Effects of Indole Fatty Alcohols on the Differentiation of Neural Stem Cell Derived Neurospheres

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In a search for inducers of neuronal differentiation to treat neurodegenerative diseases such as Alzheimer's disease, a series of indole fatty alcohols (IFAs) were prepared. **13c** ($n = 18$) was able to promote the differentiation of neural stem cell derived neurospheres into neurons at a concentration of 10 nM. Analysis of the expression of the Notch pathway genes in neurospheres treated during the differentiation phase with **13c** ($n = 18$) revealed a significant decrease in the transcription of the Notch 4 receptor.

Introduction

Neuronal disorders have common pathological features that result in defects of nervous system functionality. Loss of neurons and/or glia contributes to this degeneration.

Over the past several years, protein neurotrophic factors (pNFs) have been investigated as a treatment for these disorders.[1] Endogenous molecules such as nerve growth factor (NGF) or brain derived neurotrophic factor (BDNF) are crucial for the regulation of the survival, differentiation, and plasticity of neural cells during development.[2,3] However, early attempts to develop clinical treatments with pNFs were unsuccessful.[4] The main obstacle was the blood−brain barrier.[5] The use of recombinant forms of pNFs by subcutaneous application avoided the direct injection of these proteins into the brain. Nevertheless this procedure did not prevent severe side effects.[4]

In parallel, efforts were made to develop functional mimics of pNFs. Synthetic lipophilic compounds were prepared to promote neuronal survival and differentiation. Drugs such as lactacystin,[6] epolactaene,[7] FK506,[8] and *N*-glyoxyl derivatives[9] have trophic capacities in vitro and in vivo. In a previous study, we reported the neurotrophic activity of cyclohexenonic long-chain ω-alkanols.[10,11] This class of molecules promotes neurite outgrowth and the survival of fetal neurons in vitro. The length of the side chain fatty alcohol appeared to be determinant for the neuritogenic activity. However until now, apart from the potential that these synthetic molecules offer, no clinical proof of efficacy on neurodegenerative disorders has ever been established.

A new approach to the repair of the nervous system could target the stimulation of endogenous neural stem cells[12] and seek to orientate them toward the production of neurons by promoting both neuronal cell fate decision[13] and differentiation. Neural stem cells are multipotential progenitor cells, which can self-renew and generate both neurons and glia depending on the presence of growth factors, hormones, and environmental cues.[14,15] These neural stem cells are substantially present in the adult brain as a quiescent population of cells,[16] and recent data show that they may be able to proliferate and migrate to sites of damaged tissue where they may have potential therapeutic roles in the treatment of brain injuries. Nakatomi and colleagues have demonstrated that endogenous adult brain progenitors in the presence of exogenous growth factors were capable of replacing hippocampal pyramidal neurons that were lost due to ischemia.[17] Compounds like retinoic acid[18] and TWS119[19] (Figure 1) control the processes that regulate stem cell fate and were able to induce neuronal differentiation of embryonic stem cells. The aim of our research was to construct synthetic trophic drugs, capable of stimulating endogenous neural stem cells and of promoting their differentiation into neurons. These small lipophilic molecules must have systemic activity, oral bioavailability, and the ability to cross the blood−brain barrier.

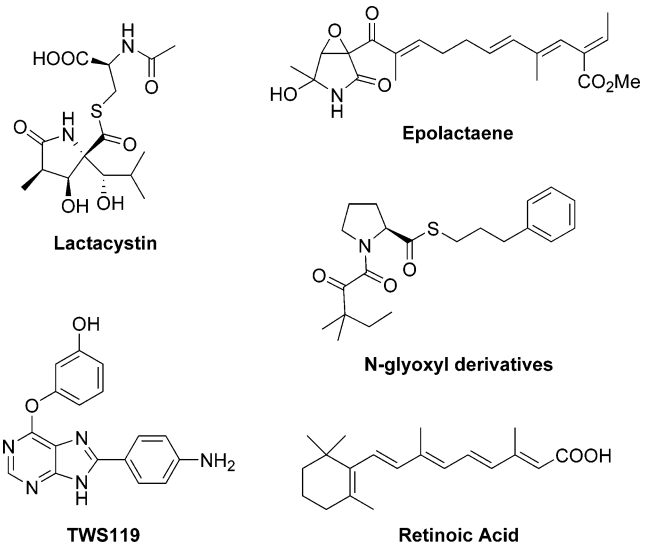

Figure 1. Molecules acting either on the differentiation or on the specification of neuronal lineage.

Neural stem cells can be isolated through the selective action of EGF. They can be grown in vitro as clones of

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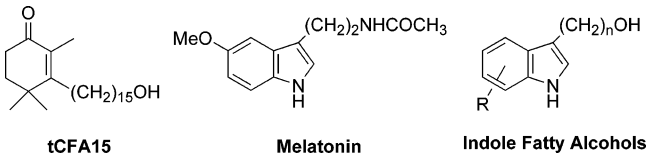


Figure 2. Structures of **tCFA15**, melatonin, and IFAs.

undifferentiated cells floating in the medium, the so-called neurospheres.[20] When provided with a solid support, neurospheres differentiate to give rise to neurons, oligodendrocytes, and astrocytes in proportions that are genetically and epigenetically defined.[21] Neurospheres represent an interesting in vitro model system for investigating the influence of factors on the differentiation potential of neural stem cells.

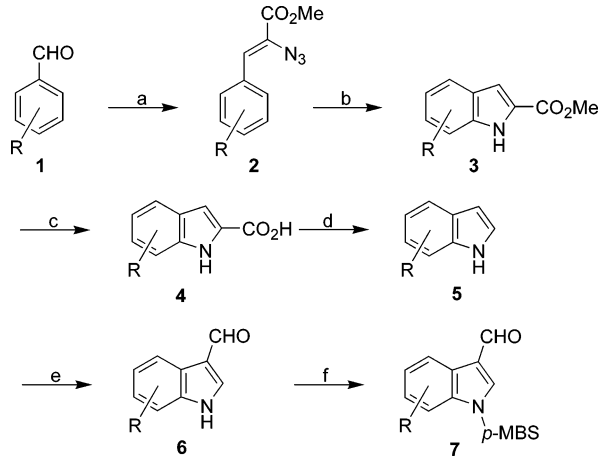
In a parallel work, we have shown that **tCFA15** (Figure 2) was able to promote neuronal cell fate decision and differentiation of neural stem cell derived neurospheres at 10^{-7} M.[22] The putative structure−activity relationship was further investigated with the aim of combining the trophic activity of the long-chain ω-alkanols and a neuroprotective moiety. Oxidative stress has been linked to neuronal cell death resulting either in acute insults due to ischemia or trauma or in chronic neurodegenerative diseases such as Alzheimer's disease.[23] Synergistic effects have been observed in drug combination studies of trophic factors with antioxidants.[24] For this reason, molecules were synthesized which combine a long-chain ω-alkanol with an antioxidant core. Since melatonin is a well-known antioxidant,[25] the indole ring bearing methoxy groups was chosen. In this study, we analyzed the effects of indole fatty alcohols (IFAs) as free radical scavengers and as stimulants of neural stem cell derived neurospheres.

Chemistry

Indole carboxaldehydes **7** were the first intermediates needed to prepare the desired indole fatty alcohols by means of a Wittig reaction. The protocol chosen to prepare gram scale quantities of methoxyindoles utilizes Moody azide pyrolysis.[26] Our procedure started with commercial methoxybenzaldehydes **1**. They were transformed to azidocinnamates **2**, which were then cyclized in hot xylene (Scheme 1). Cyclization of unsymmetrical azidocinnamates provided two regioisomers in proportions depending upon the substitution position. The 5-methoxy- and 7-methoxyindole carboxylates were obtained from the 3-methoxy-azidocinnamate in a 1:1 ratio, whereas the reaction with the 3,4-dimethoxy derivative gave preferentially the 5,6-dimethoxyindole carboxylate with a yield of 86%. Base hydrolysis of **3** followed by Cu/quinoline mediated decarboxylation of the acid **4** afforded the methoxyindoles **5**. Vilsmeier formylation[27] followed by indole protection produced the indole carboxaldehydes **7**.

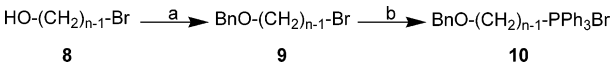
The phosphonium salts **10** were prepared from O-protected bromo alcohols **9** (Scheme 2). 11-Bromoundecan-1-ol is commercially available whereas 9-bromononan-1-ol was obtained by monobromination of nonanediol in a mixture of HBr−cyclohexane. The C-13 and C-15 bromo alcohols were prepared by reduction of the corresponding diacid and lactone with lithium aluminum hydride followed by monobromination of the diols.[28] The C-17 bromo alcohol was obtained in four

Scheme 1[a]



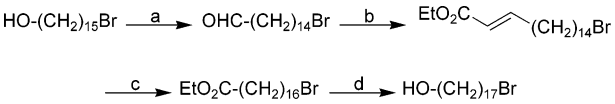
[a] Reagents and conditions: (a) $N_3CH_2CO_2Me$, NaOMe, MeOH, −10 °C. (b) Xylene, reflux. (c) NaOH 2 N, reflux. (d) Cu powder, quinoline, reflux. (e) (i) $P_2O_3Cl_4$, DMF, 0 °C to 40 °C. (ii) Ice, NaOH 1 N. (iii) reflux. (f) NaOH, *p*-methoxybenzenesulfonyl chloride, CH_2Cl_2, room temperature.

Scheme 2[a]



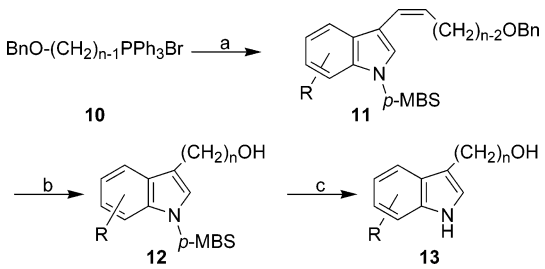
[a] Reagents and conditions: (a) NaH, BnBr, THF, reflux. (b) PPh_3, CH_3CN, reflux.

Scheme 3[a]



[a] Reagents and conditions: (a) PCC, CH_2Cl_2, room temperature. (b) NaH, $(EtO)_2P(O)CH_2CO_2Et$, THF, 0 °C to room temperature. (c) H_2, Pd/C, EtOH, room temperature. (d) $LiAlH_4$, THF, 0 °C to room temperature.

Scheme 4[a]



[a] Reagents and conditions: (a) (i) nBuLi, THF, −78 °C to room temperature. (ii) *t*BuOK, 0 °C. (iii) Aldehyde **7**, THF, −78 °C to 0 °C. (b) H_2, Pd/C, EtOH, room temperature. (c) Na/Hg 6%, Na_2HPO_4, MeOH, 0 °C to room temperature.

steps from 15-bromopentadecan-1-ol by homologation (Scheme 3).

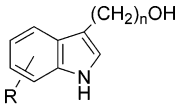
The next step in the synthesis involved the Wittig reaction between the phosphonium salts **10** and the indole carboxaldehydes **7** using Schlosser's base[29] (Scheme 4). Catalytic hydrogenation of **11** with palladium on charcoal followed by indole deprotection led to the IFAs **13**. Compound **14** was obtained according to the same procedure with the tetradecylphosphonium salt as reagent. Table 2 presents the structures evaluated in this study.

Table 1. Radical Scavenging Activity of IFAs against ABTS[a]

compd	R	*n*	ABTS IC_{50} (μM)
melatonin			211
Trolox			600
tCFA15			na[b]
13b	4-MeO	16	23
13c	5-MeO	16	34
13d	6-MeO	16	34
13e	7-MeO	16	26
13f	4,5-MeO	16	17
13g	5,6-MeO	16	34
14	5-MeO	15 (without ω-OH)	207

[a] After 2 h of reaction, the free radical scavenging activity of each antioxidant was quantified by the decolorization of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid radical cation) at 405 nm. The IC_{50} value was determined as the concentration of each sample required for a 50% diminution of the $ABTS^{\bullet+}$ formed. Each value is the average of triplicate determinations; variation was generally ±5%. [b] No free radical scavenging activity.

Table 2. Neuronal Differentiation Activity of IFAs[a]



13a-g

compd (10 nM)	R	*n*	% neurons relative to control	diff[b]
control			100	+
retinoic acid			198	++
melatonin			119	+
13a	H	10	115	+
	H	12	124	+
	H	14	118	+
	H	16	144	++
	H	18	113	+
13b	4-MeO	10	95	+
	4-MeO	12	98	+
	4-MeO	14	118	+
	4-MeO	16	133	+
	4-MeO	18	110	++
13c	5-MeO	10	109	+
	5-MeO	12	108	+
	5-MeO	14	143	++
	5-MeO	16	168	++
	5-MeO	18	169	++
13d	6-MeO	10	110	+
	6-MeO	12	67	+
	6-MeO	14	82	+
	6-MeO	16	135	++
	6-MeO	18	156	++
13f	4,5-MeO	16	118	+
	4,5-MeO	18	106	+
13g	5,6-MeO	16	113	+
	5,6-MeO	18	116	++
14	5-MeO	15 (without ω-OH)	105	+

[a] Details in the Experimental Section. Each compound was evaluated in at least three independent experiments; variation was generally ±10%. [b] Diff stands for qualitative result on the neuronal differentiation; + indicates that neurons differentiate with morphology similar to that of the control; ++ neurons elicit larger morphologies with longer processes than in control.

Free-Radical Scavenging Activity

The free-radical scavenging activity of IFAs was confirmed in the 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) competition assay.[30] In the presence of hydroxyl radicals, ABTS is oxidized to the stable ABTS cation radical ($ABTS^{\bullet+}$) observed by its absorbance at 405 nm. This method measures the relative ability of IFAs to scavenge $^{\bullet}OH$ and thus inhibit the formation of $ABTS^{\bullet+}$ as measured by a decrease of its absorbance at 405 nm. The results are presented in Table 1.

Surprisingly these IFAs caused stronger suppression of $ABTS^{\bullet+}$ than melatonin and than Trolox, a water soluble antioxidant derivative of α-tocopherol. **tCFA15** was unable to scavenge the hydroxyl radicals. Compound **13f**, bearing two methoxy groups on the C-4 and C-5 positions of the benzene moiety, was the most potent. The relative efficacy of these compounds was independent of the length of the side chain (n = 10−18, data not shown). However, the presence of the hydroxyl group was essential for the high potency of these IFAs. The activity of compound **14**, an analogue of compound **13c** with the terminal $-CH_2OH$ of the side chain removed, was reduced to that of melatonin.

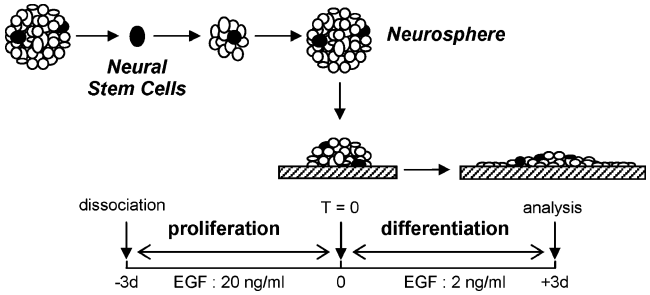


Figure 3. Experimental protocol.

Effect of IFAs on the Differentiation of Neural Stem Cell Derived Neurospheres

In an attempt to establish a structure−function relationship between our compounds, we analyzed each of the synthetic products described above for its ability to influence the cell fate decision and differentiation of neural stem cell derived neurospheres into neurons and astrocytes.

The experimental protocol was that described by Grandbarbe et al.[21] (Figure 3). Briefly, dissociated neurospheres, floating in the culture medium, were grown for 3 days (proliferation phase) and allowed to differentiate on polyornithine support (differentiation phase). The IFAs were added just after plating onto polyornithine cover slips (T = 0), the IFA in the control being replaced by the same volume of ethanol. Neurospheres were then analyzed by immunocytochemistry, using the antibodies specific for mitotic neurons (microtubule associated protein 2, MAP2ab), for postmitotic neurons (monoclonal antibody against neuronal class III tubulin, TuJ1) and an astrocyte-specific intermediate filament protein (glial fibrillary acidic protein, GFAP). The present study does not take into account oligodendrocytes which are difficult to visualize in neurospheres. A nuclear marker, TO-PRO-3-iodide, was used to quantify the total number of cells. First of all, we determined quantitatively that the strongest effect on neuronal population was observed at 10 nM. Higher concentrations (1000 nM, 100 nM) were cytotoxic, and lower concentrations (1 nM) were inefficient. The efficiency of each product on neuron differentiation was estimated from qualitative morphological criteria (aspect) including the size of the neurons and the length of their processes (Table 2).

To investigate the structural requirement of IFAs, we examined whether the length of the hydrocarbon chain was a determining factor for the biological activity as

well as the position of the methoxy group on the benzene moiety. Indole fatty alcohols bearing a side chain containing at least 16 carbon atoms enhanced the neuronal production. Compounds **13c** ($n = 16$) and **13c** ($n = 18$), bearing a methoxy group at the C-5 position of the indole ring, led to a substantial increase of 70% in the production of neurons from the neurospheres as compared to the untreated cells. This activity was comparable to that observed with retinoic acid at the same dose (98%), which remains the highest value obtained in our experiments. The neuronal morphology was similar in both cases; neurons were larger with longer processes indicating a more mature state of differentiation. The presence of the long-chain ω-alkanol is essential for the neurotrophic activity as confirmed by the low increase in neuronal production observed with melatonin (19%). Also, the poor result (5%) obtained with compound **14** confirmed the necessity of the hydroxyl group on the side chain. We also analyzed the dimethoxy series **13f** and **13g** for their effect on neuronal fate and differentiation, which proved to be ineffective (<20%) despite their good antioxidant capacity. We therefore concluded that there was no correlation between the antioxidant property of the indole fatty alcohols and their capacity to promote the production of neurons. In contrast, neither qualitative nor quantitative effects were observed on astrocytes, suggesting that IFAs might be acting specifically on neuronal progenitors, by promoting their proliferation and differentiation.

In order to further characterize the effects of IFAs, we focused on **13c** ($n = 18$), since the compound **13c** ($n = 16$) was slightly more toxic at higher concentrations. We examined the dependence of its effect on the differentiation of neurospheres on the time of administration. This procedure allowed us to define whether a factor is acting on the cell fate decision, on cell differentiation, or on both.[21] When the product is administered during the proliferation phase, an increase in the number of neurons accompanied by a decrease of GFAP-positive cells would suggest that the test compound acts on a cell fate decision. **13c** ($n = 18$) was added every day at a concentration of 10 nM to the neurospheres during the proliferation phase, the differentiation phase, or both. In all cases, the results were similar and showed an increase in the number of MAP2-=positive cells up to 90%, the neurons being larger with longer processes. In contrast, neither qualitative nor quantitative effects were observed on astrocytes (Figure 4). Together these results suggest that the increase in neurons was not taking place at the expense of astrocytes and therefore that **13c** ($n = 18$) was specifically acting on neuronal progenitors by increasing their proliferation and differentiation. We analyzed DNA synthesis by 5-bromo-2′-deoxyuridine (BrdU) incorporation to investigate the possible effect of **13c** ($n = 18$) on the proliferation potential of neurospheres. Although there was a slight increase in the number of BrdU incorporating cells (data not shown), there was no increase in the number of cells colocalizing MAP2ab and BrdU. This result suggests that compound **13c** ($n = 18$) has no mitotic effect on neuronal precursors. However, we cannot exclude the possibility that it may act on early neuronal progenitors which do not yet express MAP2ab. Overall, these data are consistent with the notion that

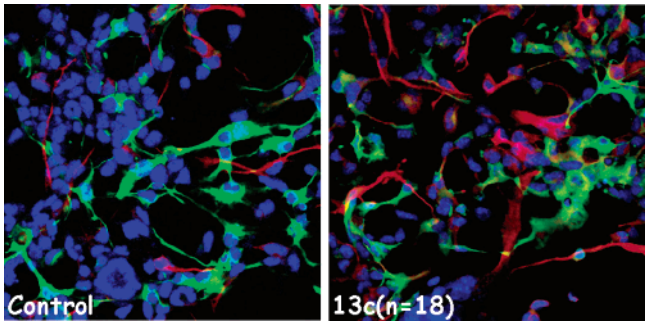


Figure 4. Neurospheres were allowed to differentiate for 3 days without (control) or with **13c** ($n = 18$) at 10 nM. Triple immunostaining involved anti-MAP2ab and Tuj1 coupled to Cy3 for neurons (red), anti-GFAP coupled to Alexa 488 for astrocytes (green), and TO-PRO, a marker specific for nuclei (blue). Colocalized markers are rarely observed and are likely to result from overlapping cells.

13c ($n = 18$) has a promoting effect on the differentiation of neurons and is not acting on cell fate decision.

In a first attempt to elucidate the mechanism of action of IFAs and to identify their targets, we investigated the possibility that the action of **13c** ($n = 18$) might be mediated through Notch signaling. Notch signaling controls a wide variety of cell fate decisions, including the differentiation of neural stem cells during neurogenesis.[21,31] In molecular terms it depends on transmembrane receptors encoded by the Notch genes (from Notch1 to Notch4 in vertebrates) activated by transmembrane ligands of the Delta and Jagged families. We thus compared the level of expression of the four Notch genes and of other genes of the Notch pathway by semiquantitative RT-PCR in neurospheres treated with **13c** ($n = 18$) at 10 nM during the differentiation phase. The Notch1−3 messengers (>70%) showed a slight decrease which was insignificant; in contrast the levels of the Notch4 messengers (19%) are consistently and significantly diminished, suggesting that it might be a biological specific target for **13c** ($n = 18$) (Figure 5). Unfortunately, contrary to other Notch genes, the function of Notch4 in the nervous system remains largely unknown, thereby precluding further correlation between the biological effect of **13c** ($n = 18$) and its mechanism of action.

In order to study the putative role of Notch4 on the production of neurons versus glia in neurospheres, we resorted to the antisense oligonucleotide technique which allows the repression of the Notch4 mRNA translation to the protein. The addition of antisense to Notch4 to neurospheres during the proliferation and the differentiation phase, relative to neurospheres treated with the sense oligonucleotides as control, resulted in an increase of the neuronal population (>300%) with no effect on astrocytes (Figure 6). The phenotypic response was similar to that obtained when the neurospheres were treated with **13c** ($n = 18$) at 10 nM. These results indicate that the **13c** ($n = 18$) promoting effect on the differentiation of neurons might strongly be due to the decrease in Notch4 level expression.

Conclusion

In conclusion, the indole fatty alcohol **13c** ($n = 18$) promotes the differentiation of neural stem cell derived neurospheres into neurons. Our results suggest that this

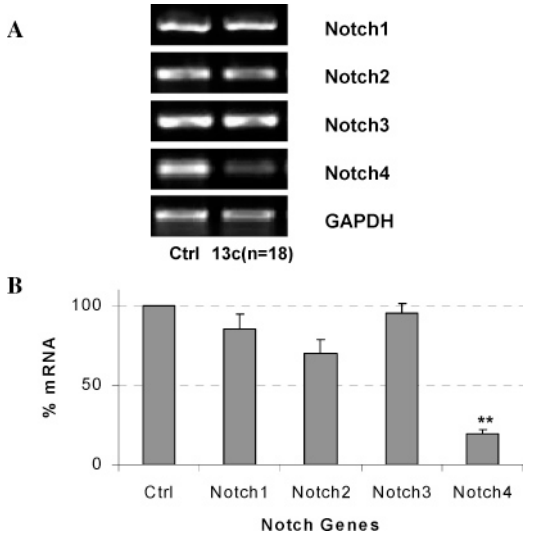


Figure 5. Expression of Notch1 to Notch4 mRNAs by semi-quantitative RT-PCR in neurospheres. Cells were treated without (Ctrl) or with **13c** (n = 18) at 10 nM during the differentiation phase. Total RNA was isolated and RT-PCR was performed using gene specific primers for Notch genes or GAPDH as an internal control. The products were analyzed by electrophoresis on agarose gels containing ethidium bromide as pictured above (A). The relative level of mRNA expressed was quantified by densitometry analysis software NIHImage1.36 (B). Any variation between 0% and 50% was not considered to be significant; $^{**}p \leq 0.01$ by multiple pairwise comparisons using Turkey−Krammer method.

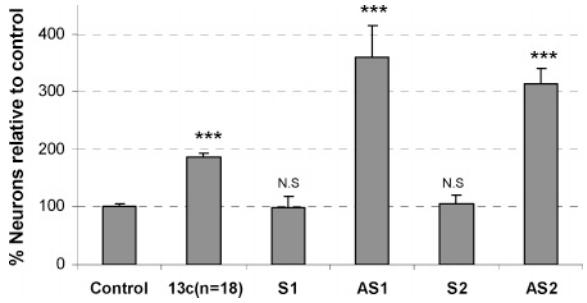


Figure 6. Effect of Notch4 antisense oligonucleotides on the production of neurons in neurospheres. **13c** (n = 18), antisense oligonucleotides (**AS1** and **AS2**), or sense oligonucleotides (**S1** and **S2**, control to AS1 and AS2) were added to neurospheres during the proliferation and the differentiation phase. Results were quantified as percentages of the total number of cells as described in the Experimental Section. Data are representative of three independent experiments. $^{***}p \leq 0.001$ by multiple pairwise comparisons using Turkey−Krammer method.

effect is probably exerted on neuronal progenitors rather than on bipotential neuronal/glial precursors, with **13c** (n = 18) promoting their differentiation. This finding supports the notion that this molecule acts as a neurotrophic factor. More work is needed for elucidating its mechanism of action. However, our results support largely the hypothesis that the compound **13c** (n = 18) may act through the Notch signaling so as to increase the production of neurons from neural stem cells. These results suggest that this compound may have interesting implications in the treatment of neuronal disorders.

Experimental Section

General Procedures. Tetrahydrofuran was distilled from sodium/benzophenone under argon prior to use. Dichloromethane, methanol, and xylene were distilled from calcium hydride. DMF was distilled from P_2O_5. All reactions involving moisture sensitive reactants were executed under argon atmosphere using oven dried and/or flame dried glassware. ^{1}H NMR spectra were recorded on Bruker AC 200 (200 MHz) and Bruker Advance 300 (300 MHz) spectrometers as solutions in $CDCl_3$ or DMSO-d_6. Chemical shifts are expressed in parts per million (ppm, δ) downfield from tetramethylsilane (TMS) and are referenced to $CHCl_3$ (7.26 ppm) or DMSO (2.54 ppm) as internal standard. J values are expressed in hertz (Hz). ^{13}C NMR spectra were recorded on Bruker AC 200 (50 MHz) and Bruker Advance 300 (75 MHz) spectrometers as solutions in $CDCl_3$ or DMSO-d_6. Chemical shifts are expressed in parts per million (ppm, δ) downfield from TMS and are referenced to $CDCl_3$ (77.4 ppm) or DMSO-d_6 (40.5 ppm) as internal standard. The attribution of the different carbons (C, CH, CH_2, CH_3) was determined by ^{13}C to ^{1}H polarization transfer (DEPT). Infrared spectra (IR, cm^{-1}) were measured on a Perkin-Elmer 881 FT-IR spectrometer. UV spectra were obtained in acetonitrile solution using a Kontron-Uvikon 810 UV−vis spectrometer. Mass spectra (MS) were measured on a Trio 2000 Fisons apparatus by direct introduction (an ionization potential of 70 eV was used, m/z relative intensities (in %) are noted in brackets). Analyses were performed by the "Service Central de Microanalyse du CNRS", and all new synthesized compounds agreed within ±0.4% of calculated values. Routine monitoring of reactions was performed using 60 F_{254} silica gel TLC plates (Merck), which were dipped in a solution of vanillin (1 g) in EtOH/H_2SO_4 (95/5) and heated on a hot plate. Flash chromatography was conducted using 60 F_{254} silica gel (Merck) with the indicated solvent.

2-Azido-3-(2′-methoxy-phenyl)acrylic Acid Methyl Ester (2b). A solution of sodium methoxide (30% w/w, 22 mL, 0.12 mol, 4 equiv) in methanol (40 mL) was cooled at −10 °C. A mixture of 2-methoxybenzaldehyde **1b** (4 g, 29.38 mmol, 1 equiv) and methyl azidoacetate (13.5 g, 0.12 mol, 4 equiv) in methanol (10 mL) was added dropwise to it over 1.5 h. The mixture was stirred at −10 °C for an additional 1.5 h, poured into ice−water (100 mL), and extracted with ethyl ether (3 × 100 mL). The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated. Flash chromatography on silica gel using hexane−AcOEt (90−10) gave a yellow solid (4.7 g, 68%). ^{1}H NMR (300 MHz): δ 3.87 (s, 3H, $-CO_2CH_3$), 3.91 (s, 3H, $-OCH_3$), 6.89 (d, J = 7.9 Hz, 1H, H-2′), 6.99 (t, J = 7.7 Hz, 1H, H-5′), 7.32 (dt, J = 7.9 Hz, J = 1.5 Hz, 1H, H-4′), 7.40 (s, 1H, H-3), 8.19 (dd, J = 7.7 Hz, J = 1.5 Hz, 1H, H-6′). ^{13}C NMR (75 MHz): δ 52.8 ($-OCH_3$), 55.6 ($-CO_2CH_3$), 110.4 (C-3′), 119.6, 120.4 (C-3,5′), 122.0 (C-1′), 125.1 (C-2), 130.6, 130.9 (C-4′,6′), 157.6 (C-2′), 164.2 (C-1).

2-Azido-3-(3′-methoxy-phenylacrylic Acid Methyl Ester (2c). Yield: 45%. ^{1}H NMR (300 MHz): δ 3.84 (s, 3H, $-CO_2CH_3$), 3.91 (s, 3H, $-OCH_3$), 6.89 (s, 1H, H-3), 6.92 (m, 1H, H-4′), 7.30 (s, 1H, H-2′), 7.33 (m, 1H, H-6′), 7.43 (m, 1H, H-5′). ^{13}C NMR (75 MHz): δ 52.9 ($-OCH_3$), 55.3 ($-CO_2CH_3$), 115.3, 115.5 (C-2′,4′), 123.4 (C-6′), 125.4 (C-3), 125.5 (C-2), 129.4 (C-5′), 134.3 (C-1′), 159.4 (C-3′), 163.9 (C-1).

2-Azido-3-(4′-methoxy-phenyl)acrylic Acid Methyl Ester (2d). Yield: 72%. ^{1}H NMR (300 MHz): δ 3.84 (s, 3H, $-CO_2CH_3$), 3.89 (s, 3H, $-OCH_3$), 6.89 (s, 1H, H-3), 6.91 (d, J = 9.1 Hz, 2H, H-2′,6′), 7.79 (d, J = 9.1 Hz, 2H, H-3′,5′). ^{13}C NMR (75 MHz): δ 52.7 ($-OCH_3$), 55.3 ($-CO_2CH_3$), 113.9 (C-3′,5′), 123.1 (C-1′), 125.7 (C-3), 125.9 (C-2), 132.4 (C-2′,6′), 160.5 (C-4′), 164.3 (C-1).

2-Azido-3-(2′,3′-dimethoxy-phenyl)acrylic Acid Methyl Ester (2e). Yield: 57%. ^{1}H NMR (300 MHz): δ 3.84 (s, 3H, $-CO_2CH_3$), 3.87 (s, 3H, $-OCH_3$), 3.92 (s, 3H, $-OCH_3$), 6.93 (d, J = 8.1 Hz, 1H, H-4′), 7.09 (t, J = 8.1 Hz, 1H, H-5′), 7.33 (s, 1H, H-3), 7.81 (d, J = 8.1 Hz, 1H, H-6′). ^{13}C NMR (75 MHz): δ 52.9 ($-CO_2CH_3$), 55.8 ($-OCH_3$), 61.4 ($-OCH_3$), 113.6 (C-4′), 119.5 (C-6′), 122.2 (C-3), 123.7 (C-5′), 126.1 (C-1′), 127.3 (C-2), 148.1 (C-2′), 152.5 (C-3′), 164.1 (C-1).

2-Azido-3-(3′,4′-dimethoxy-phenyl)acrylic Acid Methyl Ester (2f). Yield: 76%. ^{1}H NMR (300 MHz): δ 3.90 (s, 3H, $-CO_2CH_3$), 3.91, 3.92 (2s, 6H, $-OCH_3$), 6.87 (d, J = 8.4 Hz, 1H, H-5′), 6.88 (s, 1H, H-3), 7.35 (d, J = 8.4 Hz, 1H, H-6′),

7.51 (s, 1H, H-2′). ^{13}C NMR (75 MHz): δ 52.8 (−CO_2CH_3), 55.9 (−OCH_3), 110.7 (C-2′), 112.9 (C-5′), 123.2 (C-1′), 124.9 (C-3), 125.9 (C-6′), 126.2 (C-2), 148.6 (C-4′), 150.2 (C-3′), 164.2 (C-1).

4-Methoxy-1*H*-indole-2-carboxylic Acid Methyl Ester (3b). Azido ester **2b** (3.7 g, 15.86 mmol, 1 equiv) dissolved in xylene (100 mL) was added dropwise to refluxing xylene (150 mL) under argon. The solution was refluxed for 1 h, after which it was evaporated under reduced pressure. The resulting solid was flash chromatographed on silica gel using hexane−AcOEt (80−20) to give a white solid (2.9 g, 88%). Mp: 143−144 °C. ^{1}H NMR (300 MHz): δ 3.95 (s, 3H, −OCH_3), 3.96 (s, 3H, −CO_2CH_3), 6.51 (d, J = 8.1 Hz, 1H, H-5), 7.03 (d, J = 8.1 Hz, 1H, H-7), 7.24 (t, J = 8.1 Hz, 1H, H-6), 7.36 (s, 1H, H-3), 9.17 (s, 1H, H-1). ^{13}C NMR (75 MHz): δ 51.9 (−OCH_3), 55.3 (−CO_2CH_3), 99.7 (C-5), 104.8 (C-7), 106.5 (C-3), 118.9 (C-3′), 125.8 (C-2), 126.5 (C-6), 138.3 (C-7′), 154.6 (C-4), 162.5 (−CO_2CH_3). Anal. Calcd C: 64.38 H: 5.40 N: 6.83. Found C: 64.35 H: 5.36 N: 6.65. IR (CsI): 3320 (s br, N−H), 2992 (w, =C−H), 2933, 2841 (w, C−H), 1699 (s, C=O), 1522 (m, C−N), 1254 (m, C−O), 755 (s, C−H arom).

5-Methoxy-1*H*-indole-2-carboxylic Acid Methyl Ester (3c). Yield: 37%. Mp: 177−178 °C. ^{1}H NMR (300 MHz): δ 3.85 (s, 3H, −CO_2CH_3), 3.94 (s, 3H, −OCH_3), 7.00 (dd, J = 8.9 Hz, J = 2.2 Hz, 1H, H-6), 7.08 (d, J = 2.2 Hz, 1H, H-4), 7.14 (s, 1H, H-3), 7.32 (d, J = 8.9 Hz, 1H, H-7), 8.91 (s, 1H, H-1). ^{13}C NMR (75 MHz): δ 51.9 (−OCH_3), 55.7 (−CO_2CH_3), 102.5 (C-4), 108.3 (C-3), 112.8 (C-6), 117.1 (C-7), 127.5 (C-2), 127.8 (C-3′), 132.2 (C-7′), 154.7 (C-5), 162.4 (−CO_2CH_3). Anal. Calcd C: 64.38 H: 5.40 N: 6.83. Found C: 64.44 H: 5.44 N: 6.88. IR (CsI): 3314 (s, N−H), 2997 (w, =C−H), 2938, 2836 (w, C−H), 1688 (s, C=O), 1527 (m, C−N), 1250 (m, C−O), 1027 (m, C−O), 766 (s, C−H arom).

6-Methoxy-1*H*-indole-2-carboxylic Acid Methyl Ester (3d). Yield: 94%. Mp: 118−119 °C. ^{1}H NMR (300 MHz): δ 3.85 (s, 3H, −OCH_3), 3.94 (s, 3H, −CO_2CH_3), 6.83 (dd, J = 9.5 Hz, J = 2.2 Hz, 1H, H-5), 6.84 (s, 1H, H-3), 7.17 (d, J = 2.2 Hz, 1H, H-7), 7.55 (d, J = 9.5 Hz, 1H, H-4), 9.02 (s, 1H, H-1). ^{13}C NMR (75 MHz): δ 51.8 (−OCH_3), 55.5 (−CO_2CH_3), 93.7 (C-7), 109.2 (C-3), 112.3 (C-5), 121.8 (C-3′), 123.4 (C-4), 126.0 (C-2), 138.0 (C-7′), 158.9 (C-6), 162.5 (−CO_2CH_3). IR (CsI): 3321 (s, N−H), 2998 (w, =C−H), 2935, 2832 (w, C−H), 1695 (s, C=O), 1525 (m, C−N), 1253 (m, C−O), 1091 (m, C−O), 773 (s, C−H arom).

7-Methoxy-1*H*-indole-2-carboxylic Acid Methyl Ester (3e). Yield: 28%. Mp: 115−116 °C. ^{1}H NMR (300 MHz): δ 3.95 (s, 3H, −OCH_3), 3.98 (s, 3H, −CO_2CH_3), 6.74 (d, J = 7.9 Hz, 1H, H-6), 7.08 (t, J = 7.9 Hz, 1H, H-5), 7.21 (s, 1H, H-3), 7.29 (d, J = 7.9 Hz, 1H, H-4), 9.09 (s, 1H, H-1). ^{13}C NMR (75 MHz): δ 52.1 (−OCH_3), 55.5 (−CO_2CH_3), 104.2 (C-6), 109.1 (C-3), 114.9 (C-5), 121.3 (C-4), 126.9 (C-7′), 128.2 (C-3′), 128.7 (C-2), 146.6 (C-7), 162.3 (−CO_2CH_3). Anal. Calcd C: 64.38 H: 5.40 N: 6.83. Found C: 65.14 H: 5.46 N: 7.25. IR (CsI): 3340 (s, N−H), 2997 (w, =C−H), 2933, 2836 (w, C−H), 1709 (s, C=O), 1579 (m, C−N), 1263 (m, C−O), 1090 (m, C−O), 782 (s, C−H arom).

4,5-Dimethoxy-1*H*-indole-2-carboxylic Acid Methyl Ester (3f). Yield: 63%. Mp: 120−121 °C. ^{1}H NMR (300 MHz): δ 3.90 (s, 3H, −CO_2CH_3), 3.94 (s, 3H, −OCH_3), 4.08 (s, 3H, −OCH_3), 7.07 (s br, 2H, H-6,7), 7.32 (d, J = 1.8 Hz, 1H, H-3), 8.95 (s, 1H, H-1). ^{13}C NMR (75 MHz): δ 50.1 (−CO_2CH_3), 56.5 (−OCH_3), 58.9 (−OCH_3), 104.3, 104.7 (C-6,7), 114.2 (C-3), 120.4 (C-3′), 125.5 (C-2), 132.3 (C-7′), 141.0 (C-4), 142.8 (C-5), 160.4 (−CO_2CH_3). Anal. Calcd C: 61.27 H: 5.57 N: 5.95. Found C: 61.43 H: 5.60 N: 5.95. IR (CsI): 3328 (s, N−H), 2997 (w, =C−H), 2938, 2836 (w, C−H), 1695 (s, C=O), 1530 (m, C−N), 1256 (m, C−O), 1061 (w, C−O), 753 (s, C−H arom).

5,6-Dimethoxy-1*H*-indole-2-carboxylic Acid Methyl Ester (3g). Yield: 86%. Mp: 167−168 °C. ^{1}H NMR (300 MHz): δ 3.92 (s, 9H, −OCH_3, −CO_2CH_3), 6.85 (s, 1H, H-4), 7.04 (s, 1H, H-7), 7.11 (d, J = 2.1 Hz, 1H, H-3), 8.89 (s, 1H, H-1). ^{13}C NMR (75 MHz): δ 51.8 (−CO_2CH_3), 56.1 (−OCH_3), 93.7 (C-4), 102.5 (C-3), 108.8 (C-7), 120.4 (C-3′), 125.6 (C-2), 132.1 (C-7′), 146.2 (C-5), 150.1 (C-6), 162.3 (−CO_2CH_3). IR (CsI): 3334 (s, N−H), 2943, 2836 (w, C−H), 1686 (s, C=O), 1531 (m, C−N), 1255 (m, C−O), 766 (s, C−H arom).

4-Methoxy-1*H*-indole-2-carboxylic Acid (4b). Carboxylate **3b** (4 g, 19.49 mmol, 1 equiv) was added to a solution of aqueous sodium hydroxide (2 M, 98 mL, 0.20 mol, 10 equiv). The suspension was stirred and heated until the reaction mixture became homogeneous, after which the solution was heated at reflux for 30 min. The mixture was acidified, and the precipitate formed was extracted with ethyl acetate (3 × 100 mL). The combined extracts were washed with water, dried over $MgSO_4$, and concentrated to give a white solid (3.7 g, 99%). Mp: 240−241 °C. ^{1}H NMR (300 MHz): δ 3.91 (s, 3H, −OCH_3), 6.55 (d, J = 7.9 Hz, 1H, H-5), 7.06 (d, J = 7.9 Hz, 1H, H-7), 7.09 (s, 1H, H-3), 7.19 (t, J = 7.9 Hz, 1H, H-6), 11.79 (s, 1H, H-1), 12.87 (s, 1H, −CO_2H). ^{13}C NMR (75 MHz): δ 55.4 (−OCH_3), 99.7 (C-5), 104.9, 105.8 (C-3,7), 118.4 (C-3′), 125.8 (C-6), 127.4 (C-2), 138.9 (C-7′), 154.1 (C-4), 163.0 (−CO_2H). IR (CsI): 3325 (s br, N−H, O−H), 2997 (w, =C−H), 1703 (s, C=O), 1519 (m, C−N), 1205 (m, C−O), 1019 (m, C−O), 731 (s, C−H arom).

5-Methoxy-1*H*-indole-2-carboxylic Acid (4c). Yield: 99%. Mp: 199−201 °C. ^{1}H NMR (300 MHz): δ 3.79 (s, 3H, −OCH_3), 6.93 (dd, J = 8.9 Hz, J = 2.2 Hz, 1H, H-6), 7.03 (s, 1H, H-3), 7.13 (d, J = 2.2 Hz, 1H, H-4), 7.36 (d, J = 8.9 Hz, 1H, H-7), 11.64 (s, 1H, −CO_2H), 12.86 (s, 1H, H-1). ^{13}C NMR (75 MHz): δ 55.1 (−OCH_3), 101.9 (C-4), 106.8 (C-3), 113.3 (C-6), 115.7 (C-7), 127.1 (C-2), 128.5 (C-3′), 132.5 (C-7′), 153.7 (C-4), 162.7 (−CO_2H). Anal. Calcd C: 62.82 H: 4.74 N: 7.33. Found C: 62.79 H: 4.73 N: 7.08. IR (CsI): 3335 (s br, N−H, O−H), 2998 (w, =C−H), 1715 (s, C=O), 1523 (m, C−N), 1213 (m, C−O), 1028 (m, C−O), 736 (s, C−H arom).

6-Methoxy-1*H*-indole-2-carboxylic Acid (4d). Yield: 99%. Mp: 204−205 °C. ^{1}H NMR (300 MHz): δ 3.81 (s, 3H, −OCH_3), 6.75 (dd, J = 8.8 Hz, J = 1.9 Hz, 1H, H-5), 6.90 (s, 1H, H-3), 7.05 (d, J = 1.9 Hz, 1H, H-7), 7.55 (d, J = 8.8 Hz, 1H, H-4), 11.58 (s, 1H, H-1), 12.73 (s, 1H, −CO_2H). ^{13}C NMR (75 MHz): δ 55.0 (−OCH_3), 93.9 (C-7), 107.7 (C-3), 111.5 (C-5), 121.1 (C-3′), 122.7 (C-4), 127.1 (C-2), 138.2 (C-7′), 157.6 (C-6), 162.6 (−CO_2H). IR (CsI): 3345 (s large, N−H, O−H), 2996 (w, =C−H), 1693 (s, C=O), 1526 (m, C−N), 1211 (m, C−O), 1031 (m, C−O), 745 (s, C−H arom).

7-Methoxy-1*H*-indole-2-carboxylic Acid (4e). Yield: 96%. ^{1}H NMR (300 MHz): δ 3.94 (s, 3H, −OCH_3), 6.80 (d, J = 7.8 Hz, 1H, H-6), 7.02 (d, J = 7.8 Hz, 1H, H-5), 7.11 (s, 1H, H-3), 7.25 (d, J = 7.8 Hz, 1H, H-4), 11.65 (s, 1H, −CO_2H), 12.82 (s, 1H, H-1). ^{13}C NMR (75 MHz): δ 56.2 (−OCH_3), 105.0 (C-6), 109.1 (C-3), 115.1 (C-5), 121.6 (C-4), 129.1, 129.2, 129.5 (C-2, 3′, 7′), 147.7 (C-7), 163.5 (−CO_2H). Anal. Calcd C: 62.82 H: 4.74 N: 7.33. Found C: 62.68 H: 4.80 N: 7.31. IR (CsI): 3312 (s br, N−H, O−H), 2998 (w, =C−H), 1674 (s, C=O), 1549 (m, C−N), 1249 (m, C−O), 1087 (m, C−O), 730 (s, C−H arom).

4,5-Dimethoxy-1*H*-indole-2-carboxylic Acid (4f). Yield: 93%. ^{1}H NMR (300 MHz): δ 3.82 (s, 3H, −OCH_3), 3.97 (s, 3H, −OCH_3), 7.09−7.14 (m, 2H, H-6,7), 7.12 (s, 1H, H-3); 11.70 (s, 1H, −CO_2H), 12.95 (s, 1H, H-1). ^{13}C NMR (75 MHz): δ 58.5 (−OCH_3), 60.6 (−OCH_3), 104.7 (C-7), 107.8 (C-6), 116.0 (C-3), 121.9 (C-3′), 129.0 (C-2), 135.0 (C-7′), 142.3 (C-4), 144.2 (C-5), 163.0 (−CO_2H). Anal. Calcd C: 59.73 H: 5.01 N: 6.33. Found C: 59.73 H: 5.18 N: 5.88. IR (CsI): 3394 (s br, N−H, O−H), 2998 (w, =C−H), 1647 (s, C=O), 1523 (m, C−N), 1215 (m, C−O), 1064 (m, C−O), 741 (s, C−H arom).

5,6-Dimethoxy-1*H*-indole-2-carboxylic Acid (4g). Yield: 85%. ^{1}H NMR (300 MHz): δ 3.71, 3.74 (s, 6H, −OCH_3), 6.83 (s, 1H, H-3), 6.91 (s, 1H, H-4), 7.03 (s, 1H, H-7), 11.40 (s, 1H, −CO_2H), 12.53 (s, 1H, H-1). ^{13}C NMR (75 MHz): δ 55.8, 56.0 (−OCH_3), 94.8 (C-4), 103.0 (C-3), 107.9 (C-7), 120.2 (C-3′), 126.9 (C-2), 132.8 (C-7′), 145.9 (C-5), 149.6 (C-6), 163.1 (−CO_2H). IR (CsI): 3391 (s br, N−H, O−H), 2997 (w, =C−H), 1652 (s, C=O), 1539 (m, C−N), 1227 (m, C−O), 1015 (m, C−O), 724 (s, C−H arom).

4-Methoxy-1*H*-indole (5b). The carboxylic acid **4b** (3.7 g, 19.09 mmol, 1 equiv), copper powder (850 mg, 13.36 mmol, 0.7 equiv), and freshly distilled quinoline (50 mL) were brought to reflux for 2 h. The mixture was then cooled and filtered on

Celite. The filtrate was poured on ice, and the solution was brought to pH 4 with concentrated HCl and extracted with ethyl acetate (3 × 100 mL). The combined extracts were washed with HCl 2 M (3 × 100 mL), saturated $NaHCO_3$, and brine. The organic solution was dried over $MgSO_4$ and concentrated. The residue was flash chromatographed on silica gel using hexane−AcOEt (85−15) to give a white solid (2.6 g, 94%). Mp: 69−70 °C. ^{1}H NMR (300 MHz): δ 3.99 (s, 3H, −OC*H*$_3$), 6.57 (d, J = 7.9 Hz, 1H, H-5), 6.70 (t, J = 2.5 Hz, 1H, H-3), 7.03 (t, J = 7.9 Hz, 1H, H-7), 7.10 (t, J = 2.5 Hz, 1H, H-2), 7.16 (t, J = 7.9 Hz, 1H, H-6), 8.13 (s, 1H, H-1). ^{13}C NMR (75 MHz): δ 53.3 (−O*C*H$_3$), 99.6, 99.8 (C-3,5), 104.5 (C-7), 118.6 (C-3′), 122.7, 122.8 (C-2,6), 137.3 (C-7′), 153.4 (C-4). IR (CsI): 3415 (s, N−H), 2993 (w, =C−H), 2941, 2835 (w, C−H), 1599 (s, C−N), 1486 (m, C−H), 1239 (s, C−O), 1053 (m, C−O), 725 (s, C−H arom).

5-Methoxy-1*H*-indole (5c). Yield: 77%. Mp: 52−53 °C. ^{1}H NMR (300 MHz): δ 3.87 (s, 3H, −OC*H*$_3$), 6.50 (t, J = 2.8 Hz, 1H, H-3), 6.89 (dd, J = 8.8 Hz, J = 2.4 Hz, 1H, H-6), 7.13 (d, J = 2.4 Hz, 1H, H-4), 7.19 (t, J = 2.8 Hz, 1H, H-2), 7.28 (d, J = 8.8 Hz, 1H, H-6), 8.06 (s, 1H, H-1). ^{13}C NMR (75 MHz): δ 53.9 (−O*C*H$_3$), 102.3, 102.4 (C-3,6), 111.7 (C-4), 112.4 (C-7), 124.9 (C-2); 130.1 (C-3′), 131.0 (C-7′), 154.2 (C-5). IR (CsI): 3411 (s, N−H), 2997 (w, =C−H), 2943, 2832 (w, C−H), 1625 (s, C−N), 1480 (m, C−H), 1224 (s, C−O), 1028 (m, C−O), 725 (s, C−H arom).

6-Methoxy-1*H*-indole (5d). Yield: 87%. Mp: 92−94 °C. ^{1}H NMR (300 MHz): δ 3.87 (s, 3H, −OC*H*$_3$), 6.52 (d, J = 2.6 Hz, 1H, H-3), 6.85 (m, 2H, H-5,7), 7.08 (t, J = 2.6 Hz, 1H, H-2), 7.55 (d, J = 9.1 Hz, 1H, H-4), 7.99 (s, 1H, H-1). ^{13}C NMR (75 MHz): δ 55.7 (−O*C*H$_3$), 94.6 (C-7), 102.4 (C-3), 109.9 (C-5), 121.3 (C-4), 122.2 (C-3′), 123.1 (C-2), 136.6 (C-7′), 156.4 (C-6). IR (CsI): 3395 (s, N−H), 3009 (w, =C−H), 2955, 2841 (w, C−H), 1631 (s, C−N), 1509 (m, C−H), 1249 (s, C−O), 1041 (m, C−O), 815 (s, C−H arom).

7-Methoxy-1*H*-indole (5e). Yield: 71%. ^{1}H NMR (300 MHz): δ 3.98 (s, 3H, −OC*H*$_3$), 6.56 (t, J = 2.7 Hz, 1H, H-3), 6.67 (d, J = 7.7 Hz, 1H, H-6), 7.06 (t, J = 7.7 Hz, 1H, H-5), 7.19 (t, J = 2.7 Hz, 1H, H-2), 7.29 (d, J = 7.7 Hz, 1H, H-4), 8.39 (s, 1H, H-1). ^{13}C NMR (75 MHz): δ 57.6 (−O*C*H$_3$), 104.0 (C-6),105.2 (C-3), 115.8 (C-5), 122.5 (C-4), 126.0 (C-2), 128.8 (C-3′), 131.5 (C-7′), 148.5 (C-7). Anal. Calcd C: 73.45 H: 6.16 N: 9.52. Found C: 73.53 H: 6.16 N: 9.54. IR (CsI): 3418 (s, N−H), 3046 (w, =C−H), 2937, 2836 (w, C−H), 1580 (s, C−N), 1490 (m, C−H), 1255 (s, C−O), 1081 (m, C−O), 725 (s, C−H arom).

4,5-Dimethoxy-1*H*-indole (5f). Yield: 83%. Mp: 160−161 °C. ^{1}H NMR (300 MHz): δ 3.91 (s, 3H, −OC*H*$_3$), 4.08 (−OCH$_3$), 6.64 (t, J = 2.6 Hz, 1H, H-3), 6.94 (d, J = 8.6 Hz, 1H, H-6), 7.07 (d, J = 8.6 Hz, 1H, H-7), 7.14 (t, J = 2.6 Hz, 1H, H-2), 8.18 (s, 1H, H-1). ^{13}C NMR (75 MHz): δ 58.4 (−O*C*H$_3$), 60.7 (−O*C*H$_3$), 99.8 (C-3), 105.8 (C-6), 111.8 (C-7), 122.2 (C-3′), 124.6 (C-2), 133.3 (C-7′), 141.9 (C-5), 144.5 (C-4). Anal. Calcd C: 67.78 H: 6.26 N: 7.90. Found C: 67.57 H: 6.28 N: 7.82. IR (CsI): 3339 (s, N−H), 2995 (w, =C−H), 2932, 2836 (w, C−H), 1638 (s, C−N), 1490 (m, C−H), 1224 (s, C−O), 1039 (m, C−O), 742 (s, C−H arom).

5,6-Dimethoxy-1*H*-indole (5g). Yield: 61%. Mp: 155−157 °C. ^{1}H NMR (300 MHz): δ 3.90, 3.93 (s, 6H, −OC*H*$_3$), 6.45 (t, J = 2.8 Hz, 1H, H-3), 6.89 (s, 1H, H-7), 7.08 (t, J = 2.8 Hz, 1H, H-2), 7.10 (s, 1H, H-4), 8.05 (s, 1H, H-1). ^{13}C NMR (75 MHz): δ 56.2, 56.3 (−O*C*H$_3$), 94.4 (C-3), 102.3, 102.3 (C-4,7), 120.5 (C-3′), 122.7 (C-2), 130.1 (C-7′), 145.1 (C-5), 147.0 (C-6). IR (CsI): 3353 (s, N−H), 2997 (w, =C−H), 2961, 2830 (w, C−H), 1629 (s, C−N), 1478 (s, C−H), 1221 (s, C−O), 997 (m, C−O), 757 (s, C−H arom).

4-Methoxy-1*H*-indole-3-carbaldehyde (6b). Pyrophosphoryl chloride (1.9 mL, 13.86 mmol, 1.2 equiv) was added dropwise to a stirred mixture of *N,N*-dimethylformamide (4.5 mL, 57.76 mmol, 5 equiv) and indole **5b** (1.7 g, 11.55 mmol, 1 equiv) at 0 °C. The resulting syrup was stirred at 0 °C for 0.5 h then at 40 °C for 1 h. Ice was added, followed by a solution of sodium hydroxide (2 M), and the mixture was heated under reflux. On cooling, the solution was extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$, and concentrated. Flash chromatography with hexane−AcOEt (60−40) gave a white solid (1.7 g, 85%). Mp: 162−163 °C. ^{1}H NMR (300 MHz): δ 4.00 (s, 3H, −OC*H*$_3$), 6.72 (d, J = 8.1 Hz, 1H, H-5), 7.09 (d, J = 8.1 Hz, 1H, H-7), 7.21 (t, J = 8.1 Hz, 1H, H-6), 7.92 (d, J = 3.1 Hz, 1H, H-2), 9.36 (s, 1H, H-1), 10.50 (s, 1H, −C*H*O). ^{13}C NMR (75 MHz): δ 55.2 (−O*C*H$_3$), 102.2 (C-5), 105.7 (C-7), 115.5 (C-3), 118.0 (C-3′), 123.5 (C-6), 129.5 (C-2), 137.8 (C-7′), 153.8 (C-4), 186.2 (−*C*HO).

1*H*-Indole-3-carbaldehyde (6a). Yield: 91%. Mp: 188−189 °C. ^{1}H NMR (300 MHz): δ 7.19−7.28 (m, 2H, H-5,6), 7.53 (dd, J = 7.2 Hz, J = 1.6 Hz, 1H, H-7), 8.04 (dd, J = 6.6 Hz, J = 1.5 Hz, 1H, H-4), 8.29 (s, 1H, H-2), 9.94 (s, 1H, −C*H*O), 12.13 (s, 1H, H-1). ^{13}C NMR (75 MHz): δ 112.9 (C-7), 118.6 (C-3), 121.3 (C-5), 122.6 (C-4), 123.9 (C-6), 124.5 (C-3′), 137.5 (C-7′), 138 9 (C-2), 185.4 (−*C*HO). IR (CsI): 3391 (s br, N−H), 2927, 2819 (w, C−H), 1634 (s, C=O), 1447 (m, C−H), 1244 (m, C−H), 761 (s, C−H arom).

5-Methoxy-1*H*-indole-3-carbaldehyde (6c). Yield: 90%. Mp: 182−183 °C. ^{1}H NMR (200 MHz): δ 3.83 (s, 3H, −OC*H*$_3$), 6.92 (dd, J = 8.9 Hz, J = 2.5 Hz, 1H, H-6), 7.44 (d, J = 8.9 Hz, 1H, H-7), 7.63 (d, J = 2.5 Hz, 1H, H-4), 8.25 (s, 1H, H-2), 9.93 (s, 1H, −C*H*O), 12.06 (s, 1H, H-1). ^{13}C NMR (50 MHz): δ 55.2 (−O*C*H$_3$), 102.5 (C-4), 113.1, 113.2 (C-6,7), 118.0 (C-3), 124.8 (C-3′), 131.7 (C-7′), 138.3 (C-2), 155.6 (C-5), 184.7 (−*C*HO). IR (CsI): 3339 (s br, N−H), 2926, 2839 (w, C−H), 1635 (s, C=O), 1489 (m, C−H), 1263 (s, C−O), 1052 (m, C−O), 776 (s, C−H arom).

6-Methoxy-1*H*-indole-3-carbaldehyde (6d). Yield: 89%. Mp: 195−196 °C. ^{1}H NMR (300 MHz): δ 3.82 (s, 3H, −OC*H*$_3$), 6.85 (dd, J = 8.6 Hz, J = 2.2 Hz, 1H, H-5), 6.99 (d, J = 2.2 Hz, 1H, H-7), 7.98 (d, J = 8.6 Hz, 1H, H-4), 8.05 (s, 1H, H-2), 9.89 (s, 1H, −C*H*O), 12.08 (s, 1H, H-1). ^{13}C NMR (75 MHz): δ 55.0 (−O*C*H$_3$), 95.2 (C-7), 111.6 (C-5), 117.9, 118.2 (C-3,3′), 121.3 (C-4), 137.0 (C-2), 137.9 (C-7′), 156.7 (C-6), 184.3 (−*C*HO). IR (CsI): 3187 (s br, N−H), 2824 (w, C−H), 1638 (s, C=O), 1423 (m, C−H), 1228 (s, C−O), 1036 (m, C−O), 769 (s, C−H arom).

7-Methoxy-1*H*-indole-3-carbaldehyde (6e). Yield: 78%. Mp: 162−163 °C. ^{1}H NMR (300 MHz): δ 3.97 (s, 3H, −OC*H*$_3$), 6.77 (d, J = 7.9 Hz, 1H, H-6), 7.23 (t, J = 7.9 Hz, 1H, H-5), 7.81 (d, J = 3.1 Hz, 1H, H-2), 7.88 (d, J = 7.9 Hz, 1H, H-4), 9.23 (s, 1H, H-1), 10.06 (s, 1H, −C*H*O). ^{13}C NMR (75 MHz): δ 55.4 (−O*C*H$_3$), 104.2 (C-6), 114.2 (C-4), 120.0 (C-3), 123.7 (C-5), 125.8 (C-7′), 127.2 (C-3′), 134.6 (C-2), 146.0 (C-7), 185.4 (−*C*HO). Anal. Calcd C: 68.56 H: 5.18 N: 8.00. Found C: 68.41 H: 5.18 N: 7.90. IR (CsI): 3401 (s br, N−H), 2889 (w, C−H), 1615 (s, C=O), 1467 (m, C−H), 1239 (s, C−O), 1059 (m, C−O), 750 (s, C−H arom).

4,5-Dimethoxy-1*H*-indole-3-carbaldehyde (6f). Yield: 57%. Mp: 116−117 °C. ^{1}H NMR (300 MHz): δ 3.93 (s, 3H, −OC*H*$_3$), 4.01 (s, 3H, −OC*H*$_3$), 6.98 (d, J = 8.7 Hz, 1H, H-6), 7.18 (d, J = 8.7 Hz, 1H, H-7), 7.93 (d, J = 3.1 Hz, 1H, H-2), 9.82 (s, 1H, H-1), 10.38 (s, 1H, −C*H*O). ^{13}C NMR (75 MHz): δ 57.6 (−O*C*H$_3$), 60.8 (−O*C*H$_3$), 107.9 (C-6), 112.1 (C-7), 118.5 (C-3), 120.6 (C-3′), 131.7 (C-2), 133.2 (C-7′), 142.5 (C-4), 147.8 (C-5), 187.3 (−*C*HO). Anal. Calcd C: 64.38 H: 5.40 N: 6.83. Found C: 64.02 H: 5.40 N: 6.73. IR (CsI): 3268 (s br, N−H), 2938, 2836 (w, C−H), 1644 (s, C=O), 1505 (m, C−H), 1296 (s, C−O), 1062 (m, C−O), 793 (s, C−H arom).

5,6-Dimethoxy-1*H*-indole-3-carbaldehyde (6g). Yield: 86%. Mp: 167−168 °C. ^{1}H NMR (300 MHz): δ 3.75 (s, 6H, −OC*H*$_3$), 6.98 (s, 1H, H-4), 7.51 (s, 1H, H-7), 8.03 (s, 1H, H-2), 9.83 (s, 1H, H-1), 10.45 (s, 1H, −C*H*O). ^{13}C NMR (75 MHz): δ 56.1 (−O*C*H$_3$), 96.1 (C-4), 103.0 (C-7), 117.3 (C-3), 118.8 (C-3′), 131.6 (C-7′), 137.0 (C-2), 146.9 (C-5), 147.9 (C-6), 185.1 (−*C*HO). IR (CsI): 3401 (s br, N−H), 2965, 2841 (w, C−H), 1635 (s, C=O), 1487 (m, C−H), 1296 (s, C−O), 1074 (m, C−O), 723 (s, C−H arom).

4-Methoxy-1-(4′-methoxy-benzenesulfonyl)-1*H*-indole-3-carbaldehyde (7b). To a solution of **6b** (1.7 g, 9.59 mmol, 1 equiv) in dichloromethane (20 mL) were added sodium hydroxide pellets (574 mg, 14.34 mmol, 1.5 equiv), and the

mixture was stirred for 30 min at room temperature. 4-Methoxybenzenesulfonyl chloride (3.0 g, 14.34 mmol, 1.5 equiv) was then added, and the mixture was stirred for 12 h at room temperature. A saturated solution of NH_4Cl (100 mL) was then added, and the mixture was extracted with ethyl acetate (3 × 100 mL). The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified by flash chromatography using hexane−AcOEt (70−30) to give a white solid (3.2 g, 97%). Mp: 137−138 °C. ^{1}H NMR (300 MHz): δ 3.84 (s, 3H, −OC*H*$_3$), 3.95 (s, 3H, −OC*H*$_3$), 6.99 (d, J = 8.2 Hz, 1H, H-5), 7.15 (d, J = 9.0 Hz, 2H, H-3″,5″), 7.41 (t, J = 8.2 Hz, 1H, H-6), 7.61 (d, J = 8.2 Hz, 1H, H-7), 8.12 (d, J = 9.0 Hz, 2H, H-2″,6″), 8.40 (s, 1H, H-2), 10.40 (s, 1H, −C*H*O). ^{13}C NMR (75 MHz): δ 55.7, 55.9 (−O*C*H$_3$), 105.5, 106.0 (C-5,7), 115.2 (C-3″,5″), 116.4 (C-3′), 121.4 (C-3), 126.8 (C-6), 127.2 (C-7′), 129.1 (C-2), 129.9 (C-2″,6″), 135.3 (C-1″), 153.9 (C-4), 164.3 (C-4″), 187.0 (−*C*HO). IR (KBr): 3008 (w, =C−H), 2956, 2835 (w, C−H), 1677 (s, C=O), 1475 (m, C−H), 1254 (s, C−O), 1058 (m, C−O).

1-(4-Methoxy-benzenesulfonyl)-1*H*-indole-3-carbaldehyde (7a). Yield: 87%. Mp: 138−139 °C. ^{1}H NMR (200 MHz): δ 3.81 (s, 3H, −OC*H*$_3$), 6.94 (d, J = 9.1 Hz, 2H, H-3″,5″), 7.32−7.45 (m, 2H, H-5,6), 7.91 (d, J = 9.1 Hz, 2H, H-2″,6″), 7.87−7.96 (m, 1H, H-4), 8.23 (s, 1H, H-2), 8.25 (dd, J = 6.9 Hz, J = 2.7 Hz, 1H, H-7), 10.09 (s br, 1H, −C*H*O). ^{13}C NMR (50 MHz): δ 55.8 (−O*C*H$_3$), 113.3 (C-7), 114.9 (C-3″,5″), 122.3 (C-3), 122.6 (C-4,6), 125.0 (C-5), 126.3 (C-2″,6″), 128.6 (C-3′), 129.6 (C-7′), 135.2 (C-1″), 136.2 (C-2), 164.5 (C-4″), 185.3 (−*C*HO). IR (KBr): 3015 (w, =C−H), 2948, 2831 (w, C−H), 1676 (s, C=O), 1491 (m, C−H).

5-Methoxy-1-(4-methoxy-benzenesulfonyl)-1*H*-indole-3-carbaldehyde (7c). Yield: 95%. Mp: 125−126 °C. ^{1}H NMR (300 MHz): δ 3.81 (s, 3H, −OC*H*$_3$), 3.84 (s, 3H, −OC*H*$_3$), 6.93 (d, J = 8.9 Hz, 2H, H-3″,5″), 6.99 (dd, J = 9.1 Hz, J = 2.5 Hz, 1H, H-6), 7.71 (d, J = 2.5 Hz, 1H, H-4), 7.81 (d, J = 9.1 Hz, 1H, H-7), 7.88 (d, J = 8.9 Hz, 2H, H-2″,6″), 8.17 (s, 1H, H-2), 10.05 (s, 1H, −C*H*O). ^{13}C NMR (75 MHz): δ 55.8 (−O*C*H$_3$), 104.1 (C-4), 114.1 (C-6), 114.9 (C-3″,5″), 116.2 (C-7), 122.2 (C-3), 127.4 (C-3′), 128.7 (C-7′), 129.6 (C-2″,6″), 135.2 (C-1″), 136.7 (C-2), 157.8 (C-5), 164.5 (C-4″), 185.5 (−*C*HO). Anal. Calcd C: 59.12 H: 4.38 N: 4.06 O: 23.16. Found C: 59.07 H: 4.40 N: 4.14 O: 23.90. IR (KBr): 3010 (w, =C−H), 2965, 2843 (w, C−H), 1683 (s, C=O), 1480 (m, C−H), 1262 (s, C−O), 1035 (m, C−O).

6-Methoxy-1-(4-methoxy-benzenesulfonyl)-1*H*-indole-3-carbaldehyde (7d). Yield: 97%. Mp: 118−119 °C. ^{1}H NMR (300 MHz): δ 3.85 (s, 3H, −OC*H*$_3$), 3.99 (s, 3H, −OC*H*$_3$), 7.07 (dd, J = 8.8 Hz, J = 2.2 Hz, 1H, H-5), 7.18 (d, J = 8.9 Hz, 2H, H-3″,5″), 7.44 (d, J = 2.2 Hz, 1H, H-7), 8.01 (d, J = 8.8 Hz, 1H, H-4), 8.10 (d, J = 8.9 Hz, 2H, H-2″,6″), 8.77 (s, 1H, H-2), 10.05 (s, 1H, −C*H*O). ^{13}C NMR (75 MHz): δ 55.6, 55.9 (−O*C*H$_3$), 97.2 (C-7), 113.5 (C-5), 115.3 (C-3″,5″), 119.1 (C-3′), 121.5 (C-3), 122.4 (C-4), 127.2 (C-7′), 129.7 (C-2″,6″), 135.4 (C-1″), 137.3 (C-2), 158.2 (C-6), 164.3 (C-4″), 186.5 (−*C*HO). IR (KBr): 2995 (w, =C−H), 2946, 2829 (w, C−H), 1674 (s, C=O), 1475 (m, C−H), 1249 (s, C−O), 1067 (m, C−O).

7-Methoxy-1-(4-methoxy-benzenesulfonyl)-1*H*-indole-3-carbaldehyde (7e). Yield: 30%. Mp: 118−119 °C. ^{1}H NMR (300 MHz): δ 3.77 (s, 3H, −OC*H*$_3$), 3.88 (s, 3H, −OC*H*$_3$), 6.98 (d, J = 7.9 Hz, 1H, H-6), 7.20 (d, J = 8.9 Hz, 2H, H-3″,5″), 7.33 (t, J = 7.9 Hz, 1H, H-5), 7.77 (d, J = 7.9 Hz, 1H, H-4), 7.97 (d, J = 8.9 Hz, 2H, H-2″,6″), 8.92 (s, 1H, H-2), 10.15 (s, 1H, −C*H*O). ^{13}C NMR (75 MHz): δ 55.8, 56.4 (−O*C*H$_3$), 108.8 (C-6), 114.1 (C-4), 115.1 (C-3″,5″), 121.0 (C-3), 126.7 (C-5), 128.6 (C-3′), 129.1 (C-7′), 130.8 (C-2″,6″), 141.0 (C-2), 147.1 (C-1″), 155.2 (C-7), 164.6 (C-4″), 187.4 (−*C*HO). IR (KBr): 3017 (w, =C−H), 2955, 2836 (w, C−H), 1659 (s, C=O), 1493 (s, C−H), 1244 (s, C−O), 1059 (m, C−O).

4,5-Dimethoxy-1-(4-methoxy-benzenesulfonyl)-1*H*-indole-3-carbaldehyde (7f). Yield: 96%. Mp: 132−133 °C. ^{1}H NMR (300 MHz): δ 3.81 (s, 3H, −OC*H*$_3$), 3.91 (s, 3H, −OC*H*$_3$), 3.96 (s, 3H, −OC*H*$_3$), 6.93 (d, J = 9.0 Hz, 2H, H-3″,5″), 7.03 (d, J = 9.0 Hz, 1H, H-6), 7.65 (d, J = 9.0 Hz, 1H, H-7), 7.87 (d, J = 9.0 Hz, 2H, H-2″,6″), 8.22 (s, 1H, H-2); 10.43 (s, 1H, −C*H*O). ^{13}C NMR (75 MHz): δ 55.8 (−O*C*H$_3$), 57.1 (−O*C*H$_3$), 60.8 (−O*C*H$_3$), 109.1 (C-6), 112.8 (C-7), 114.8 (C-3″,5″), 121.5 (C-3), 122.2 (C-3′), 128.6 (C-7′), 129.6 (C-2″,6″), 130.7 (C-1″), 130.8 (C-2), 142.5 (C-4), 149.0 (C-5), 164.4 (C-4″), 187.4 (−*C*HO). Anal. Calcd C: 57.59 H: 4.56 N: 3.73 S: 8.54. Found C: 57.27 H: 4.59 N: 3.92 S: 8.35. IR (KBr): 3021 (w, =C−H), 2940, 2838 (w, C−H), 1674 (s, C=O), 1495 (m, C−H), 1263 (s, C−O), 1056 (m, C−O).

5,6-Dimethoxy-1-(4-methoxy-benzenesulfonyl)-1*H*-indole-3-carbaldehyde (7g). Yield: 26%. Mp: 198−199 °C. ^{1}H NMR (300 MHz): δ 3.74 (s, 3H, −OC*H*$_3$), 3.77 (s, 3H, −OC*H*$_3$), 3.84 (s, 3H, −OC*H*$_3$), 7.10 (d, J = 8.9 Hz, 2H, H-3″,5″), 7.38 (s, 1H, H-4), 7.49 (s, 1H, H-7), 8.01 (d, J = 8.9 Hz, 2H, H-2″,6″), 8.62 (s, 1H, H-2); 9.96 (s, 1H, −C*H*O). ^{13}C NMR (75 MHz): δ 55.3 (−O*C*H$_3$), 56.1, 56.4 (−O*C*H$_3$), 96.9 (C-4), 103.3 (C-7), 115.8 (C-3″,5″), 122.1 (C-3′), 127.8 (C-3), 129.0 (C-7′), 130.1 (C-2″,6″), 130.8 (C-1″), 137.0 (C-2), 148.4 (C-6), 149.1 (C-5), 164.8 (C-4″), 187.2 (−*C*HO). IR (KBr): 2998 (w, =C−H), 2945, 2829 (w, C−H), 1676 (s, C=O), 1492 (m, C−H), 1260 (s, C−O), 1087 (m, C−O).

3-(10-Benzyloxy-dec-1-enyl)-4-methoxy-1-(4-methoxy-benzenesulfonyl)-1*H*-indole (11b, *n* = 10). To a solution of 9-benzyloxynonyltriphenylphosphonium bromide (600 mg, 1.04 mmol, 1.2 equiv) in THF (12 mL) was added dropwise a solution of n-BuLi (1.5 M in hexane, 0.7 mL, 1.04 mmol, 1.2 equiv) under argon at −78 °C. After 15 min stirring at room temperature, potassium *tert*-butoxide (117 mg, 1.04 mmol, 1.2 equiv) was added at 0 °C. The solution was stirred for 15 min at 0 °C. It was then cooled to −78 °C, and a solution of aldehyde **7b** (300 mg, 0.87 mmol, 1 equiv) in THF (7 mL) was added slowly. The solution was stirred for 1 h at −78 °C, then for 1 h 30 min at 0 °C. The mixture was poured on a saturated solution of NH_4Cl (50 mL) and extracted with ethyl ether (3 × 50 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified on silica gel (eluting with hexane−AcOEt 90−10 to 85−15) to give a white solid (362 mg, 74%). ^{1}H NMR (300 MHz): δ 1.33 (s br, 8H, H-12 to 15), 1.47 (m, 2H, H-16), 1.62 (m, 2H, H-11), 2.32 (q, J = 6.9 Hz, 2H, H-10), 3.47 (t, J = 6.9 Hz, 2H, H-17), 3.77 (s, 3H, −OC*H*$_3$), 3.86 (s, 3H, −OC*H*$_3$), 4.51 (s, 2H, H-18), 5.70 (dt, J = 10.6 Hz, J = 6.9 Hz, 1H, H-9), 6.63 (d, J = 7.8 Hz, 1H, H-5), 6.80 (d, J = 10.6 Hz, 1H, H-8), 6.86 (d, J = 8.9 Hz, 2H, H-3″,5″), 7.16−7.37 (m, 6H, H-7, H-2‴ to 6‴), 7.38 (s, 1H, H-2), 7.58 (t, J = 7.8 Hz, 1H, H-6), 7.80 (d, J = 8.9 Hz, 2H, H-2″,6″). ^{13}C NMR (75 MHz): δ 26.2 (C-10), 29.2−29.8 (C-11 to 16), 55.4, 55.6 (−O*C*H$_3$), 70.5 (C-17), 72.9 (C-18), 103.9, 104.1 (C-3,5), 106.6 (C-7), 114.4 (C-3″,5″), 119.5 (C-3′), 120.5 (C-2), 122.5 (C-6), 125.6 (C-8), 127.4 (C-4‴), 127.6 (C-2‴,6‴), 128.3 (C-2″,6″), 129.0 (C-3‴,5‴), 131.7 (C-1″), 132.7 (C-9), 136.6 (C-7′), 138.7 (C-1‴), 154.9 (C-4), 163.7 (C-4″).

	yield, %					
n	**11a**	**11b**	**11c**	**11d**	**11f**	**11g**
10	70	74	65	91		
12	87	71	62	78		
14	88	45	84	77		
16	90	69	94	85	81	49
18	69	68	71	66	73	71

3-(10-Benzyloxy-dec-1-enyl)-1-(4-methoxy-benzenesulfonyl)-1*H*-indole (11a, *n* = 10). Yield: 70%. ^{1}H NMR (200 MHz): δ 1.31 (s br, 8H, H-12 to 15), 1.42−1.61 (m, 4H, H-11,-16), 2.31 (q, J = 6.9 Hz, 2H, H-10), 3.46 (t, J = 6.6 Hz, 2H, H-17), 3.77 (s, 3H, −OC*H*$_3$), 4.50 (s, 2H, H-18), 5.81 (dt, J = 11.2 Hz, J = 6.9 Hz, 1H, H-9), 6.40 (d, J = 11.2 Hz, 1H, H-8), 6.86 (d, J = 8.9 Hz, 2H, H-3″,5″), 7.24−7.35 (m, 7H, H-5,6, H-2‴-6‴); 7.50 (s, 1H, H-2), 7.53 (d, J = 6.9 Hz, 1H, H-4), 7.82 (d, J = 8.9 Hz, 2H, H-2″,6″), 7.98 (d, J = 7.6 Hz, 1H, H-7). ^{13}C NMR (50 MHz): δ 26.3 (C-10), 29.9 (C-11 to 16), 55.7 (−O*C*H$_3$), 70.7 (C-17), 73.0 (C-18), 113.7 (C-7), 114.5 (C-9), 117.6 (C-3″,5″), 119.4 (C-3), 119.8 (C-5), 123.3 (C-4), 123.6 (C-6), 124.9 (C-2″,6″), 127.6 (C-4‴), 127.7 (C-3‴,5‴), 128.5 (C-2‴,6‴), 129.2 (C-8), 129.9 (C-3′), 131.2 (C-7′), 134.8 (C-1″), 135.0 (C-2), 139.0 (C-1‴), 163.9 (C-4″).

3-(10-Benzyloxy-dec-1-enyl)-5-methoxy-1-(4-methoxy-benzenesulfonyl)-1*H*-indole (11c, *n* = 10). Yield: 65%. ^{1}H

NMR (200 MHz): δ 1.31 (s br, 8H, H-12 to 15), 1.48 (m, 2H, H-16), 1.61 (m, 2H, H-11), 2.30 (q, J = 6.9 Hz, 2H, H-10), 3.46 (t, J = 6.4 Hz, 2H, H-17), 3.77 (s, 3H, −OCH_3), 3.82 (s, 3H, −OCH_3), 4.50 (s, 2H, H-18), 5.80 (dt, J = 11.3 Hz, J = 6.9 Hz, 1H, H-9), 6.34 (d, J = 11.3 Hz, 1H, H-8), 6.85 (d, J = 8.9 Hz, 2H, H-3′′,5′′), 6.89−6.94 (m, 2H, H-4,6), 7.33 (m, 5H, H-2′′′ to 6′′′), 7.45 (s, 1H, H-2), 7.78 (d, J = 8.9 Hz, 2H, H-2′′,6′′), 7.87 (d, J = 9.6 Hz, 1H, H-7). ^{13}C NMR (50 MHz): δ 26.2 (C-10), 29.5−29.8 (C-11 to 16), 55.7 (−OCH$_3$), 70.6 (C-17), 72.9 (C-18), 102.0 (C-4), 113.9 (C-6), 114.4 (C-3′′,5′′), 114.6 (C-3), 117.6 (C-7), 119.4 (C-3′), 124.3 (C-2), 127.5 (C-8), 127.7 (C-2′′′,6′′′), 128.4 (C-3′′′,5′′′), 129.0 (C-2′′,6′′), 129.2 (C-4′′′), 129.6 (C-7′), 131.9 (C-1′′), 134.9 (C-9), 138.5 (C-1′′′), 156.4 (C-5), 163.7 (C-4′′).

3-(10-Benzyloxy-dec-1-enyl)-6-methoxy-1-(4-methoxy-benzenesulfonyl)-1*H*-indole (11d, *n* = 10). Yield: 91%. ^{1}H NMR (300 MHz): δ 1.31 (s br, 8H, H-12 to 15), 1.48 (m, 2H, H-16), 1.61 (m, 2H, H-11), 2.30 (q, J = 6.7 Hz, 2H, H-10), 3.46 (t, J = 6.6 Hz, 2H, H-17), 3.78 (s, 3H, −OCH_3), 3.88 (s, 3H, −OCH_3), 4.50 (s, 2H, H-18), 5.79 (dt, J = 11.5 Hz, J = 6.7 Hz, 1H, H-9), 6.35 (d, J = 11.5 Hz, 1H, H-8), 6.87 (d, J = 8.9 Hz, 2H, H-3′′,5′′), 6.88 (m, 1H, H-5), 7.33 (m, 6H, H-4, H-2′′′ to 6′′′), 7.39 (s, 1H, H-2), 7.52 (d, J = 2.0 Hz, 1H, H-7), 7.80 (d, J = 8.9 Hz, 2H, H-2′′,6′′). ^{13}C NMR (75 MHz): δ 26.2 (C-10), 29.3−29.8 (C-11 to 16), 55.6, 55.8 (−OCH$_3$), 70.5 (C-17), 72.8 (C-18), 97.9 (C-7), 112.3 (C-5), 114.4 (C-3′′,5′′), 117.5 (C-4), 119.2 (C-3), 120.0 (C-2), 122.2 (C-8), 124.7 (C-3′), 127.4−128.9 (C-2′′,6′′, C-2′′′ to 6′′′), 129.6 (C-1′′), 134.7 (C-9), 135.6 (C-7′), 138.7 (C-1′′′), 158.1 (C-6), 163.7 (C-4′′). IR (CsI): 2925, 2853 (s, C−H), 1642, 1595 (m, C═C), 1497 (m, C−H), 1264 (m, C−O), 1095 (m, C−O), 676 (m, C−H).

3-(16-Benzyloxy-hexadec-1-enyl)-7-methoxy-1-(4-methoxy-benzenesulfonyl)-1*H*-indole (11e, *n* = 16). Yield: 77%. ^{1}H NMR (300 MHz): δ 1.25 (s br, 20H, H-12 to 21), 1.51 (m, 2H, H-22), 1.60 (m, 2H, H-11), 2.37 (q, J = 6.9 Hz, 2H, H-10), 3.46 (t, J = 6.6 Hz, 2H, H-23), 3.73 (s, 3H, −OCH_3), 3.83 (s, 3H, −OCH_3), 4.50 (s, 2H, H-24), 5.82 (dt, J = 11.3 Hz, J = 6.9 Hz, 1H, H-9), 6.41 (d, J = 11.3 Hz, 1H, H-8), 6.70 (d, J = 6.7 Hz, 1H, H-6), 6.92 (d, J = 8.9 Hz, 2H, H-3′′,5′′), 7.15 (d, J = 6.7 Hz, 1H, H-4), 7.28−7.33 (m, 5H, H-2′′′ to 6′′′), 7.35 (s, 1H, H-2), 7.79 (m, 1H, H-5), 7.80 (d, J = 8.9 Hz, 2H, H-2′′,6′′). ^{13}C NMR (75 MHz): δ 26.2 (C-10), 29.4−29.8 (C-11 to 22), 55.6 (−OCH$_3$), 70.5 (C-23), 72.8 (C-24), 107.1 (C-6), 112.1 (C-4), 113.9 (C-3′′,5′′), 117.5 (C-5), 123.8 (C-8), 126.0 (C-3), 127.4 (C-4′′′), 127.6 (C-2′′′,6′′′), 128.3 (C-2′′,6′′), 129.6 (C-3′′′,5′′′), 131.7 (C-1′′), 132.6 (C-7′), 133.7 (C-3′), 134.4 (C-9), 138.7 (C-1′′′), 147.4 (C-7), 163.2 (C-4′′). Anal. Calcd C: 72.52 H: 7.96 N: 2.17. Found C: 72.13 H: 7.81 N: 2.06.

3-(16-Benzyloxy-hexadec-1-enyl)-4,5-dimethoxy-1-(4-methoxy-benzenesulfonyl)-1*H*-indole (11f, *n* = 16). Yield: 81%. ^{1}H NMR (300 MHz): δ 1.25 (s br, 20H, H-12 to 21), 1.47 (m, 2H, H-22), 1.58 (m, 2H, H-11), 2.30 (q, J = 6.9 Hz, 2H, H-10), 3.46 (t, J = 6.6 Hz, 2H, H-23), 3.79 (s, 3H, −OCH_3), 3.82 (s, 3H, −OCH_3), 3.87 (s, 3H, −OCH_3), 4.50 (s, 2H, H-24), 5.73 (dt, J = 11.6 Hz, J = 6.9 Hz, 1H, H-9), 6.74 (d, J = 11.6 Hz, 1H, H-8), 6.86 (d, J = 8.9 Hz, 2H, H-3′′,5′′), 6.95 (d, J = 8.9 Hz, 1H, H-6), 7.28−7.36 (m, 6H, H-2, 2′′′ to 6′′′), 7.65 (d, J = 8.9 Hz, 1H, H-7), 7.78 (d, J = 8.9 Hz, 2H, H-2′′,6′′). ^{13}C NMR (75 MHz): δ 26.2 (C-10), 29.5−29.7 (C-11 to 22), 55.6 (−OCH$_3$), 57.1 (−OCH$_3$), 61.3 (−OCH$_3$), 70.5 (C-23), 72.8 (C-24), 109.0 (C-3,6), 111.9 (C-7), 114.4 (C-3′′,5′′), 119.7 (C-2), 124.3 (C-3′), 127.4−129.3 (C-7′,8,2′′,6′′,2′′′ to 6′′′), 130.8 (C-1′′), 133.3 (C-9), 138.7 (C-1′′′), 143.0 (C-4), 148.6 (C-5), 163.7 (C-4′′).

3-(16-Benzyloxy-hexadec-1-enyl)-5,6-dimethoxy-1-(4-methoxy-benzenesulfonyl)-1*H*-indole (11g, *n* = 16). Yield: 49%. ^{1}H NMR (300 MHz): δ 1.26 (s br, 20H, H-12 to 21), 1.48 (m, 2H, H-22), 1.61 (m, 2H, H-11), 2.29 (q, J = 6.9 Hz, 2H, H-10), 3.46 (t, J = 6.6 Hz, 2H, H-23), 3.78 (s, 3H, −OCH_3), 3.89 (s, 3H, −OCH_3), 3.97 (s, 3H, −OCH_3), 4.50 (s, 2H, H-24), 5.79 (dt, J = 11.3 Hz, J = 6.9 Hz, 1H, H-9), 6.32 (d, J = 11.3 Hz, 1H, H-8), 6.85 (d, J = 8.9 Hz, 2H, H-3′′,5′′), 6.90 (s, 1H, H-7), 7.25−7.35 (m, 6H, H-2, H-2′′′ to 6′′′), 7.55 (s, 1H, H-4), 7.77 (d, J = 8.9 Hz, 2H, H-2′′,6′′). ^{13}C NMR (75 MHz): δ 26.2 (C-10), 29.4−29.7 (C-11 to 22), 55.6 (−OCH$_3$), 56.1, 56.4 (−OCH$_3$), 70.5 (C-23), 72.8 (C-24), 97.4 (C-7), 100.9 (C-4), 114.3 (C-3′′,5′′), 117.6 (C-2), 119.6 (C-3), 122.2 (C-8), 123.8 (C-3′), 127.6−128.8 (C-2′′,6′′,2′′′ to 6′′′), 29.0 (C-7′), 129.6 (C-1′′), 134.8 (C-9), 138.7 (C-1′′′), 147.1 (C-6); 148.2 (C-5), 163.7 (C-4′′). Anal. Calcd C: 71.08 H: 7.90 N: 2.07. Found C: 71.20 H: 8.07 N: 2.16. IR (CsI): 2925, 2853 (s, C−H), 1648, 1596 (m, C═C), 1486 (m, C−H), 1263 (m, C−O), 1094 (m, C−O), 675 (m, C−H).

5-Methoxy-1-(4-methoxy-benzenesulfonyl)-3-pentadec-1-enyl-1*H*-indole (11h). Yield: 76%. ^{1}H NMR (300 MHz): δ 0.87 (t, J = 6.7 Hz, 3H, H-22), 1.25 (s br, 20H, H-12 to 21), 1.47 (m, 2H, H-11), 2.30 (q, J = 4.4 Hz, 2H, H-10), 3.77 (s, 3H, −OCH_3), 3.82 (s, 3H, −OCH_3), 5.80 (dt, J = 11.4 Hz, J = 6.9 Hz, 1H, H-9), 6.35 (d, J = 11.4 Hz, 1H, H-8), 6.86 (d, J = 9.0 Hz, 2H, H-3′′,5′′), 6.89−6.94 (m, 2H, H-4,6), 7.45 (s, 1H, H-2), 7.77 (d, J = 9.0 Hz, 2H, H-2′′,6′′), 7.87 (d, J = 9.6 Hz, 1H, H-7). ^{13}C NMR (50 MHz): δ 14.6 (C-22), 26.2 (C-10), 29.3−29.8 (C-11 to 21), 55.6 (−OCH$_3$), 56.2 (−OCH$_3$), 102.1 (C-4), 113.9 (C-6), 114.4 (C-3′′,5′′), 114.6 (C-3), 117.5 (C-7), 119.4 (C-3′), 124.2 (C-2), 127.5 (C-8), 128.9 (C-2′′,6′′), 129.8 (C-7′), 131.9 (C-1′′), 134.7 (C-9), 156.4 (C-5), 163.6 (C-4′′). Anal. Calcd C: 70.82 H: 8.24 N: 2.66. Found C: 70.93 H: 8.31 N: 2.70.

10-[4-Methoxy-1-(4-methoxy-benzenesulfonyl)-1*H*-indol-3-yl]decan-1-ol (12b, *n* = 10). To a solution of alkene **11b (*n* = 10)** (350 mg, 0.62 mmol, 1 equiv) in ethanol (6 mL) was added palladium on charcoal (5%, 35 mg, 10% w/w). The mixture was stirred under 1 atm of hydrogen for 4 h at room temperature. The mixture was then filtered on Celite and then concentrated. The residue was purified by flash chromatography using hexane−AcOEt (70−30) to give a white solid (277 mg, 93%). ^{1}H NMR (300 MHz): δ 1.27 (s br, 12H, H-10 to 15), 1.59 (m, 4H, H-9,16), 2.76 (t, J = 7.3 Hz, 2H, H-8), 3.64 (t, J = 6.2 Hz, 2H, H-17), 3.78 (s, 3H, −OCH_3), 3.85 (s, 3H, −OCH_3), 6.61 (d, J = 8.2 Hz, 1H, H-5), 6.85 (d, J = 8.9 Hz, 2H, H-3′′,5′′), 7.15 (s, 1H, H-2), 7.18 (t, J = 8.2 Hz, 1H, H-6), 7.56 (d, J = 8.2 Hz, 1H, H-7), 7.77 (d, J = 8.9 Hz, 2H, H-2′′,6′′). ^{13}C NMR (75 MHz): δ 25.7 (C-15), 26.9 (C-9), 29.3−29.8 (C-8,10 to 14), 32.8 (C-16), 55.2, 55.6 (−OCH$_3$), 63.1 (C-17), 103.6 (C-5), 106.6 (C-7), 114.3 (C-3′′,5′′), 120.6 (C-3), 121.2 (C-6), 124.1 (C-3′), 125.3 (C-2), 128.9 (C-2′′,6′′), 129.8 (C-1′′), 137.0 (C-7′), 154.6 (C-4), 163.5 (C-4′′).

	yield, %					
n	**12a**	**12b**	**12c**	**12d**	**12f**	**12g**
10	92	93	91	80		
12	92	94	83	85		
14	93	57	91	92		
16	92	47	91	47	89	94
18	46	50	94	85	93	90

10-[1-(4-Methoxy-benzenesulfonyl)-1*H*-indol-3-yl]decan-1-ol (12a, *n* = 10). Yield: 92%. ^{1}H NMR (200 MHz): δ 1.29 (s br, 12H, H-10 to 15), 1.62 (m, 4H, H-9,16), 2.63 (t, J = 7.4 Hz, 2H, H-8), 3.64 (t, J = 6.4 Hz, 2H, H-17), 3.77 (s, 3H, −OCH_3), 6.85 (d, J = 9.1 Hz, 2H, H-3′′,5′′), 7.21−7.33 (m, 3H, H-2,5,6), 7.47 (d, J = 6.9 Hz, 1H, H-4), 7.78 (d, J = 9.1 Hz, 2H, H-2′′,6′′), 7.97 (d, J = 7.6 Hz, 1H, H-7). ^{13}C NMR (50 MHz): δ 24.9 (C-15), 25.8 (C-9), 28.9 (C-8), 29.7 (C-10 to 14), 32.8 (C-16), 55.6 (−OCH$_3$), 63.1 (C-17), 113.8 (C-7), 114.4 (C-9), 119.6 (C-3′′,5′′), 122.7 (C-4), 122.9 (C-6), 123.7 (C-3), 124.5 (C-5), 129.0 (C-2′′,6′′), 131.3 (C-7′), 134.9 (C-1′′), 135.4 (C-2), 163.6 (C-4′′).

10-[5-Methoxy-1-(4-methoxy-benzenesulfonyl)-1*H*-indol-3-yl]decan-1-ol (12c, *n* = 10). Yield: 91%. ^{1}H NMR (200 MHz): δ 1.29 (s br, 12H, H-10 to 15), 1.60 (m, 4H, H-9,16), 2.58 (t, J = 7.4 Hz, 2H, H-8), 3.64 (t, J = 6.4 Hz, 2H, H-17), 3.77 (s, 3H, −OCH_3), 3.82 (s, 3H, −OCH_3), 6.83 (d, J = 8.9 Hz, 2H, H-3′′,5′′), 6.88−6.93 (m, 2H, H-4,6), 7.24 (s, 1H, H-2), 7.75 (d, J = 8.9 Hz, 2H, H-2′′,6′′), 7.86 (d, J = 9.6 Hz, 1H, H-7). ^{13}C NMR (50 MHz): δ 24.9 (C-15), 25.8 (C-9), 28.7 (C-8), 29.4−29.6 (C-10 to 14), 32.9 (C-16), 55.6, 55.8 (−OCH$_3$), 63.1 (C-17), 102.3 (C-4), 113.2 (C-6), 114.3 (C-3′′,5′′), 114.7 (C-7), 123.6 (C-2), 124.0 (C-3), 128.9 (C-2′′,6′′), 129.9 (C-7′), 130.2 (C-3′), 132.4 (C-1′′), 156.3 (C-5), 163.6 (C-4′′).

10-[6-Methoxy-1-(4-methoxy-benzenesulfonyl)-1*H*-indol-3-yl]decan-1-ol (12d, *n* = 10). Yield: 80%. ^{1}H NMR (300

MHz): δ 1.28 (s br, 12H, H-10 to 15), 1.58 (m, 4H, H-9,16), 2.58 (t, *J* = 7.3 Hz, 2H, H-8), 3.63 (t, *J* = 6.6 Hz, 2H, H-17), 3.78 (s, 3H, −OC*H*$_3$), 3.87 (s, 3H, −OC*H*$_3$), 6.85 (d, *J* = 8.9 Hz, 2H, H-3″,5″), 6.86 (m, 1H, H-5), 7.17 (s, 1H, H-2), 7.32 (d, *J* = 8.6 Hz, 1H, H-4), 7.52 (d, *J* = 2.2 Hz, 1H, H-7), 7.77 (d, *J* = 8.9 Hz, 2H, H-2″,6″). ^{13}C NMR (75 MHz): δ 24.9 (C-15), 25.7 (C-9), 28.8 (C-8), 29.3−29.5 (C-10 to 14), 32.8 (C-16), 55.6, 55.8 (−O*C*H$_3$), 63.0 (C-17), 98.2 (C-7), 111.9 (C-5), 114.3 (C-3″,5″), 119.9 (C-4), 121.3 (C-2), 123.6 (C-3), 125.1 (C-3′), 128.9 (C-2″,6″), 129.8 (C-1″), 136.4 (C-7′), 157.9 (C-6), 163.5 (C-4″). IR (CsI): 3368 (w br, O−H), 2919, 2850 (s, C−H), 1595 (m, C=C), 1497 (m, C−H), 1264 (m, C−O), 1113 (m, C−O), 676 (m, C−H).

16-[7-Methoxy-1-(4-methoxy-benzenesulfonyl)-1*H*-indol-3-yl]hexadecan-1-ol (12e, *n* = 16). Yield: 52%. ^{1}H NMR (300 MHz): δ 1.27 (s br, 24H, H-10 to 21), 1.56 (m, 2H, H-22), 1.71 (m, 2H, H-9), 2.66 (t, *J* = 7.5 Hz, 2H, H-8), 3.64 (t, *J* = 6.6 Hz, 2H, H-23), 3.71 (s, 3H, −OC*H*$_3$), 3.83 (s, 3H, −OC*H*$_3$), 6.67 (m, 1H, H-6), 6.90 (d, *J* = 9.0 Hz, 2H, H-3″,5″), 7.10 (m, 2H, H-4,5), 7.57 (s, 1H, H-2), 7.78 (d, *J* = 9.0 Hz, 2H, H-2″,6″). ^{13}C NMR (75 MHz): δ 24.9 (C-21), 25.7 (C-9), 29.0 (C-8), 29.4−29.7 (C-10 to 20), 32.8 (C-22), 55.6 (−O*C*H$_3$), 63.1 (C-23), 106.9 (C-6), 112.1 (C-4), 113.8 (C-3″,5″), 121.0 (C-3), 123.5 (C-5), 124.9 (C-7′), 125.1 (C-2), 129.5 (C-2″,6″), 132.1 (C-3′), 134.1 (C-1″), 153.3 (C-7), 163.1 (C-4″).

16-[4,5-Dimethoxy-1-(4-methoxy-benzenesulfonyl)-1*H*-indol-3-yl]hexadecan-1-ol (12f, *n* = 16). Yield: 89%. ^{1}H NMR (300 MHz): δ 1.26 (s br, 24H, H-10 to 21), 1.59 (m, 4H, H-9,22), 2.73 (t, *J* = 7.3 Hz, 2H, H-8), 3.64 (t, *J* = 6.6 Hz, 2H, H-23), 3.79 (s, 3H, −OC*H*$_3$), 3.86 (s, 3H, −OC*H*$_3$), 3.87 (s, 3H, −OC*H*$_3$), 6.85 (d, *J* = 9.0 Hz, 2H, H-3″,5″), 6.92 (d, 1H, H-6), 7.15 (s, 1H, H-2), 7.64 (d, *J* = 8.8 Hz, 1H, H-7), 7.75 (d, *J* = 9.0 Hz, 2H, H-2″,6″). ^{13}C NMR (75 MHz): δ 25.7 (C-21), 26.4 (C-9), 29.4−29.7 (C-8,10 to 20), 32.8 (C-22), 55.6 (−O*C*H$_3$); 57.0 (−O*C*H$_3$), 61.3 (−O*C*H$_3$), 63.1 (C-23), 109.0 (C-6), 111.6 (C-7), 114.2 (C-3″,5″), 123.2 (C-2), 123.7 (C-3), 128.9 (C-2″,6″), 129.7 (C-3′), 131.6 (C-7′), 142.7 (C-1″), 148.3 (C-4), 155.8 (C-5), 163.5 (C-4″). IR (CsI): 3401 (w br, O−H), 2925, 2853 (s, C−H), 1595 (m, C=C), 1495 (s, C−H), 1263 (s, C−O), 1095 (m, C−O), 675 (m, C−H).

16-[5,6-Dimethoxy-1-(4-methoxy-benzenesulfonyl)-1*H*-indol-3-yl]hexadecan-1-ol (12g, *n* = 16). Yield: 94%. ^{1}H NMR (300 MHz): δ 1.26 (s br, 24H, H-10 to 21), 1.58 (m, 4H, H-9,22), 2.57 (t, *J* = 7.5 Hz, 2H, H-8), 3.64 (t, *J* = 6.6 Hz, 2H, H-23), 3.78 (s, 3H, −OC*H*$_3$), 3.89 (s, 3H, −OC*H*$_3$), 3.96 (s, 3H, −OC*H*$_3$), 6.84 (s, 1H, H-4), 6.85 (d, *J* = 9.0 Hz, 2H, H-3″,5″), 7.14 (s, 1H, H-7), 7.55 (s, 1H, H-2), 7.73 (d, *J* = 9.0 Hz, 2H, H-2″,6″). ^{13}C NMR (75 MHz): δ 24.9 (C-21), 25.7 (C-9), 28.7 (C-8), 29.4−29.7 (C-10 to 20), 32.8 (C-22), 55.6, 56.2, 56.4 (−O*C*H$_3$), 63.1 (C-23), 97.7 (C-7), 109.0 (C-4), 114.2 (C-3″,5″), 121.3 (C-2), 124.0, 124.1 (C-3,3′), 128.7 (C-2″,6″), 129.4 (C-7′), 129.8 (C-1″), 146.9 (C-6), 148.0 (C-5), 163.5 (C-4″). IR (CsI): 3400 (w br, O−H), 2924, 2852 (s, C−H), 1596 (m, C=C), 1486 (m, C−H), 1263 (m, C−O), 1094 (m, C−O), 676 (m, C−H).

5-Methoxy-1-(4-methoxy-benzenzsulfonyl)-3-pentadecyl-1*H*-indole 12h. Yield: 91%. ^{1}H NMR (300 MHz): δ 0.87 (t, *J* = 6.7 Hz, 3H, H-22), 1.26 (s br, 24H, H-10 to 21), 1.59 (m, 2H, H-9), 2.59 (q, *J* = 7.4 Hz, 2H, H-8), 3.77 (s, 3H, −OC*H*$_3$), 3.82 (s, 3H, −OC*H*$_3$), 6.83 (d, *J* = 9.0 Hz, 2H, H-3″,5″), 6.89−6.96 (m, 2H, H-4,6), 7.25 (s, 1H, H-2), 7.76 (d, *J* = 9.0 Hz, 2H, H-2″,6″), 7.86 (d, *J* = 9.6 Hz, 1H, H-7). ^{13}C NMR (50 MHz): δ 14.6 (C-22), 25.9 (C-9), 28.7 (C-8), 29.5−29.8 (C-10 to 21), 55.6 (−O*C*H$_3$), 55.9 (−O*C*H$_3$), 102.3 (C-4), 113.6 (C-6), 114.4 (C-3″,5″), 114.6 (C-7), 123.6 (C-2), 124.1 (C-3), 128.9 (C-2″,6″), 129.8 (C-7′), 130.2 (C-3′), 132.4 (C-1″), 156.4 (C-5), 163.6 (C-4″).

10-(4-Methoxy-1*H*-indol-3-yl)decan-1-ol (13b, *n* = 10). To a solution of indole **12b (*n* = 10)** (260 mg, 0.55 mmol, 1 equiv) in dry methanol (18 mL) were added disodium hydrogenphosphate (156 mg, 1.10 mmol, 2 equiv) and sodium amalgam (6%, 4 g) at 0 °C under argon. The mixture was stirred at room temperature for 12 h, then quenched with a saturated solution of NH_4Cl (50 mL), and extracted with ethyl ether (3 × 50 mL). The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified on silica gel (eluting with hexane−AcOEt from 80−20 to 75−25) to give white crystals (142 mg, 85%). ^{1}H NMR (300 MHz): δ 1.31 (s br, 12H, H-10 to 15), 1.57 (m, 2H, H-16), 1.68 (m, 2H, H-9), 2.87 (t, *J* = 7.5 Hz, 2H, H-8), 3.64 (t, *J* = 6.2 Hz, 2H, H-17), 3.92 (s, 3H, −OC*H*$_3$), 6.48 (d, *J* = 7.8 Hz, 1H, H-5), 6.82 (s, 1H, H-2), 6.94 (d, *J* = 7.8 Hz, 1H, H-7), 7.07 (t, *J* = 7.8 Hz, 1H, H-6), 7.88 (s br, 1H, H-1). ^{13}C NMR (75 MHz): δ 25.7 (C-15), 26.9 (C-9), 29.4−29.6 (C-10 to 14), 31.2 (C-8), 32.8 (C-16), 55.1 (−O*C*H$_3$), 63.1 (C-17), 99.3 (C-7), 104.3 (C-5), 117.5 (C-3), 117.8 (C-3′), 119.7 (C-6), 122.5 (C-2), 138.1 (C-7′), 155.0 (C-4). UV (acetonitrile): λ_{max} 225 nm (ε 301465), 270 nm (ε 71091), 283 nm (ε 67263), 293 nm (ε 68010). IR (KBr): 3377 (s, O−H, N−H), 3038 (w, =C−H), 2925, 2850 (s, C−H), 1615, 1585 (m, C=C), 1467 (m, C−H), 1094 (m, C−O), 724 (w, C−H).

13b	*n*	analysis
4-MeO	10	Mp: 44−45 °C. MS (EI): 303 (M$^+$, 25), 160 ($C_{10}H_{10}NO$, 100), 130 (C_9H_8N, 17). Anal. Calcd C: 75.21, H: 9.63, N: 4.62. Found C: 75.45, H: 9.71, N: 4.54.
	12	Mp: 45−46 °C. MS (EI): 331 (M$^+$, 32), 160 ($C_{10}H_{10}NO$, 100), 130 (C_9H_8N, 16). Anal. Calcd C: 76.09, H: 10.03, N: 4.23. Found C: 76.02, H: 10.10, N: 4.12.
	14	Mp: 48−49 °C. MS (EI): 360 (M$^+$, 35), 160 ($C_{10}H_{10}NO$, 100), 130 (C_9H_8N, 16). Anal. Calcd C: 76.83, H: 10.37, N: 3.90. Found C: 77.15, H: 10.52, N: 3.77.
	16	Mp: 53−54 °C. MS (EI): 388 (M$^+$, 43), 160 ($C_{10}H_{10}NO$, 100), 130 (C_9H_8N, 15). Anal. Calcd C: 77.47, H: 10.66, N: 3.61. Found C: 77.62, H: 10.76, N: 3.54.
	18	Mp: 65−66 °C. MS (EI): 416 (M$^+$, 41), 160 ($C_{10}H_{10}NO$, 100), 130 (C_9H_8N, 15). Anal. Calcd C: 78.02, H: 10.91, N: 3.37. Found C: 78.01, H: 10.98, N: 3.29.

	yield, %					
n	**13a**	**13b**	**13c**	**13d**	**13f**	**13g**
10	90	85	84	84		
12	83	82	95	80		
14	70	99	87	85		
16	75	92	72	89	98	97
18	91	96	85	95	89	98

10-(1*H*-indol-3-yl)decan-1-ol (13a, *n* = 10). Yield: 90%. ^{1}H NMR (200 MHz): δ 1.28 (s br, 12H, H-10 to 15), 1.57 (m, 2H, H-16), 1.71 (m, 2H, H-9), 2.75 (t, *J* = 7.6 Hz, 2H, H-8), 3.64 (t, *J* = 6.4 Hz, 2H, H-17), 6.97 (s, 1H, H-2), 7.16 (m, 2H, H-5,6), 7.35 (d, *J* = 7.6 Hz, 1H, H-4), 7.61 (d, *J* = 7.6 Hz, 1H, H-7), 7.95 (s br, 1H, H-1). ^{13}C NMR (50 MHz): δ 25.3 (C-15), 25.9 (C-9), 29.8 (C-10 to 14), 30.3 (C-8), 32.9 (C-16), 63.3 (C-17), 111.2 (C-7), 117.4 (C-3), 119.2 (C-4,6), 121.1 (C-5), 121.9 (C-2), 127.8 (C-3′), 136.5 (C-7′). UV (acetonitrile): λ_{max} 204 nm (ε 19629), 222 nm (ε 28322), 281 nm (ε 6314). IR (KBr): 3416 (s, O−H, N−H), 3049 (w, =C−H), 2916, 2849 (w, C−H), 1638, 1618 (m, C=C), 1456 (m, C−H), 1059 (w, C−O), 741 (m, C−H).

13a	*n*	analysis
H	10	Mp: 55−56 °C. MS (EI): 273 (M$^+$, 22), 144 ($C_{10}H_{10}N$, 3), 130 (C_9H_8N, 100). Anal. Calcd C: 79.07, H: 9.95, N: 5.12. Found C: 79.08, H: 10.05, N: 5.05.
	12	Mp: 66−67 °C. MS (EI): 301 (M$^+$, 27), 144 ($C_{10}H_{10}N$, 4), 130 (C_9H_8N, 100). Anal. Calcd C: 79.68, H: 10.36, N: 4.65. Found C: 79.77, H: 10.47, N: 4.62.
	14	Mp: 72−73 °C. MS (EI): 329 (M$^+$, 32), 144 ($C_{10}H_{10}N$, 4), 130 (C_9H_8N, 100). Anal. Calcd C: 80.19, H: 10.71, N: 4.25. Found C: 80.41, H: 10.80, N: 4.21.
	16	Mp: 79−80 °C. MS (EI): 357 (M$^+$, 37), 144 ($C_{10}H_{10}N$, 4), 130 (C_9H_8N, 100). Anal. Calcd C: 80.62, H: 10.99, N: 3.92. Found C: 80.61, H: 11.10, N: 3.85.
	18	Mp: 83−84 °C. MS (EI): 385 (M$^+$, 33), 144 ($C_{10}H_{10}N$, 3), 130 (C_9H_8N, 100). Anal. Calcd C: 80.98, H: 11.24, N: 3.63. Found C: 81.34, H: 11.46, N: 3.54.

10-(5-Methoxy-1*H*-indol-3-yl)decan-1-ol (13c, *n* = 10). Yield: 84%. ^{1}H NMR (200 MHz): δ 1.30 (s br, 12H, H-10 to 15), 1.57 (m, 2H, H-16), 1.71 (m, 2H, H-9), 2.71 (t, J = 7.4 Hz, 2H, H-8), 3.64 (t, J = 6.4 Hz, 2H, H-17), 3.88 (s, 3H, −OC*H*$_3$), 6.85 (dd, J = 8.9 Hz, J = 2.2 Hz, 1H, H-6), 6.94 (s, 1H, H-2), 7.05 (d, J = 2.2 Hz, 1H, H-4), 7.24 (d, J = 8.9 Hz, 1H, H-7), 7.86 (s br, 1H, H-1). ^{13}C NMR (50 MHz): δ 25.2 (C-15), 25.8 (C-9), 29.5−29.6 (C-10 to 14), 30.0 (C-8), 32.9 (C-16), 56.1 (−O*C*H$_3$), 63.2 (C-17), 101.2 (C-4), 111.8, 111.9 (C-6,7), 117.0 (C-3), 122.0 (C-2), 128.1 (C-3′), 131.7 (C-7′), 153.8 (C-5). UV (acetonitrile): λ_{max} 206 nm (ϵ 208300), 225 nm (ϵ 218030), 278 nm (ϵ 57697), 297 nm (ϵ 45465). IR (KBr): 3424; 3277 (w, O−H, N−H), 3001 (w, =C−H), 2920, 2846 (w, C−H), 1630, 1578 (w, C=C), 1466 (m, C−H), 1070 (m, C−O), 708 (w, C−H).

13c	*n*	analysis
5-MeO	10	Mp: 75−76 °C. MS (EI): 304 (M$^+$, 25), 160 ($C_{10}H_{10}NO$, 100), 145 (C_9H_7NO, 7). Anal. Calcd C: 75.21, H: 9.63, N: 4.62. Found C: 75.10, H: 9.73, N: 4.53.
	12	Mp: 82−83 °C. MS (EI): 331 (M$^+$, 28), 160 ($C_{10}H_{10}NO$, 100), 145 (C_9H_7NO, 7). Anal. Calcd C: 76.09, H: 10.03, N: 4.23. Found C: 76.09, H: 10.10, N: 4.01.
	14	Mp: 87−88 °C. MS (EI): 360 (M$^+$, 35), 160 ($C_{10}H_{10}NO$, 100), 145 (C_9H_7NO, 6). Anal. Calcd C: 76.83, H: 10.37, N: 3.90. Found C: 76.86, H: 10.49, N: 3.81.
	16	Mp: 92−93 °C. MS (EI): 388 (M$^+$, 35), 160 ($C_{10}H_{10}NO$, 100), 145 (C_9H_7NO, 5). Anal. Calcd C: 77.47, H: 10.66, N: 3.61. Found C: 77.76, H: 10.78, N: 3.57.
	18	Mp: 94−95 °C. MS (EI): 416 (M$^+$, 38), 160 ($C_{10}H_{10}NO$, 100), 145 (C_9H_7NO, 5). Anal. Calcd C: 78.02, H: 10.91, N: 3.37. Found C: 77.88, H: 10.99, N: 3.29.

10-(6-Methoxy-1*H*-indol-3-yl)decan-1-ol (13d, *n* = 10). Yield: 84%. ^{1}H NMR (300 MHz): δ 1.30 (s br, 12H, H-10 to 15), 1.56 (m, 2H, H-16), 1.69 (m, 2H, H-9), 2.71 (t, J = 7.3 Hz, 2H, H-8), 3.64 (m, 2H, H-17), 3.85 (s, 3H, −OC*H*$_3$), 6.79 (dd, J = 8.6 Hz, J = 2.2 Hz, 1H, H-5), 6.85 (s, 1H, H-2), 6.87 (m, 1H, H-7), 7.47 (d, J = 8.6 Hz, 1H, H-4), 7.81 (s br, 1H, H-1). ^{13}C NMR (75 MHz): δ 25.2 (C-15), 25.7 (C-9), 29.4−29.6 (C-10 to 14), 30.1 (C-8), 32.8 (C-16), 55.7 (−O*C*H$_3$), 63.1 (C-17), 94.6 (C-7), 109.0 (C-5), 117.1 (C-3), 119.6, 119.7 (C-2,4), 122.1 (C-3′), 137.0 (C-7′), 156.4 (C-6). UV (acetonitrile): λ_{max} 206 nm (ϵ 189323), 228 nm (ϵ 279970), 275 nm (ϵ 56545), 292 nm (ϵ 57990). IR (KBr): 3424 (s, N−H, O−H), 3012 (w, =C−H), 2918, 2848 (s, C−H), 1631, 1581 (m, C=C), 1463 (m, C−H), 1083 (m, C−O), 725 (w, C−H).

13d	*n*	analysis
6-MeO	10	Mp: 88−89 °C. MS (EI): 303 (M$^+$, 31), 160 ($C_{10}H_{10}NO$, 100), 145 (C_9H_7NO, 8). Anal. Calcd C: 75.21, H: 9.63, N: 4.62. Found C: 75.51, H: 9.80, N: 4.48.
	12	Mp: 95−96 °C. MS (EI): 331 (M$^+$, 32), 160 ($C_{10}H_{10}NO$, 100), 145 (C_9H_7NO, 7). Anal. Calcd C: 76.09, H: 10.03, N: 4.23. Found C: 76.31, H: 10.16, N: 4.17.
	14	Mp: 97−98 °C. MS (EI): 359 (M$^+$, 59), 160 ($C_{10}H_{10}NO$, 100), 145 (C_9H_7NO, 9). Anal. Calcd C: 76.83, H: 10.37, N: 3.90. Found C: 76.51, H: 10.44, N: 3.81.
	16	Mp: 102−103 °C. MS (EI): 387 (M$^+$, 33), 160 ($C_{10}H_{10}NO$, 100), 145 (C_9H_7NO, 8). Anal. Calcd C: 77.47, H: 10.66, N: 3.61. Found C: 77.18, H: 10.81, N: 3.55.
	18	Mp: 105−106 °C. MS (EI): 416 (M$^+$, 35), 160 ($C_{10}H_{10}NO$, 100), 145 (C_9H_7NO, 8). Anal. Calcd C: 78.02, H: 10.91, N: 3.37. Found C: 77.26, H: 10.91, N: 3.28.

16-(7-Methoxy-1*H*-indol-3-yl)hexadecan-1-ol (13e, *n* = 16). Yield: 54%. ^{1}H NMR (300 MHz): δ 1.26 (s br, 24H, H-10 to 21), 1.57 (m, 2H, H-22), 1.70 (m, 2H, H-9), 2.73 (t, J = 7.5 Hz, 2H, H-8), 3.64 (t, J = 6.6 Hz, 2H, H-23), 3.95 (s, 3H, −OC*H*$_3$), 6.64 (d, J = 7.7 Hz, 1H, H-6), 6.94 (s, 1H, H-2), 7.02 (t, J = 7.7 Hz, 1H, H-5), 7.22 (d, J = 7.7 Hz, 1H, H-4), 8.13 (s br, 1H, H-1). ^{13}C NMR (75 MHz): δ 25.3 (C-21), 25.7 (C-9), 29.6 (C-10 to 20), 30.2 (C-8), 32.8 (C-22), 55.3 (−O*C*H$_3$), 63.1 (C-23), 101.7 (C-6), 111.8 (C-4), 117.6 (C-3), 119.3 (C-5), 120.6 (C-2), 129.0 (C-7′), 146.1 (C-3′), 152.4 (C-7). UV (acetonitrile): λ_{max} 223 nm (ϵ 30060), 269 nm (ϵ 4350), 291 nm (ϵ 2930). IR (KBr): 3447, 3340 (w, O−H, N−H), 3049 (w, =C−H), 2914, 2846 (w, C−H), 1581 (m, C=C), 1463 (m, C−H), 1060 (w, C−O). MS (EI): 387 (M$^+$, 40), 160 ($C_{10}H_{10}NO$, 100), 145 (C_9H_8-NO, 11). Anal. Calcd C: 77.47, H: 10.66, N: 3.61. Found C: 77.25, H: 10.73, N: 3,86.

16-(4,5-Dimethoxy-1*H*-indol-3-yl)hexadecan-1-ol (13f, *n* = 16). Yield: 98%. ^{1}H NMR (300 MHz): δ 1.26 (s br, 24H, H-10 to 21), 1.57 (m, 2H, H-22), 1.70 (m, 2H, H-9), 2.84 (t, J = 7.5 Hz, 2H, H-8), 3.64 (t, J = 6.4 Hz, 2H, H-23), 3.90 (s, 3H, −OC*H*$_3$), 3.96 (s, 3H, −OC*H*$_3$), 6.87 (s, 1H, H-2), 6.89 (d, J = 8.8 Hz, 1H, H-6), 7.01 (d, J = 8.8 Hz, 1H, H-7), 7.80 (s br, 1H, H-1). ^{13}C NMR (75 MHz): δ 25.7 (C-21), 26.5 (C-9), 29.4−29.7 (C-10 to 20), 31.0 (C-8), 32.8 (C-22), 58.2 (−O*C*H$_3$), 61.3 (−O*C*H$_3$), 63.1 (C-23), 106.1 (C-6), 111.7 (C-7), 117.0 (C-3), 121.7 (C-3′), 121.9 (C-2), 133.9 (C-7′), 143.3 (C-4), 145.3 (C-5). UV (acetonitrile): λ_{max} 194 nm (ϵ 24361), 226 nm (ϵ 27481), 274 nm (ϵ 7505), 296 nm (ϵ 5372). IR (KBr): 3452, 3228 (w, O−H, N−H), 3051 (w, =C−H), 2917, 2845 (w, C−H), 1582 (m, C=C), 1464 (m, C−H), 1066 (w, C−O).

13f	*n*	analysis
4,5-MeO	16	Mp: 79−80 °C. MS (EI): 418 (M$^+$, 71), 190 ($C_{11}H_{12}NO_2$, 100), 175 ($C_{10}H_{10}NO_2$, 50). Anal. Calcd C: 74.77, H: 10.38, N: 3.35. Found C: 74.51, H: 10.50, N: 3.33.
	18	Mp: 82−83 °C. MS (EI): 446 (M$^+$, 50), 190 ($C_{11}H_{12}NO_2$, 100), 175 ($C_{10}H_{10}NO_2$, 53). Anal. Calcd C: 75.46, H: 10.63, N: 3.14. Found C: 75.09, H: 10.69, N: 3.20

16-(5,6-Dimethoxy-1*H*-indol-3-yl)hexadecan-1-ol (13g, *n* = 16). Yield: 97%. ^{1}H NMR (300 MHz): δ 1.25 (s br, 24H, H-10 to 21), 1.56 (m, 2H, H-22), 1.69 (m, 2H, H-9), 2.69 (t, J = 6.9 Hz, 2H, H-8), 3.64 (t, J = 6.6 Hz, 2H, H-23), 3.90 (s, 3H, −OC*H*$_3$), 3.94 (s, 3H, −OC*H*$_3$), 6.84, 6.86 (2s, 2H, H-4,7), 7.01 (s, 1H, H-2), 7.76 (s br, 1H, H-1). ^{13}C NMR (75 MHz): δ 25.2 (C-21), 25.7 (C-9), 29.4−29.7 (C-10 to 20), 30.0 (C-8), 32.8 (C-22), 56.2, 56.4 (−O*C*H$_3$), 63.1 (C-23), 94.6 (C-7), 100.8 (C-4), 117.0 (C-3), 119.5 (C-2), 120.3 (C-3′), 130.5 (C-7′), 144.7 (C-5), 147.0 (C-6). UV (acetonitrile): λ_{max} 205 nm (ϵ 24468), 226 nm (ϵ 25619), 280 nm (ϵ 7979), 297 nm (ϵ 9190). IR (KBr): 3394, 3260 (w, O−H, N−H), 3035 (w, =C−H), 2916, 2846 (w, C−H), 1579 (m, C=C), 1468 (m, C−H), 1070 (w, C−O).

13g	*n*	analysis
5,6-MeO	16	Mp: 72−83 °C. MS (EI): 418 (M$^+$, 71), 190 ($C_{11}H_{12}NO_2$, 100), 176 ($C_{10}H_{10}NO_2$, 15). Anal. Calcd C: 74.77, H: 10.38, N: 3.35. Found C: 75.02, H: 9.69, N: 3.00.
	18	Mp: 85−86 °C. MS (EI): 446 (M$^+$, 54), 190 ($C_{11}H_{12}NO_2$, 100), 176 ($C_{10}H_{10}NO_2$, 17). Anal. Calcd C: 75.46, H: 10.63, N: 3.14. Found C: 75.01, H: 10.59, N: 3.16.

5-Methoxy-3-pentadecyl-1*H*-indole (14). Yield: 71%. ^{1}H NMR (300 MHz): δ 0.88 (t, J = 6.7 Hz, 3H, H-22), 1.25 (s br, 24H, H-10 to 21), 1.60 (m, 2H, H-9), 2.71 (q, J = 7.6 Hz, 2H, H-8), 3.87 (s, 3H, −OC*H*$_3$), 6.85 (dd, J = 8.8 Hz, J = 2.5 Hz, 1H, H-6), 6.94 (s, 1H, H-4), 7.04 (s, 1H, H-2), 7.23 (d, J = 8.8 Hz, 1H, H-7), 7.80 (s br, 1H, H-1). ^{13}C NMR (50 MHz): δ 14.6 (C-22), 25.8 (C-9), 29.5−29.6 (C-10 to 21), 30.1 (C-8), 56.1 (−O*C*H$_3$), 101.3 (C-4), 111.6, 111.8 (C-6,7) 117.0 (C-3), 122.1 (C-2), 128.1 (C-3′), 131.6 (C-7′), 153.8 (C-5). IR (KBr): 3424 (w, N−H), 3001 (w, =C−H), 2920, 2846 (w, C−H), 1630, 1578 (w, C=C), 1466 (m, C−H), 1070 (m, C−O), 708 (w, C−H). MS (EI): 358 (M$^+$). Anal. Calcd C: 80.61, H: 10.99, N: 3.92. Found C: 80.63, H: 11.02, N: 3.97.

Biology. Drug was dissolved in ethanol at the concentration of 10^{-2} M and diluted in culture medium to render a final concentration of 0.1% ethanol. Control cells were incubated under the same conditions. Neurospheres have been prepared as described by Grandbarbe et al.[20]

Differentiation and Analysis of Neurospheres. After 3 days of proliferation, 50−100 neurospheres were plated onto polyornithine (poly-L-ornithine Sigma)-coated (14 mm) cover slips, in a 24 well plate (Nunc) to differentiate. Differentiation of neurospheres is often described as requiring the withdrawal of EGF, which is replaced by fetal bovine serum (FBS). In order to avoid the hazardous effect of FBS, while preserving cell survival, we reduced EGF concentration to 2 ng/mL during

the differentiation phase, which is sufficient to allow cell survival but minimizes proliferation. Under these conditions, the differentiating sphere remains a dynamic structure where generation of new cells (stem cells and progenitors) is diminished but not totally abolished.

Immunostaining and Quantification. Neurospheres were fixed for 20 min in 4% paraformaldehyde in PBS (pH 7.4), washed in PBS, and permeabilized for 5 min with PBS/0.5% Triton X-100 (Sigma). The neurospheres were incubated for 30 min in PBS containing 3% BSA and then for 2 h with the appropriate mixture of antibodies. Primary antibodies used were mouse monoclonal anti-MAP2 (2a+2b) (1/600, Sigma) and mouse monoclonal TUJ1 (1/400, Convance) specific for immature and postmitotic neurons, respectively, and rabbit polyclonal anti-GFAP (1/1000, Dako) for astrocytes. After washing in PBS, differentiating spheres were incubated for 1 h with anti-mouse Cy3-conjugated secondary antibodies (1/1000, Jackson ImmunoResearch) and anti-rabbit Alexa 488-conjugated antibodies (1/600, Molecular Probes). Preparations were counterstained with TO-PRO-3-iodide (1/15000, Molecular Probes), mounted in Aquamount (Polyscience), and viewed for triple immunofluorescence using a Zeiss LSM 510 confocal microscope. Data are based on three or four independent experiments in which averages of more than 10 spheres were analyzed per condition, per experiment. Under the conditions used for cell concentration after full dissociation (3×10^4 cells/mL) and plating (50−100 spheres/cover slip), each neurosphere may be considered as the clonal progeny of a single neural stem cell. For cell type quantitative estimation, neurospheres were chosen of approximately the same size. The confocal plane was at the basis of the neurosphere, i.e. where the cells differentiate. The optical slice was ≤2 μm. The results are expressed in percentage of total cell number assessed from TO-PRO-3-iodide staining.

Reverse Transcriptase Polymerase Chain Reaction Analysis. The cDNA was synthesized from 3 μg of total RNA for neurospheres at 37 °C for 1 h, in a 20 μL reaction mixture, containing 200 units of M-MLV reverse transcriptase (Invitrogen), 0.5 mM dNTPs (QBiogen), 5 mM DTT (Invitrogen), 0.01 μg/μL random hexamer (Invitrogen), and 20 units of RNaseOUT (Invitrogen). One-tenth of total cDNA was amplified in a 20 μL reaction mixture containing 1.25 units of Taq DNA polymerase (Invitrogen, 0.5 mM dNTPs (QBiogen)) and 0.5 μL of each primer (IGBMC, Strasbourg). PCR conditions have been adjusted for each primer. Typical cycle conditions were 30 s at 94 °C, 30 s at 55 °C, and 30 s at 72 °C for all the primers studied. Then 10 μL of the reaction mixture was separated on a 1% agarose gel and visualized by ethidium bromide staining. The graphed results are shown as means ± SEM. Group changes were assessed by multiple pairwise comparisons using the Turkey−Krammer method. Statistical differences were obtained between groups at $P \leq 0.01$ for **.

genes	sequence
Notch1 F	5′-TGCCAAATGCCTGCCAGAAT-3′
Notch1 R	5′-CATGGATCTTGTCCATGCAG-3′
Notch2 F	5′-GAGGCGACTCTTCTGCTGTTGAAGA-3′
Notch2 R	5′-ATAGAGTCACTGAGCTCTCGGACAG-3′
Notch3 F	5′-ACACTGGGAGTTCTCTGTGAG-3′
Notch3 R	5′-GCTGTCTGCTGGCATGGGATA-3′
Notch4 F	5′-CTTCTCGTCCTCCAGCTCAT-3′
Notch4 R	5′-GCTGACATCAGGGGTGTCAC-3′

Treatment with Notch4 Antisense Oligonucleotides. Notch4 antisense (**AS1** and **AS2**) and control sense (**S1** and **S2**) oligonucleotides have been designed with OLIGO 6.1 software (Molecular Biology Insights), and sequences are as follows: **AS1**, CCA TGA GCT TTC GGG TTC; **S1**, GAA CCC GAA AGC TCA TGG; **AS2**, AAG GCG TTG GCT AAA; and **S2**, TTT AGC CAA CGC CTT. Neurospheres have been treated with 10 μM concentrations of sense (control conditions) or antisense oligonucelotides or with **13c** ($n = 18$) at 10 nM during differentiation and proliferation phases. The graphed results are shown as means ± SEM. When statistical differences were obtained between groups at $P \leq 0.001$ for ***, group changes were assessed by multiple pairwise comparisons using the Turkey−Krammer method.

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