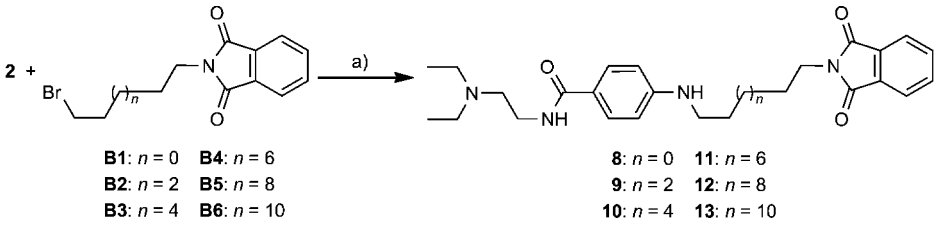
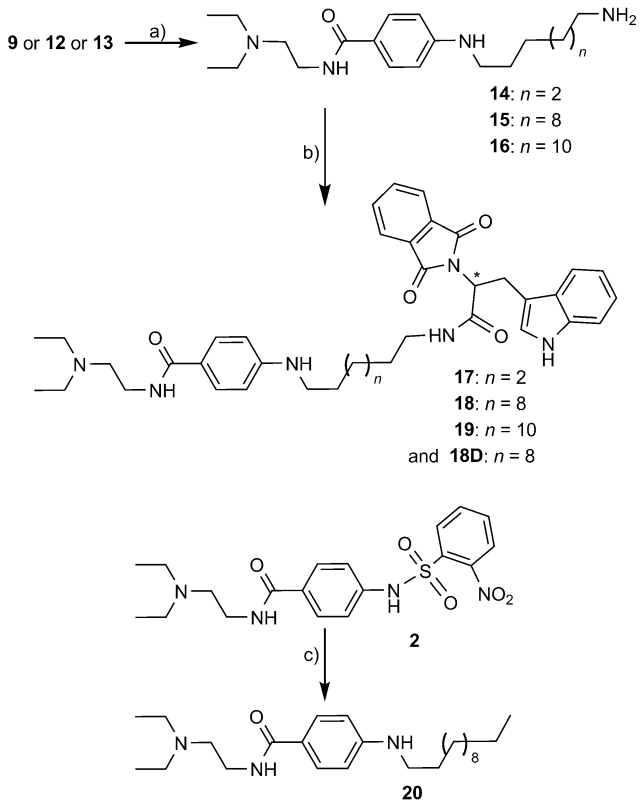


Figure 1. Normalized methylation activity (%) expressed relative to activity in the absence of inhibitor ("mock") for A) DNMT1 and B) Dnmt3A/3L in the presence of compounds (10 μM). Data are mean ± SE of three experiments.

active than **12**. Compound **20**, in which the procainamide is attached to the simple dodecane linker, was among the most potent compounds, while the presence of a primary amine (in **15**) decreased the activity, especially on DNMT1 (Figure 1). This suggests that the presence of a hydrophobic group may be necessary for the activity. In order to exclude the possibility that the inhibition was due to aggregation of the molecules (and thus a non-specific effect),[28] **12**, **18** and **20** were tested in the presence of detergent.[29,30] No significant effect was observed (Figure S2).



Scheme 3. a) K_2CO_3, KI, CH_3CN, 18 h, reflux, then thiophenol, K_2CO_3, CH_3CN, 3 h, reflux, 79% for $n=0$, 82% for $n=2$, 75% for $n=4$, 83% for $n=6$, 89% for $n=8$, 85% for $n=10$.



Scheme 4. a) NH_2-$NHCH_3$, MeOH, 18 h, RT, 69% for $n=8$. b) L-RG108, DCC, NHS, DMF, 4 h, RT, 58% for $n=2$ over two steps, 72% for $n=8$, 46% for $n=10$ over the two steps; D-RG108 was used to afford 62% **18D**. c) 1-Bromododecane, K_2CO_3, KI, CH_3CN, 18 h, reflux, then thiophenol, 3 h, RT, 94%.

Study of the mechanism of action

Next we investigated by saturation transfer difference (STD) NMR spectroscopy experiments which moiety of the conjugates interacts with the enzyme. In this context, it has been recently proposed by modelling that procainamide could interact with DNMT1 as well as the DNA substrate.[31] The STD experiments showed that procainamide did not interact with DNMT1, Dnmt3A or Dnmt3A/3L, whereas phthalimide and L-RG108 interacted with the enzymes (Figure 2 and Figure S3).

These results suggest that the procainamide moiety of the conjugate acts as DNA binder and directs the action of the phthalimide or L-RG108 on the enzyme. Finally, docking simulations with Dock 6.4 for **12** (Figure 2E and Figure S5), **18** and **20** (data not shown) in the X-ray structure of the catalytic domain of Dnmt3A (PDB ID: 2QRV) positioned the phthalimide or L-RG108 moiety with the linker arm in the catalytic pocket, while the procainamide moiety protruded outwards through a cavity. In addition, the selectivity of the conjugates for the C5 DNA methyltransferases was addressed by comparison of the activity on the murine cata-

Table 1. Biochemical and biological activity of the procainamide conjugates. Inhibition (IC_{50}) of murine catalytic complex Dnmt3A/3L and human DNMT1, and cytotoxicity (TC_{50}) with DU145 and HCT116 cell lines.

	IC_{50} [μM][a]		TC_{50} [μM][b]	
Compounds	Dnmt3A/3L	DNMT1	HCT116	DU145
1	>500	>300	430±44	700±68
L-RG108	>500	>250	424±63	657±51
phthalimide	>500	>1000	n.d.	n.d.
6	81±6	>100	n.d.	n.d.
7	174±5	>100	n.d.	n.d.
8	450±50	>100	180±10	313±53
9	185±20	>100	33±10	72±13
10	26±2	>100	4.3±0.8	24±10
11	26±5	77±9	10±5	25±14
12	8.2±1.9	4.9±1.3	2.1±0.6	8.5±2.1
13	9.7±2.0	8.5±2.1	4.8±2.1	5.8±1.1
15	15±5	66±6	27±7	43±0.7
17	89±5	55±4	n.d.	n.d.
18	4.2±0.8	21±9	8.5±3.1	7.3±0.5
18D	10.5±1.7	21±2	2.9±1.3	5.9±2.6
19	9.0±2.6	18±3	3.7±1.8	2.1±0.6
20	3.3±0.9	8.2±0.9	2.2±1.2	8±0.7

[a] Concentration (mean±SE of three to five experiments) at which 50% inhibition of the enzyme activity was observed. [b] Concentration (mean±SE of three to five experiments) at which 50% inhibition of cellular proliferation was observed. n.d. = not determined

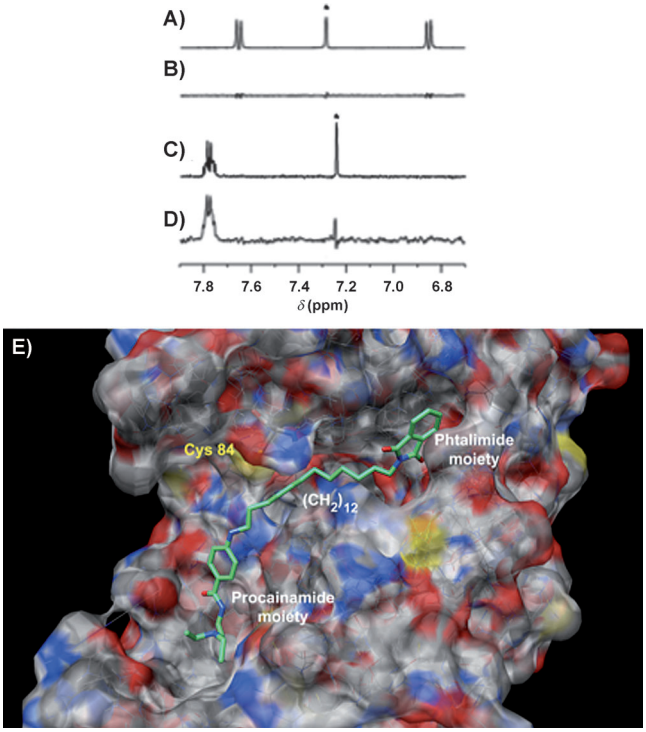


Figure 2. 1D ^{1}H NMR spectra of A) procainamide (500 μM), C) phthalimide (500 μM), and 1D STD NMR spectra of B) procainamide (500 μM), D) phthalimide (500 μM) in the presence of Dnmt3A (8.5 μM). *: contaminant. E) Docking of **12** in the X-ray crystal structure of Dnmt3A/3L (PDB ID: 2QRV, chain A).

lytic Dnmt3A/3L complex against bacterial EcoDam *N*-6 DNA methyltransferase and the catalytic domain of human G9a histone H3K9 methyltransferase, as described in [32]–[34]. As shown in Table 2, **12** and **13** were very specific for C5 DNA methyltransferases. Finally, we determined the cytotoxicity of the most potent compounds on two tumour cell lines, HCT116 (colon cancer cells) and DU145 (prostate cancer cells). The cytotoxicity profile obtained for the two cancer cell lines was similar to the inhibition profile of the methylation activity (Table 1 and Figure S4). Again, for the phthalimide family, as the length of the linker increased the cytotoxicity increased up to compound **12**. Once more, the compounds that showed the highest cytotoxicity were the phthalimide conjugates **12** and **13**, the long amido conjugates of L-RG108 (**18** and **19**) and of D-RG108 (**18 D**) and compound **20**.

Table 2. Methyltransferase selectivity. Inhibition (IC_{50}) of murine catalytic complex Dnmt3A/3L, EcoDam and G9A.[a]

	IC_{50} [μM]		
Cpds	Dnmt3A/3L	EcoDam	G9a
8	410±240	>1000	>1000
11	210±20	n.d.	n.d.
12	8.5±4.2	>1000	70±10
13	9.2±3.6	200±40	160±50

[a] Concentration (mean±SE of three to five experiments) at which 50% inhibition of the enzyme activity was observed.

Conclusion

We have developed a new rapid synthetic pathway to obtain potent inhibitors of DNMT1 and Dnmt3A/3L, based on the conjugation of procainamide to L-RG108 or phthalimide. Five conjugates were at least 50 times more active than their parent compounds. For the L-RG108 conjugates, the attachment site on L-RG108 was extremely important: conjugation to the indolo moiety at position 5 resulted in moderately active compounds, while conjugation at the carboxyl group gave among the most active compounds. We also showed that the length of the linker arm plays an important role and that the 12-carbon linker (**12** and **18**) is the most promising. STD experiments and docking simulations suggest that the procainamide moiety does not interact with the enzyme, in agreement with the hypothesis of its binding to DNA as a ligand to position the phthalimide or L-RG108 to interact with the enzyme. Interestingly, **12** and **13** showed very good selectivity for C5 DNA methyltransferases when compared to bacterial DNA methyltransferase (EcoDam) and mammalian histone G9a methyltransferase. This observation together with the fact that the presence of 0.01% Triton X-100 did not affect enzyme inhibition indicates that the inhibition is not due to non-specific aggregation as observed in several screening campaigns.[29,30] In addition, conjugates **18** and **19** presented a slight selectivity between Dnmt3A/3L and DNMT1, which can be further improved by substitution, as shown for *S*-adenosyl-L-homocystein analogues.[35] Interestingly, the most potent inhibitors, **12**, **13**, **18**, **19** and **20**, also showed micromolar cytotoxicity towards

cancerous cell lines DU145 and HCT116. This is very promising for their application as antitumor agents.

Experimental Section

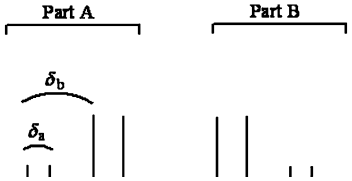
Chemicals: General *S*-adenosyl-L-methionine (SAM) was bought from Sigma; aliquots were stored at −80 °C; procainamide was from Sigma; oligonucleotides were from Eurogentec (Seraing, Belgium). All chemicals were from Sigma–Aldrich except dibromotetradecane, which was from Epsilon Chemie (Brest, France). L-RG108 and D-RG018 were synthesized from L- and D-tryptophane, respectively, according to Schirrmacher et al.[25] Compound **3**, an OH-derivatized L-RG108, was obtained as described by Schirrmacher et al.[25]

Compound purity was verified by reversed-phase HPLC on an X-terra C18 MS column (3.9×100 mm; Waters) with a linear gradient acetonitrile in 0.1% formic acid (0→95% CH_3CN).

Physical measurements: ^{1}H and ^{13}C NMR spectra were acquired on a Bruker AM400 spectrometer (400 MHz) or on a Bruker Avance II spectrometer equipped with a ^{13}C cryoprobe, at 500 MHz for ^{1}H and 125 MHz for ^{13}C; 2D experiments were performed by using standard Bruker programs. Assignments of the NMR signals are presented in the Supporting Information.

We decided to describe the ABX systems in ^{1}H NMR spectra as illustrated below. The "external" chemical displacements of the ABX system (ν8 for part B and ν1 for part A) will be pointed out, and the coupling constants J_a and J_b are defined by: $J_a = (\nu1-\nu2) = (\nu7-\nu8)$ $J_b = (\nu1-\nu3) = (\nu6-\nu8)$

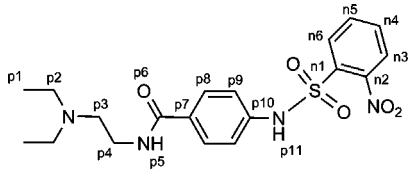
ABX system:



ν_1 and ν_8 (ABX, 2H; J_a = Hz, J_b = Hz; attribution); NMR spectra of all tested molecules are available in the Supporting Information.

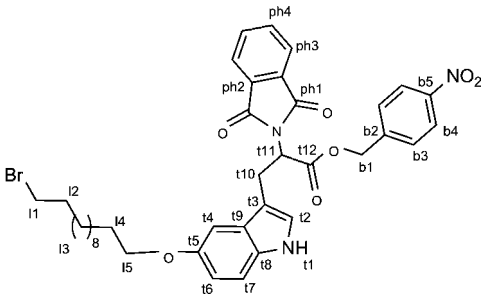
Chemical synthesis

***N*-[2-(Diethylamino)ethyl]-4-[(2-nitrobenzene)sulfonamido]benzamide (2)**:



2-Nitrophenylsufonyl chloride (5 g, 23 mmol) and DMAP (0.25 g, 2 mmol) was added to a solution of procainamide **1** (hydrochloride salt; 5 g, 18.5 mmol) in dichloromethane (50 mL) and TEA (5 mL). The mixture was stirred for 18 h at room temperature. The solvent was removed by vacuum, and the residue was purified by flash chromatography silica gel with dichloromethane/methanol (9:1 + 1.75% ammonia) to give **2** as a pale yellow amorphous solid (hygroscopic; 4.3 g, yield 55%). ^{1}H NMR (400 MHz, DMSO): δ = 8.36 (m, 1H$_{p11}$), 7.94 (m, 1H$_{n3}$), 7.82 (m, 1H$_{n6}$), 7.71 (m, 2H$_{n4+n5}$), 7.64 (d, J = 8.7 Hz, 2H$_{p8}$), 7.01 (d, J = 8.7 Hz, 2H$_{p9}$), 3.44 (m, 2H$_{p4}$), 2.94 (m, 6H$_{p2+p3}$), 1.10 (t, J = 7.2 Hz, 6H$_{p1}$); ^{13}C NMR (100 MHz, DMSO): δ = 171.5 (C_{p6}), 153.3 (C_{p7}), 151.3 (C_{n2}), 139.4 (C_{n1}), 138.4 (C_{n5}), 137.1 (C_{n4}), 134.9 (C_{n3}), 133.4 (C_{n6}), 131.8 (C_{p10}), 129.2 (C_{p8}), 124.4 (C_{p7}), 55.8 (C_{p4}) 52.0 (C_{p3}), 40.6 (C_{p2}), 14.9 (C_{p1}); HRMS-ESI (*m/z*) calcd for $C_{19}H_{24}N_4O_5S$: 421.15402 $[M+H]^+$; found: 421.15374.

(4-Nitrophenyl)methyl-3-{5-[(6-bromohexyl)oxy]-1*H*-indol-3-yl}-2-(1,3-dioxo-2,3-dihydro-1*H*-isoindol-2-yl)propanoate (4) and (4-nitrophenyl)methyl-3-{5-[(6-bromododecyl)oxy]-1*H*-indol-3-yl}-2-(1,3-dioxo-2,3-dihydro-1*H*-isoindol-2-yl)propanoate (5):

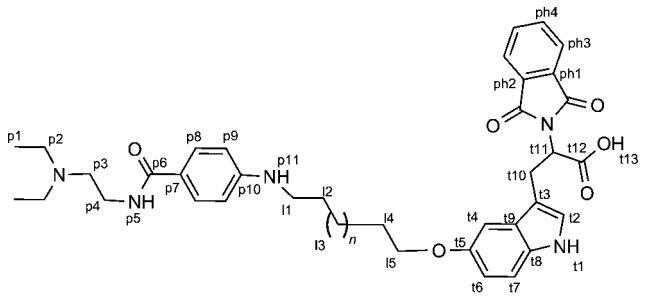


Dibromoalkyl (1,12-dibromododecane; 400 mg, 1.22 mmol) or 1,6-dibromohexane (285 mg, 1.22 mmol), K_2CO_3 (170 mg, 1.22 mmol) and a catalytic amount of potassium iodide was added to a solution of **3** (200 mg, 0.41 mmol) in acetone (5 mL). The mixture was heated at 90 °C overnight. The mixture was diluted with ethylacetate and washed with water. The organic phase was washed with brine and dried with magnesium sulfate. Solvent was removed and the residue was purified by flash chromatography on silica gel (cyclohexane/ethylacetate, 8:2) to afford **4** (154 mg, yield 58%) and **5** (184 mg, yield 61%) as dark yellow foams.

Compound 4: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.10 (dm, J = 8.9 Hz, 2H$_{b4}$), 7.84 (m, 1H$_{t1}$), 7.63 (m, 2H$_{ph3}$), 7.57 (m, 2H$_{ph4}$), 7.36 (dm, J = 8.9 Hz, 2H$_{b3}$), 7.06 (d, J = 8.7 Hz, 1H$_{t7}$), 6.32 (m, 2H$_{t2+t4}$), 6.71 (dm, J = 8.7 Hz, 1H$_{t6}$), 5.25 (m, 3H$_{b1+t11}$), 3.85 (m, 2H$_{l5}$), 3.66 (d, J = 8.0 Hz, 2H$_{t10}$), 3.33 (t, J = 6.9 Hz, 2H$_{l1}$), 1.76 (m, 2H$_{l2}$), 1.70 (m, 2H$_{l4}$), 1.22 (m, 4H$_{l3}$); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.9 (C_{ph1}), 167.5 (C_{t12}), 153.6 (C_{t5}), 147.8 (C_{b5}), 142.5 (C_{b2}), 134.2 (C_{ph4}), 131.6 (C_{ph2}), 131.1 (C_{t8}), 128.3 (C_{t2}), 127.6 (C_{t3}), 123.8 (C_{b4}), 123.5 (C_{b3}), 123.3 (C_{ph3}), 113.0 (C_t), 111.8 (C_t), 110.5 (C_{t9}), 101.1 (C_t), 68.7 (C_{b1}), 66.0 (C_{l5}), 52.6 (C_{t11}), 34.1 (C_{t10}), 32.9 (C_{l1}), 26.6–25.0 ($C_{l2+l3+l4}$); MS-ESI (*m/z*) calcd for $C_{32}H_{30}N_3O_7Br$: 648.1345–650.1325 $[M+H]^+$; found: 648.1414–650.1386.

Compound 5: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.09 (dm, J = 8.8 Hz, 2Hb4), 7.82 (m, 1H$_{t1}$), 7.65 (m, 2H$_{ph3}$), 7.57 (m, 2H$_{ph4}$), 7.36 (dm, J = 8.8 Hz, 2H$_{b3}$), 7.06 (d, J = 8.7 Hz, 1H$_{t7}$), 6.90 (m, 2H$_{t2+t4}$), 6.69 (dm, J = 8.7 Hz, 1H$_{t6}$), 5.23 (m, 3H$_{b1+t11}$), 3.83 (m, 2H$_{l5}$), 3.65 (d, J = 8.0 Hz, 2H$_{t10}$), 3.33 (t, J = 6.9 Hz, 2H$_{l1}$), 1.77 (m, 2H$_{l2}$), 1.69 (m, 2H$_{l4}$), 1.42–1.18 (m, 16H$_{l3}$); ^{13}C NMR (400 MHz, $CDCl_3$): δ = 168.9 (C_{ph1}), 167.5 (C_{t12}), 153.6 (C_{t5}), 147.8 (C_{b5}), 142.5 (C_{b2}), 134.2 (C_{ph4}), 131.6 (C_{ph2}), 131.1 (C_{t8}), 128.4 (C_{t2}), 127.6 (C_{t3}), 123.8 (C_{b4}), 123.5 (C_{b3}), 123.3 (C_{ph3}), 113.0 (C_t), 111.8 (C_t), 110.5 (C_{t9}), 101.1 (C_t), 68.7 (C_{b1}), 66.0 (C_{l5}), 52.7 (C_{t11}), 34.2 (C_{t10}), 32.9 (C_{l1}), 29.6–25.0 ($C_{l2+l3+l4}$); MS-ESI (*m/z*) calcd for $C_{16}H_{20}NO_2Br$: 732.2284–734.2264 $[M+H]^+$; found: 732.2334–734.2354.

3-[5-({6-[(4-{[2-(Diethylamino)ethyl]carbamoyl}phenyl)amino]-hexyl}oxy)-1*H*-indol-3-yl]-2-(1,3-dioxo-2,3-dihydro-1*H*-isoindol-2-yl)propanoic acid (6) and 3-[5-({6-[(4-{[2-(Diethylamino)ethyl]carbamoyl}phenyl)amino]dodecyl}oxy)-1*H*-indol-3-yl]-2-(1,3-dioxo-2,3-dihydro-1*H*-isoindol-2-yl)propanoic acid (7):



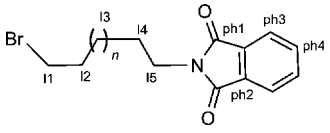
K_2CO_3 (160 mg, 1.16 mmol) and the desired L-RG108 derivative **4** (123 mg, 0.19 mmol) or **5** (140 mg, 0.19 mmol) was added to a solution of nosylate **2** (160 mg, 0.38 mmol) in dry acetonitrile (3 mL). The mixture was refluxed overnight, then thiophenol (50 μL, 0.48 mmol) was added. The reaction mixture was heated at 80 °C for 2 h, then water (50 μL) was added, and the mixture was heated at 80 °C for 0.5 h. Solvents were removed, and the residue was purified by preparative HPLC with a C-18 column and a linear gradient (0–90% acetonitrile with 0.1% TFA) to afford **6** (73 mg, yield 68%) or **7** (83 mg, yield 58%) as dark yellow foams.

Compound 6: ^{1}H NMR (400 MHz, DMSO): $\delta = 10.55$ (s, 1 H_{t13}), 9.28 (s_b, 1 H_{t1}), 8.28 (m, 1 H_{p5}), 7.77 (m, 4 $H_{ph3+ph4}$), 7.59 (d, $J = 8.8$ Hz, 2 H_{p8}), 7.07 (d, $J = 8.7$ Hz, 1 H_{t7}), 6.95 (m, 1 $H_{t2\ or\ t4}$), 6.86 (m, 1 $H_{t2\ or\ t4}$), 6.54 (m, 3 H_{t6+p9}), 5.02 (t, $J = 8.3$, 1 H_{t11}), 3.77 (m, 2 H_{l5}), 3.52–3.42 (m, 4 H_{t10+p4}), 3.17 (m, 6 H_{p2+p3}), 3.04 (m, 2 H_{l1}), 1.60 (m, 4 H_{l2+l4}), 1.40 (m, 4 H_{l3}), 1.16 (t, $J = 7.2$, 6 H_{p1}); ^{13}C NMR (100 MHz, DMSO): $\delta =$ 170.3 (C_{p6}), 167.2 (C_{t12}), 167.1 (C_{ph1}), 152.3 (C_{t5}), 151.7 (C_{p7}), 134.8 (C_{ph4}), 130.9 (C_{ph2}), 130.7 (C_{t8}), 128.8 (C_{p8}), 128.6 (C_{t2}), 127.1 (C_{t3}), 123.1 (C_{ph3}), 119.7 (C_{p10}), 112.0 ($C_{t4\ or\ t6}$), 111.6 (C_{p9}), 110.6 ($C_{t4\ or\ t6}$), 109.5 (C_{t9}), 100.5 (C_{t7}), 67.6 (C_{l5}), 52.7 (C_{t11}), 50.2 (C_{p3}), 46.7 (C_{p2}), 42.4 (C_{t10}), 34.3–25.5 ($C_{l1+l2+l3+l4+p4}$), 8.4 (C_{p1}); HRMS-ESI (*m/z*) calcd for $C_{38}H_{46}O_6N_5$: 668.34426 $[M+H]^+$; found: 668.34475.

Compound 7: ^{1}H NMR (400 MHz, DMSO): $\delta = 10.55$ (s, 1 H_{t13}), 9.33 (s_b, 1 H_{t1}), 8.28 (m, 1 H_{p5}), 7.77 (m, 4 $H_{ph3+ph4}$), 7.58 (d, $J = 8.8$ Hz, 2 H_{p8}), 7.07 (d, $J = 8.7$ Hz, 1 H_{t7}), 6.95 (m, 1 $H_{t2\ or\ t4}$), 6.85 (m, 1 $H_{t2\ or\ t4}$), 6.55 (dd, $J = 2.3$; 8.7 Hz, 1 H_{t6}), 6.51 (d, $J = 8.7$ Hz, 2 H_{p9}), 5.03 (t, $J =$ 8.4 Hz, 1 H_{t11}), 3.79–3.71 (m, 2 H_{l5}), 3.48 (m, 4 H_{t10+p4}), 3.16 (m, 6 H_{p2+p3}), 2.99 (t, $J = 8.4$ Hz, 2 H_{l1}), 1.62–1.49 (m, 4 H_{l2+l4}), 1.4–1.1 (m, 22 H_{l3+p1}); ^{13}C NMR (100 MHz, DMSO): $\delta = 170.1$ (C_{p6}), 167.0 (C_{t12}), 166.9 (C_{ph1}), 152.1 (C_{t5}), 151.5 (C_{p7}), 134.6 (C_{ph4}), 130.9 (C_{ph2}), 130.7 (C_{t8}), 128.8 (C_{p8}), 128.6 (C_{t2}), 127.1 (C_{t3}), 123.1 (C_{ph3}), 119.5 (C_{p10}), 111.8 ($C_{t4\ or\ t6}$), 111.4 (C_{p9}), 110.4 ($C_{t4\ or\ t6}$), 109.3 (C_{t9}), 100.3 (C_{t7}), 67.5 (C_{l5}), 52.5 (C_{t11}), 50.0 (C_{p3}), 46.5 (C_{p2}), 34.1–21.9 ($C_{l1+l2+l3+l4+t10+p4}$), 8.2 (C_{p1}); HRMS-ESI (*m/z*) calcd for $C_{44}H_{58}O_6N_5$ $[M+H]^+$: 752.43816; found: 752.43892.

2-(8-Bromobutyl)-2,3-dihydro-1H-isoindole-1,3-dione (B1) and 2-(8-Bromohexyl)-2,3-dihydro-1*H*-isoindole-1,3-dione (B2) were bought from Sigma–Aldrich.

Synthesis of bromoalkylphthalimides (B3–B6):



Potassium phthalimide (2.5 mmol) was added to a solution of dibromoalkyl (1.8 mmol) in DMF (5 mL), and the mixture was stirred overnight at room temperature, then diluted with ethylacetate and washed with water. The organic phase was washed with brine and dried on magnesium sulfate. Solvent was removed, and the residue was purified by flash chromatography on silica gel (cyclohexane/ethylacetate, 8:2) to afford bromoalkylphthalimides as white amorphous solids.

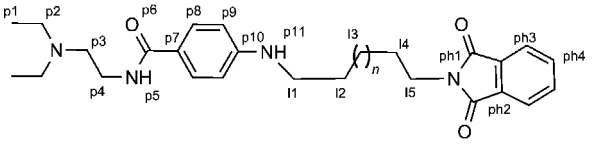
2-(8-Bromooctyl)-2,3-dihydro-1*H*-isoindole-1,3-dione (B3): $n = 4$; from 490 mg of 1.8-dibromooctane, **B3** was obtained in 54% yield. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.75$ (m, 2 H_{ph3}), 7.58 (m, 2 H_{ph4}), 3.58 (t, $J = 7.3$ Hz, 2 H_{l5}), 3.27 (t, $J = 6.8$ Hz, 2 H_{l1}), 1.76 (m, 2 H_{l4}), 1.58 (m, 2 H_{l2}), 1.39–1.15 (m, 8 H_{l3}); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 168.4$ (C_{ph1}), 133.8 (C_{ph4}), 132.2 (C_{ph2}), 123.1 (C_{ph3}), 38.0 (C_{l5}), 33.9 (C_{l1}), 32.7 (C_{l4}), 28.9–26.7 (C_{l2+l3}); MS-ESI (*m/z*) calcd for $C_{16}H_{20}NO_2Br$: 338.0756–340.0735 $[M+H]^+$; found: 338.0805–340.0784.

2-(8-Bromodecyl)-2,3-dihydro-1*H*-isoindole-1,3-dione (B4): $n = 6$; from 540 mg of 1,10-dibromodecane, **B4** was obtained in 55% yield. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.75$ (m, 2 H_{ph3}), 7.58 (m, 2 H_{ph4}), 3.58 (t, $J = 7.3$ Hz, 2 H_{l5}), 3.27 (t, $J = 6.8$ Hz, 2 H_{l1}), 1.76 (m, 2 H_{l4}), 1.58 (m, 2 H_{l2}), 1.39–1.15 (m, 12 H_{l3}); ^{13}C NMR (100 MHz, DMSO): $\delta = 168.4$ (C_{ph1}), 133.8 (C_{ph4}), 132.2 (C_{ph2}), 123.1 (C_{ph3}), 38.0 (C_{l5}), 33.9 (C_{l1}), 32.8 (C_{l4}), 29.7–25.6 (C_{l2+l3}); MS-ESI (*m/z*) calcd for $C_{18}H_{24}NO_2Br$: 366.1069–368.1048 $[M+H]^+$; found: 366.1118–368.1096.

2-(8-Bromododecyl)-2,3-dihydro-1*H*-isoindole-1,3-dione (B5): $n = 8$; from 590 mg of 1,12-dibromododecane, **B5** was obtained in 58% yield. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.73$ (m, 2 H_{ph3}), 7.62 (m, 2 H_{ph4}), 3.61 (t, $J = 7.3$ Hz, 2 H_{l5}), 3.34 (t, $J = 6.8$ Hz, 2 H_{l1}), 1.72 (m, 2 H_{l4}), 1.61 (m, 2 H_{l2}), 1.33 (m, 16 H_{l3}); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 168.4$ (C_{ph1}), 133.8 (C_{ph4}), 132.2 (C_{ph2}), 123.1 (C_{ph3}), 38.1 (C_{l5}), 34.0 (C_{l1}), 32.8 (C_{l4}), 29.5–27.0 (C_{l2+l3}); MS-ESI (*m/z*) calcd for $C_{20}H_{28}NO_2Br$: 394.1382–396.1361 $[M+H]^+$; found: 394.1417–396.1385.

2-(8-Bromotetradecyl)-2,3-dihydro-1*H*-isoindole-1,3-dione (B6): $n = 10$; from 640 mg of 1,14-dibromotetradecane, **B6** was obtained in 51% yield. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.73$ (m, 2 H_{ph3}), 7.62 (m, 2 H_{ph4}), 3.60 (t, $J = 7.3$ Hz, 2 H_{l5}), 3.35 (t, $J = 6.8$ Hz, 2 H_{l1}), 1.74 (m, 2 H_{l4}), 1.61 (m, 2 H_{l2}), 1.40–1.10 (m, 20 H_{l3}); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 168.5$ (C_{ph1}), 133.8 (C_{ph4}), 132.1 (C_{ph2}), 123.1 (C_{ph3}), 38.1 (C_{l5}), 34.1 (C_{l1}), 32.9 (C_{l4}), 29.6–26.8 (C_{l2+l3}); MS-ESI (*m/z*) calcd for $C_{22}H_{32}NO_2Br$: 422.1695–424.1674 $[M+H]^+$; found: 422.1720–424.1691.

Synthesis of phthalimido derivatives of procainamide 8 to 13: K_2CO_3 (160 mg, 1.16 mmol) and the desired bromoalkylpthtalimide



(0.19 mmol) was added to a solution of the nosylate **2** (160 mg, 0.38 mmol) in dry acetonitrile (3 mL). The mixture was refluxed overnight, then thiophenol (50 μL, 0.48 mmol) was added, and the solution was stirred at room temperature for 3 h. Solvent was removed and the residue was purified by flash chromatography on silica gel with dichloromethane/methanol (98:2 + 1.75% ammonia) as eluent.

***N*-[2-(Diethylamino)ethyl]-4-{[10-(1,3-dioxo-2,3-dihydro-1*H*-isoindol-2-yl)butyl]amino}benzamide (8)**: $n = 0$; from 54 mg of *N*-(4-bromobutyl)phthalimide, **8** was obtained as a yellow oil with a yield of 79%. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.77$ (m, 2 H_{ph3}), 7.64 (m, 2 H_{ph4}), 7.54 (dm, $J = 8.7$ Hz, 2 H_{p8}), 6.71 (m, 1 H_{p5}), 6.48 (dm, $J =$ 8.7 Hz, 2 H_{p9}), 4.02 (m, 1 H_{p11}), 3.66 (t, $J = 7.2$ Hz, 2 H_{l5}), 3.38 (dd, $J =$ 5.4, 11.4 Hz, 2 H_{p4}), 3.13 (m, 2 H_{l1}), 2.56 (t, $J = 6.1$ Hz, 2 H_{p3}), 2.49 (q,

J=7.2 Hz, 4 H_{p2}), 1.65–1.60 (m, 2 H_{l4}), 1.58–1.53 (m, 2 H_{l2}), 0.97 (t, J= 7.3 Hz, 6 H_{p1}); ^{13}C NMR (100 MHz, $CDCl_3$): δ=168.5 (C_{ph1}), 167.2 (C_{p6}), 150.7 (C_{p7}), 134.0 (C_{ph4}), 132.0 (C_{ph2}), 128.6 (C_{p8}), 123.3 (C_{ph3}), 122.9 (C_{p10}), 111.7 (C_{p9}), 51.5 (C_{p3}); 46.8 (C_{p2}), 43.0 (C_{l1}), 37.6 (C_{l5}), 37.1 (C_{p4}), 26.5; 26.2 (C_{l2+l4}), 12.0 (C_{p1}); HRMS-ESI (*m/z*) calcd for $C_{25}H_{33}O_3N_4$: 437.25472 $[M+H]^+$; found: 437.25487.

***N*-[2-(Diethylamino)ethyl]-4-{[10-(1,3-dioxo-2,3-dihydro-1*H*-isoindol-2-yl)hexyl]amino}benzamide (9)**: n=2; from 59 mg of *N*-(6-*b*romohexyl)phthalimide, **9** was obtained as a pale yellow oil with a yield of 82%. ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.75 (m, 2 H_{ph3}), 7.62 (m, 2 H_{ph4}), 7.52 (dm, J=8.7 Hz, 2 H_{p8}), 6.68 (m, 1 H_{p5}), 6.44 (dm, J= 8.7 Hz, 2 H_{p9}), 3.98 (m, 1 H_{p11}), 3.61 (t, J=7.2 Hz, 2 H_{l5}), 3.37 (dd, J= 5.4, 11.4 Hz, 2 H_{p4}), 3.04 (m, 2 H_{l1}), 2.55 (t, J=6.1 Hz, 2 H_{p3}), 2.47 (q, J=7.2 Hz, 4 H_{p2}), 1.62–1.50 (m, 4 H_{l2+l4}), 1.47–1.25 (m, 4 H_{l3}), 0.95 (t, J=7.3 Hz, 6 H_{p1}); ^{13}C NMR (100 MHz, $CDCl_3$): δ=168.5 (C_{ph1}), 167.3 (C_{p6}), 150.9 (C_{p7}), 133.9 (C_{ph4}), 132.1 (C_{ph2}), 128.6 (C_{p8}), 123.2 (C_{ph3}), 122.7 (C_{p10}), 111.6 (C_{p9}), 51.5 (C_{p3}); 46.8 (C_{p2}), 43.3 (C_{l1}), 37.8 (C_{l5}), 37.1 (C_{p4}), 29.1, 28.5, 26.5 ($C_{l2+l3+l4}$), 12.0 (C_{p1}); HRMS-ESI (*m/z*) calcd for $C_{27}H_{37}O_3N_4$: 465.28602 $[M+H]^+$; found: 465.28588.

***N*-[2-(Diethylamino)ethyl]-4-{[10-(1,3-dioxo-2,3-dihydro-1*H*-isoindol-2-yl)octyl]amino}benzamide (10)**: n=4; from 64 mg of *N*-(8-*b*romooctyl)phthalimide, **10** was obtained as a yellow foam with a yield of 75%. ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.75 (m, 2 H_{ph3}), 7.62 (m, 2 H_{ph4}), 7.54 (dm, J=8.7 Hz, 2 H_{p8}), 6.68 (m, 1 H_{p5}), 6.47 (dm, J= 8.7 Hz, 2 H_{p9}), 3.95 (m, 1 H_{p11}), 3.60 (t, J=7.2 Hz, 2 H_{l5}), 3.38 (dd, J= 5.5, 11.5 Hz, 2 H_{p4}), 3.04 (m, 2 H_{l1}), 2.56 (t, J=6.1 Hz, 2 H_{p3}), 2.48 (q, J=7.3 Hz, 4 H_{p2}), 1.61–1.48 (m, 4 H_{l2+l4}), 1.35–1.20 (m, 8 H_{l3}), 0.95 (t, J=7.3 Hz, 6 H_{p1}); ^{13}C NMR (100 MHz, $CDCl_3$): δ=168.5 (C_{ph1}), 167.3 (C_{p6}), 151.0 (C_{p7}), 133.9 (C_{ph4}), 132.2 (C_{ph2}), 128.6 (C_{p8}), 123.1 (C_{ph3}), 122.7 (C_{p10}), 111.6 (C_{p9}), 51.6 (C_{p3}); 46.8 (C_{p2}), 43.5 (C_{l1}), 38.0 (C_{l5}), 37.1 (C_{p4}), 29.3–26.7 ($C_{l2+l3+l4}$), 12.0 (C_{p1}). HRMS-ESI (*m/z*) calcd for $C_{29}H_{41}O_3N_4$ $[M+H]^+$: 493.31732; found: 493.31695.

***N*-[2-(Diethylamino)ethyl]-4-{[10-(1,3-dioxo-2,3-dihydro-1*H*-isoindol-2-yl)decyl]amino}benzamide (11)**: n=6; from 70 mg of *N*-(10-bromodecyl)phthalimide, **11** was obtained as a pale yellow solid with a yield of 83%. ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.76 (m, 2 H_{ph3}), 7.63 (m, 2 H_{ph4}), 7.55 (d, J=8.7 Hz, 2 H_{p8}), 6.70 (m, 1 H_{p5}), 6.49 (d, J=8.8 Hz, 2 H_{p9}), 3.95 (m, 1 H_{p11}), 3.60 (t, J=7.2 Hz, 2 H_{l5}), 3.38 (dd, J=5.6, 11.3 Hz, 2 H_{p4}), 3.05 (dd, J=6.8, 12.3 Hz, 2 H_{l1}), 2.55 (t, J=5.9 Hz, 2 H_{p3}), 2.47 (q, J=7.1 Hz, 4 H_{p2}), 1.64–1.49 (m, 4 H_{l2+l4}), 1.26–1.15 (m, 12 H_{l3}), 0.95 (t, J=7.1 Hz, 6 H_{p1}); ^{13}C NMR (100 MHz, $CDCl_3$): δ=168.5 (C_{ph1}), 167.3 (C_{p6}), 151.0 (C_{p7}), 133.9 (C_{ph4}), 132.2 (C_{ph2}), 128.6 (C_{p8}), 123.1 (C_{ph3}), 122.6 (C_{p10}), 111.6 (C_{p9}), 51.5 (C_{p3}); 46.8 (C_{p2}), 43.5 (C_{l1}), 38.0 (C_{l5}), 37.1 (C_{p4}), 29.4–26.8 ($C_{l2+l3+l4}$), 12.0 (C_{p1}); HRMS-ESI (*m/z*) calcd for $C_{31}H_{45}O_3N_4$: 521.34862 $[M+H]^+$; found: 521.34819.

***N*-[2-(Diethylamino)ethyl]-4-{[10-(1,3-dioxo-2,3-dihydro-1*H*-isoindol-2-yl)dodecyl]amino}benzamide (12)**: n=8; from 75 mg of *N*-(12-bromododecyl)phthalimide, **12** was obtained as a pale yellow solid with a yield of 89%. ^{1}H NMR (400 MHz, $CDCl_3$): δ= 7.74 (m, 2 H_{ph3}), 7.62 (m, 2 H_{ph4}), 7.54 (d, J=8.7 Hz, 2 H_{p8}), 6.66 (m, 1 H_{p5}), 6.48 (d, J=8.7 Hz, 2 H_{p9}), 3.97 (m, 1 H_{p11}), 3.59 (t, J=7.2 Hz, 2 H_{l5}), 3.38 (dd, J=5.6, 11.5 Hz, 2 H_{p4}), 3.05 (m, 2 H_{l1}), 2.56 (t, J= 6.0 Hz, 2 H_{p3}), 2.47 (q, J=7.1 Hz, 4 H_{p2}), 1.60–1.50 (m, 4 H_{l2+l4}), 1.28–1.10 (m, 16 H_{l3}), 0.96 (t, J=7.1, 6 H_{p1}); ^{13}C NMR (100 MHz, $CDCl_3$): δ=168.4 (C_{ph1}), 167.2 (C_{p6}), 151.0 (C_{p7}), 133.8 (C_{ph4}), 132.2 (C_{ph2}), 128.5 (C_{p8}), 123.1 (C_{ph3}), 122.6 (C_{p10}), 111.6 (C_{p9}), 51.5 (C_{p3}); 46.8 (C_{p2}), 43.5 (C_{l1}), 38.0 (C_{l5}), 37.2 (C_{p4}), 29.5–27.0 ($C_{l2+l3+l4}$), 12.0 (C_{p1}); HRMS-ESI (*m/z*) calcd for $C_{33}H_{49}O_3N_4$: 549.37992 $[M+H]^+$; found: 549.37938.

***N*-[2-(Diethylamino)ethyl]-4-{[10-(1,3-dioxo-2,3-dihydro-1*H*-isoindol-2-yl)tetradecyl]amino}benzamide (13)**: n=10; from 80 mg of *N*-(14-bromotetradecyl)phthalimide, **13** was obtained as a pale yellow solid with a yield of 85%. ^{1}H NMR (400 MHz, $CDCl_3$): δ= 7.73 (m, 2 H_{ph3}), 7.61 (m, 4 H_{ph4+p8}), 7.12 (m, 1 H_{p5}), 6.48 (d, J= 8.8 Hz, 2 H_{p9}), 4.15 (m, 1 H_{p11}), 3.58 (t, J=7.1 Hz, 2 H_{l5}), 3.46 (dd, J= 5.6, 11.3 Hz, 2 H_{p4}), 3.03 (dd, J=6.6, 12.5 Hz, 2 H_{l1}), 2.74 (t, J=5.8 Hz, 2 H_{p3}), 2.65 (q, J=7.2 Hz, 4 H_{p2}), 1.57–1.48 (m, 4 H_{l2+l4}), 1.33–1.10 (m, 20 H_{l3}), 1.04 (t, J=7.1 Hz, 6 H_{p1}); ^{13}C NMR (100 MHz, $CDCl_3$): δ= 168.4 (C_{ph1}), 167.9 (C_{p6}), 151.3 (C_{p7}), 133.8 (C_{ph4}), 132.1 (C_{ph2}), 128.5 (C_{p8}), 123.1 (C_{ph3}), 121.6 (C_{p10}), 111.5 (C_{p9}), 52.2 (C_{p3}); 47.3 (C_{p2}), 43.5 (C_{l1}), 38.0 (C_{l5}), 36.8 (C_{p4}), 29.5–26.8 ($C_{l2+l3+l4}$), 11.3 (C_{p1}); HRMS-ESI (*m/z*) calcd for $C_{35}H_{53}O_3N_4$: 577.41122 $[M+H]^+$; found: 577.41085.

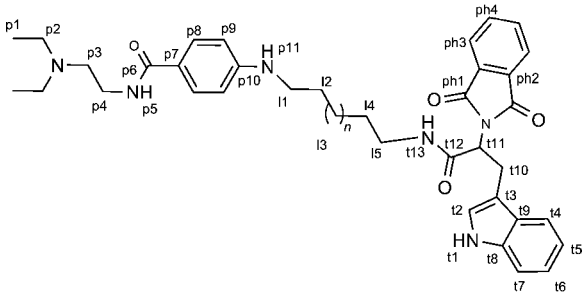
4-[(6-Aminohexyl)amino]-*N*-[2-(diethylamino)ethyl]benzamide (14), 4-[(6-Aminododecyl)amino]-*N*-[2-(diethylamino)ethyl]benzamide (15), and 4-[(6-Aminotetradecyl)amino]-*N*-[2-(diethylamino)ethyl]benzamide (16): Methylhydrazine (100 μL) was added to a solution of **9** (56 mg, 0.12 mmol) or **12** (72 mg, 0.14 mmol) or **13** (57 mg, 0.10 mmol) in ethanol (1 mL). The mixture was stirred at room temperature overnight. The solvent was removed, toluene was added and coevaporated until methylhydrazine was completely removed. The residue of the reaction with **12** was purified by flash chromatography silica gel with dichloromethane/methanol (97:3+1.75% ammonia) to give **15** (41 mg, 69%) as pale yellow foams. Compounds **14** and **16**, residues of deprotections of **9** and **13,** were used in the following step without further purification.

Compound 14: MS-ESI (*m/z*) calcd for $C_{19}H_{34}ON_4$: 335.281 $[M+H]^+$; found: 335.298.

Compound 15: ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.53 (d, J=8.7 Hz, 2 H_{p8}), 6.69 (m, 1 H_{p5}), 6.50 (d, J=8.7 Hz, 2 H_{p9}), 4.01 (m, 1 H_{p11}), 3.41 (m, 2 H_{p4}), 2.61–2.43 (m, 8 $H_{p2+l1+p3}$), 3.03 (m, 2 H_{NH_2}), 1.53 (m, 2 H_{l2}), 1.45–1.10 (m, 24 $H_{l3+l4+l5}$), 0.96 (t, J=7.1 Hz, 6 H_{p1}); ^{13}C NMR (100 MHz, $CDCl_3$): δ=167.2 (C_{p6}), 150.9 (C_{p7}), 128.6 (C_{p8}), 122.6 (C_{p10}), 111.6 (C_{p9}), 51.5 (C_{p3}); 47.0 (C_{p2}), 46.8 (C_{l1}), 37.1 (C_{p4}), 31.9 (C_{l2}), 29.5–27.1 (C_{l2+l3}), 22.7 (C_{l4}), 14.1 (C_{l5}), 12.0 (C_{p1}); MS-ESI (*m/z*) calcd for $C_{25}H_{47}ON_4$: 419.374 $[M+H]^+$; found: 419.402.

Compound 16: MS-ESI (*m/z*) calcd for $C_{27}H_{51}ON_4$: 447.41 $[M+H]^+$; found: 447.41.

***N*-[2-(Diethylamino)ethyl]-4-({6-[2-(1,3-dioxo-2,3-dihydro-1*H*-isoindol-2-yl)-3-(1*H*-indol-3-yl)propanamido]hexyl}amino)benzamide (17), *N*-[2-(Diethylamino)ethyl]-4-({6-[2-(1,3-dioxo-2,3-dihydro-1*H*-isoindol-2-yl)-3-(1*H*-indol-3-yl)propanamido]dodecyl}amino)benzamide (18 and 18D) and *N*-[2-(Diethylamino)ethyl]-4-({6-[2-(1,3-dioxo-2,3-dihydro-1*H*-isoindol-2-yl)-3-(1*H*-indol-3-yl)propanamido]tetradecyl}amino)benzamide (19)**:



NHS (*N*-hydroxysuccinimide; 41 mg, 0.36 mmol), DCC (*N,N*′-dicyclohexylcarbodiimide; 50 mg, 0.24 mmol) and DIPEA (*N,N*-diisopropylethylamine; 40 μL, 0.40 mmol) were added to a solution of L-RG108 (80 mg, 0.24 mmol) in DMF (500 μL). The mixture was stirred

at room temperature for 2 h then filtered to removed DCU (dicyclohexylurea), and then **14** (crude product from 0.05 mmol of **9**) or **15** (41 mg, 0.1 mmol) or **16** (crude product from 0.1 mmol of **13**) was added and the mixture was stirred at room for 2 h. The solvent was removed, and the residue was purified by flash chromatography on silica gel with dichloromethane/methanol (99:1+0.5% ammonia) as eluent to give **17** (from **14**) as a pale yellow solid (52 mg, yield 58% after two steps from **9**), **18** (from **15**) as a pale yellow solid (52 mg; yield 72%, $[\alpha]_D^{20>}$: −75.5 ($c=1$, MeOH)) or **19** (from **16**) as a pale yellow solid (35 mg, yield 48% over two steps from **13**). The same procedure with D-RG108 as starting materiel was applied to **15** give **18D** in 62% yield ($[\alpha]_D^{20}=+73.3$ ($c=1$, MeOH)).

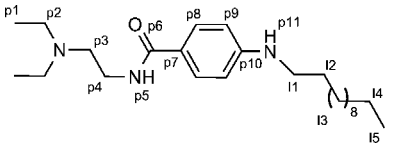
Compound 17: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=8.69$ (s_b, $1H_{t1}$), 7.70–7.51 (m, $7H_{ph4+t7+p8+ph3}$), 7.21 (m, $1H_{t4}$), 7.05–6.94 (m, $4H_{t2+t5+t6+p5}$), 6.47 (dm, $J=8.8$ Hz, $2H_{p9}$), 6.30 (m, $1H_{t13}$), 5.16 (t, $J=7.1$ Hz, $1H_{t11}$), 4.18 (m, $1H_{p11}$), 3.75–3.49 (m, $4H_{t10+p4}$), 3.13–2.54 (m, $10H_{l5+l1+p3+p2}$), 1.45 (m, $2H_{l4}$), 1.33 (m, $2H_{l2}$), 1.24–1.05 (m, $8H_{p1+l3}$); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=168.7$ (C_{t12}), 168.2 (C_{ph1}), 167.4 (C_{p6}), 151.0 (C_{p7}), 136.3 (C_{ph2}), 134.1 (C_{ph4}), 131.6 (C_{t8}), 128.6 (C_{p8}), 126.9 (C_{t3}), 123.4 (C_{ph3}), 122.9 (C_{t2}), 122.5 (C_{p10}), 122.2 ($C_{t5\ or\ t6}$), 119.6 ($C_{t5\ or\ t6}$), 118.6 (C_{t7}), 111.6 (C_{p9}), 111.3 (C_{t9}), 111.0 (C_{t4}), 55.0 (C_{t11}), 51.5 (C_{p3}); 46.8 (C_{p2}), 43.5 (C_{l1}), 39.9 (C_{l5}), 37.1 (C_{p4}), 29.5–25.5 ($C_{l2+l3+l4+t10}$), 12.0 (C_{p1}); HRMS-ESI (*m/z*) calcd for $C_{38}H_{47}O_4N_6$: 651.3653 $[M+H]^+$; found: 651.36598.

Compound 18: ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=8.31$ (s_b, $1H_{t1}$), 7.77 (m, $2H_{ph3}$), 7.67–7.59 (m, $5H_{ph4+p8+t7}$), 7.27 (d, $J=8.0$ Hz, $1H_{t4}$), 7.14 (t, $J=7.5$ Hz, $2H_{t5\ or\ t6}$), 7.08 (t, $J=7.5$ Hz, $2H_{t5\ or\ t6}$), 7.02 (d, $J=2.0$ Hz, $1H_{t2}$), 6.74 (m, $1H_{p5}$), 6.54 (dm, $J=9.0$ Hz, $2H_{p9}$), 6.25 (m, $1H_{t13}$), 5.23 (dd, $J=7.0$, 9.1 Hz, $1H_{t11}$), 4.00 (t, $J=5.0$ Hz $1H_{p11}$), 3.80–3.63 (ABX, $J_a=15.0$ Hz, $J_b=7.0$ Hz, $2H_{t10}$), 3.45 (dd, $J=5.6$, 11.6 Hz, $2H_{p4}$), 3.22 (dd, $J=7.0$ Hz, 12.9 Hz, $2H_{l5}$), 3.14 (dd, $J=7.0$, 12.6 Hz, $2H_{l1}$), 2.61 (t, $J=6.5$ Hz, $2H_{p3}$), 2.55 (q, $J=7.0$ Hz, $4H_{p2}$), 1.62 (m, $2H_{l4}$), 1.43–1.10 (m, $18H_{l3+l2}$), 0.95 (t, $J=7.0$ Hz, $6H_{p1}$); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=168.6$ (C_{t12}), 168.2 (C_{ph1}), 167.3 (C_{p6}), 150.9 (C_{p7}), 136.2 (C_{ph2}), 134.1 (C_{ph4}), 131.6 (C_{t8}), 128.5 (C_{p8}), 126.9 (C_{t3}), 123.4 (C_{ph3}), 122.7 (C_{t2}), 122.5 (C_{p10}), 122.2 ($C_{t5\ or\ t6}$), 119.6 ($C_{t5\ or\ t6}$), 118.6 (C_{t7}), 111.6 (C_{p9}), 111.2 (C_{t9}), 111.1 (C_{t4}), 55.0 (C_{t11}), 51.4 (C_{p3}); 46.7 (C_{p2}), 43.5 (C_{l1}), 39.8 (C_{l5}), 37.1 (C_{p4}), 29.7–25.4 ($C_{l2+l3+l4+t10}$), 12.0 (C_{p1}); HRMS-ESI (*m/z*) calcd for $C_{44}H_{59}O_4N_6$: 735.45923 $[M+H]^+$; found: 735.45973.

Compound 18D: ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=8.31$ (s_b, $1H_{t1}$), 7.76 (m, $2H_{ph3}$), 7.68–7.60 (m, $5H_{ph4+p8+t7}$), 7.29 (d, $J=8.0$ Hz, $1H_{t4}$), 7.14 (t, $J=7.5$ Hz, $2H_{t5\ or\ t6}$), 7.08 (t, $J=7.5$ Hz, $2H_{t5\ or\ t6}$), 7.02 (d, $J=2.0$ Hz, $1H_{t2}$), 6.74 (m, $1H_{p5}$), 6.55 (dm, $J=9.0$ Hz, $2H_{p9}$), 6.25 (m, $1H_{t13}$), 5.23 (dd, $J=7.0$ Hz, 9.1 Hz, $1H_{t11}$), 3.99 (t, $J=5.0$ Hz, $1H_{p11}$), 3.80–3.63 (ABX, $J_a=7.0$ Hz, $J_b=15.0$ Hz, $2H_{t10}$), 3.44 (dd, $J=5.6$, 11.6 Hz, $2H_{p4}$), 3.22 (dd, $J=7.0$ Hz, 12.9 Hz, $2H_{l5}$), 3.13 (dd, $J=7.0$, 12.6 Hz, $2H_{l1}$), 2.61 (t, $J=6.5$ Hz, $2H_{p3}$), 2.55 (q, $J=7.0$ Hz, $4H_{p2}$), 1.61 (m, $2H_{l4}$), 1.41–1.10 (m, $18H_{l3+l2}$), 1.03 (t, $J=7.0$ Hz, $6H_{p1}$); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=168.6$ (C_{t12}), 168.2 (C_{ph1}), 167.3 (C_{p6}), 150.9 (C_{p7}), 136.2 (C_{ph2}), 134.1 (C_{ph4}), 131.6 (C_{t8}), 128.5 (C_{p8}), 126.9 (C_{t3}), 123.4 (C_{ph3}), 122.7 (C_{t2}), 122.5 (C_{p10}), 122.2 ($C_{t5\ or\ t6}$), 119.6 ($C_{t5\ or\ t6}$), 118.6 (C_{t7}), 111.6 (C_{p9}), 111.2 (C_{t9}), 111.1 (C_{t4}), 55.0 (C_{t11}), 51.4 (C_{p3}); 46.7 (C_{p2}), 43.5 (C_{l1}), 39.8 (C_{l5}), 37.1 (C_{p4}), 29.7–25.4 ($C_{l2+l3+l4+t10}$), 12.0 (C_{p1}); HRMS-ESI (*m/z*) calcd for $C_{44}H_{59}O_4N_6$ $[M+H]^+$: 735.45923; found: 735.45954.

Compound 19: ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=8.19$ (s_b, $1H_{t1}$), 7.76 (m, $2H_{ph3}$), 7.67–7.60 (m, $5H_{ph4+t7+p8}$), 7.29 (s, $1H_{t4}$), 7.14–7.08 (m, $2H_{t5+t6}$), 7.03 (d, $J=2.2$ Hz, $1H_{t2}$), 6.70 (m, $1H_{p5}$), 6.55 (dm, $J=8.8$ Hz, $2H_{p9}$), 6.21 (m, $1H_{t13}$), 5.23 (dd, $J=7.0$ Hz, 9.1 Hz, $1H_{t11}$), 3.96 (m, $1H_{p11}$), 3.79–3.10 (ABX, $J_a=7.1$ Hz, $J_b=15.0$ Hz, $2H_{t10}$), 3.46 (dd, $J=5.6$, 11.6 Hz, $2H_{p4}$), 3.22 (dd, $J=7.1$ Hz, 12.9 Hz, $2H_{l5}$), 3.14 (dd, $J=7.0$, 12.6 Hz, $2H_{l1}$), 2.623 (t, $J=6.1$ Hz, $2H_{p3}$), 2.56 (q, $J=7.1$ Hz, $4H_{p2}$), 1.62 (m, $2H_{l4}$), 1.46–1.10 (m, $22H_{l3+l2}$), 1.04 (t, $J=7.1$ Hz, $6H_{p1}$); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=168.7$ (C_{t12}), 168.3 (C_{ph1}), 167.4 (C_{p6}), 151.0 (C_{p7}), 136.3 (C_{ph2}), 134.2 (C_{ph4}), 131.8 (C_{t8}), 128.7 (C_{p8}), 127.1 (C_{t3}), 123.64 (C_{ph3}), 122.8 (C_{t2}), 122.4 (C_{p10}), 122.2 ($C_{t5\ or\ t6}$), 119.8 ($C_{t5\ or\ t6}$), 118.6 (C_{t7}), 111.7 (C_{p9}), 111.4 (C_{t9}), 111.3 (C_{t4}), 55.2 (C_{t11}), 51.6 (C_{p3}); 46.9 (C_{p2}), 43.7 (C_{l1}), 40.0 (C_{l5}), 37.3 (C_{p4}), 29.7–25.5 ($C_{l2+l3+l4+t10}$), 12.0 (C_{p1}); HRMS-ESI (*m/z*) calcd for $C_{46}H_{63}O_4N_6$: 763.49053 $[M+H]^+$; found: 763.49146. calcd for $[M+Na]^+$: 795.51675; found: 795.51693.

***N*-[2-(Diethylamino)ethyl]-4-[(2-ethyldecyl)amino]benzamide (20):**



K_2CO_3 (160 mg, 1.16 mmol) and 1-bromododecane (47 mg, 0.19 mmol) were added to a solution of nosylate **2** (160 mg, 0.38 mmol) in dry acetonitrile (3 mL). The mixture was refluxed overnight, then thiophenol (50 μL, 0.48 mmol) was added, and the reaction mixture was stirred at room temperature for 3 h. Solvent was removed and the residue was purified by flash chromatography on silica gel with dichloromethane/methanol (98:2+1.75% ammonia) to afford **20** (72 mg; 94%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.56$ (dm, $J=8.7$ Hz, $2H_{p8}$), 6.70 (m, $1H_{p5}$), 6.44 (dm, $J=8.7$ Hz, $2H_{p9}$), 3.93 (m, $1H_{p11}$), 3.37 (dd, $J=5.5$, 11.6 Hz, $2H_{p4}$), 3.05 (dd, $J=7.04$, 12.7 Hz, $2H_{l1}$), 2.56 (t, $J=5.7$ Hz, $2H_{p3}$), 2.49 (q, $J=7.1$ Hz, $4H_{p2}$), 1.58–1.50 (m, $2H_{l2}$), 1.30–1.18 (m, $16H_{l3}$), 0.96 (t, $J=7.1$ Hz, $6H_{p1}$), 0.81 (t, $J=7.1$ Hz, $3H_{l5}$); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=167.3$ (C_{p6}), 151.0 (C_{p7}), 128.6 (C_{p8}), 122.6 (C_{p10}), 111.6 (C_{p9}), 51.5 (C_{p3}); 46.8 (C_{p2}), 43.5 (C_{l1}), 37.1 (C_{p4}), 31.9 (C_{l2}), 29.5–27.1 (C_{l2+l3}), 22.7 (C_{l4}), 14.1 (C_{l5}), 12.0 (C_{p1}); HRMS-ESI (*m/z*) calcd for $C_{25}H_{46}ON_3$: 404.36354 $[M+H]^+$; found: 404.36492.

Biological activity: C5 DNA methyltransferase activity assay: The C-terminal catalytic domain of murine Dnmt3a (aa. 623–908) and the C-terminal domain of Dnmt3L (aa. 208–421), obtained as described in [36], were preincubated together (0.6 and 1 μM, respectively) for 20–30 min. at room temperature. Tested compounds were added, together with [^{3}H]methyl *S*-adenosyl-L-methionine (SAM; 280 nM, 0.15 μCi) and unmethylated duplex (5′-GATCG CCGAT GCGCG AATCG CGATC GATGC GAT-3′/5′-ATCGC ATCGA TCGCG ATTCG CGCAT CGGCG ATC-3′, 0.2 μM) for 1 h at 37 °C in reaction buffer (50 μL, HEPES (20 mM, pH 7.2), KCl (50 mM), EDTA (1 mM), BSA (25 μg mL^{-1})). The human DNMT1 was produced in H19 cells by the bacculovirus system as described in ref. [13]. The enzyme (20 nM) was incubated in reaction buffer (50 μL, HEPES (20 mM, pH 7.2), KCl (50 mM), EDTA (1 mM), BSA (25 μg mL^{-1})) in the presence of the same hemimethylated DNA duplex (5′-GATMeCG CMeCGAT GMeCGMeCG AATMeCGMe CGATMeC GATGMeC GAT-3′/5′-ATCGC ATCGA TCGCG ATTCG CGCAT CGGCG ATC-3′, 0.2 μM) and the tested compounds with [^{3}H]methyl SAM (280 nM, 0.15 μCi) and unlabeled SAM (1.28 μM) for 2 h at 37 °C. After incubation, we removed the unincorporated SAM and the enzymes with P-30 Tris Micro Bio-Spin Biospin chromatography columns (Bio-rad), and determined incorporation of radioactivity by liquid scintillation counting (Wallac Microbeta 1450 Trilux, PerkinElmer). Background levels were determined in samples lacking the DNA substrate. The results were plotted as relative methylation activity

(expressed in % normalized to samples in the absence of tested compounds) against the logarithm of the inhibitor concentration, and fitted by non-linear regression with GraphPad Prism (GraphPad Software, La Jolla, CA).

Methyltransferase assays: A comparison was carried out between the murine catalytic Dnmt3A/3L complex, the bacterial EcoDam N-6 DNA methyltransferase and the catalytic domain of the human G9a histone H3K9 methyltransferase according to the protocols described in refs. [32] and [33].

NMR saturation transfer difference (STD) experiments: NMR experiments were recorded on a Bruker Avance III NMR spectrometer at a ^{1}H frequency of 500 MHz and equipped with a TCI cryoprobe. Ligands (between 0.1 and 1.0 mM) were tested with enzyme (0.1 to 8.5 μM), in HEPES (20 mM, pH 7.2), KCl (50 mM), EDTA (1 mM), and DTT (0.1 mM) containing 10% D_2O. The saturation scheme consisted of a cascade of Gaussian-shaped pulses (50 ms, total duration 3 s), applied at −1 ppm and 30 ppm, alternately. A double-pulsed field gradient spin echo was applied before acquisition with a selective 90° G4 pulse (4 ms) and 180° REBURP (3 ms) pulses to observe selectively the ^{1}H aromatic region.

Docking: Ligand structures were prepared for the docking process with Marvin software (ChemAxon, Budapest, Hungary). Dock 6.4 (DOCK, University of San Francisco, CA) was used for the molecular docking of the ligand in the X-ray crystal structure of Dnmt3A/3L (PDB: 2QRV, chain A). Default parameters were used. Chimera (RBVI, University of San Francisco, CA) was used for the graphical visualisation.

Cell lines: DU145, a human prostate cancer cell line, and HCT116, a human breast cancer cell line, were obtained from ATCC (LGC Standards, Molsheim, France).

MTS assay: The DU145 cell line was maintained in RPMI medium (Invitrogen) and the HCT116 cell line in DMEM (Invitrogen), supplemented with 10% foetal bovine serum (Perbio, Belgium) and glutamine (2 mM). Cells were incubated at 37 °C in a humidified atmosphere (5% CO_2). DU145 cells (3×10^4) or HCT116 cells (1.5×10^4) were seeded in 96-well plates in medium (80 μL per well) and incubated after seeding with 20 μL of increasing concentrations of inhibitor. Absorbance at 490 nm was determined after 72 h incubation by using CellTiter96 Aqueous Non-Radioactive Cell Proliferation Assay following the manufacturer's instructions (Promega).

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