Serotonin Dimers: Application of the Bivalent Ligand Approach to the Design of New Potent and Selective 5-$HT_{1B/1D}$ Agonists

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Received July 29, 1996[⊗]

A series of serotonin dimers of formula **4** in which two serotonin moeities are linked together through their 5-hydroxyl residue has been prepared and evaluated as 5-$HT_{1B/1D}$ receptor agonists. Binding experiments at cloned human 5-HT_{1B}, 5-HT_{1D}, and 5-HT_{1A} receptors show that all of these dimers are very potent ligands at 5-$HT_{1B/1D}$ receptors with increased binding selectivity vs the 5-HT_{1A} receptor when compared to serotonin. Studies of inhibition of the forskolin-stimulated c-AMP formation mediated by the human 5-HT_{1B} receptor (formerly the 5-$HT_{1D\beta}$ receptor) demonstrate that all of these serotonin dimers behave as full agonists. Among them, the piperazide derivatives of bis-serotonin, **4g**,**j**, were also identified as very potent agonists in contracting the New Zealand white rabbit saphenous vein ($pD_2 = 7.6$ in each case compared to 5.8 for sumatriptan). Results analysis supports the hypothesis that the important increase in potency of the serotonin dimers can be attributed to the presence of two serotonin pharmacophores in the same molecule, while the enhanced selectivity for 5-$HT_{1B/1D}$ receptor subtypes may be due to the position of the spacer attachment to serotonin.

The now well-recognized existence of multiple, structurally, and functionally distinct receptors for a single neurotransmitter or hormone has stimulated the search for potent and selective receptor subtype ligands, including full agonists or silent antagonists. Indeed, such types of compounds are particularly useful as pharmacological tools to assess and furthermore better characterize the physiological roles of each receptor subtype, and moreover, their development can result in new, more effective, and selective therapeutic agents with fewer side effects since activation (with agonists) or inactivation (with antagonists) of individual receptor subtypes may function to affect specific actions closely related to pathophysiological states. This applies particularly well to the case of the neurotransmitter serotonin **2** (5-HT). 5-HT is involved in numerous physiological (e.g., thermoregulation, hemodynamics, feeding, sleeping) and pathophysiological (e.g., depression, hypertension, migraine, anxiety) processes[1] and interacts with various distinct membrane receptors. These receptors have been divided into at least seven classes: 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, 5-HT_5, 5-HT_6, and 5-HT_7. The 5-HT_1 receptors have been further subdivided in 5-HT_{1A}, 5-HT_{1B} (previously 5-$HT_{1D\beta}$), 5-HT_{1D} (previously 5-$HT_{1D\alpha}$), 5-HT_{1E}, and 5-HT_{1F} subtypes.[2]

The human 5-HT_{1B} and 5-HT_{1D} receptor subtypes differ from each other on the basis of molecular biology studies and recent pharmacological characterization.[3] To date, functional distinction between 5-HT_{1B} and 5-HT_{1D} receptor subtypes is under extensive investigation, and their respective importance in mediating vascular constriction and inhibiting neurogenic inflammation (two mechanisms relevant to migraine pathology) remains to be determined.[4] The introduction of the 5-$HT_{1B/1D}$ receptor agonist sumatriptan (**1**) for the acute treatment of migraine[5] has stimulated a strong interest and an extensive effort in the fields of chemical synthesis, pharmacological characterization, and clinical evaluation of new 5-$HT_{1B/1D}$ receptor agonists. Most of the compounds reported to date (including sumatriptan, zolmitriptan,[6] or rizatriptan[7]) are tryptamine derivatives which differ from each other either by changes in the nature of the 5-substituent or by changes at the aminoethyl side-chain level, including conformationally restricted analogs like naratriptan.[8] It is noteworthy that most of these tryptamine derivatives reported as 5-$HT_{1B/1D}$ receptor agonists have almost no affinity for 5-HT_2, 5-HT_3, or 5-HT_4 receptor subtypes but generally recognize 5-HT_{1A} binding sites with nonnegligible affinities. Thus, one of the most serious problems in designing 5-$HT_{1B/1D}$ receptor ligands is their lack of selectivity relative to 5-HT_{1A} receptors.

Among the different methods currently available for medicinal chemists to design potent and selective receptor subtype ligands, the so-called "bivalent ligand" approach[9] appears very promising since many examples of molecules including two pharmacophores in a single ligand have been found to have enhanced activity and selectivity over their respective monomer counterparts. The field of opioid research has been successfully investigated for that purpose since dimers of peptide agonists derived from enkephalins[10] and dimers of opioid alkaloid antagonists[11] (derived from naltrexone or β-naltrexamine) have been characterized as potent opioid ligands (respectively agonists and antagonists) having increased receptor subtype selectivity when compared to their corresponding monomers. These efforts culminated in the identification of norbinaltorphimine, a potent, selective κ-opiate receptor antagonist that is widely employed in opioid research.[11,12]

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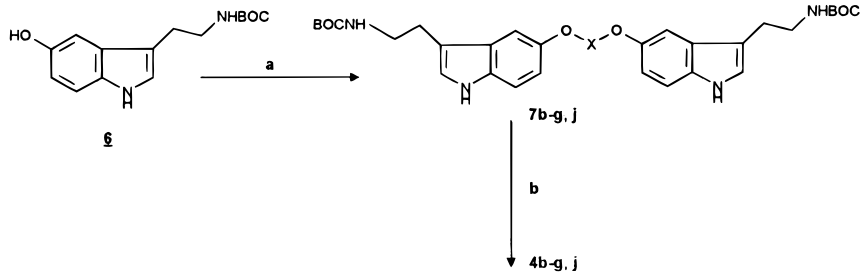
[⊗] Abstract published in *Advance ACS Abstracts,* November 15, 1996.

S0022-2623(96)00552-3 CCC: $12.00

Scheme 1[a]



[a] Reagents and conditions: (a) Cl−X−Cl or Br−X−Br (0.5 equiv), MEK, K_2CO_3 (2.5 equiv), KI (0.1 equiv), reflux, 54−56% or Cs_2CO_3 (2 equiv), DMF, 70 °C, 32−69%; (b) TFA (excess), toluene, 25 °C, 1−2 h, 49−85%.

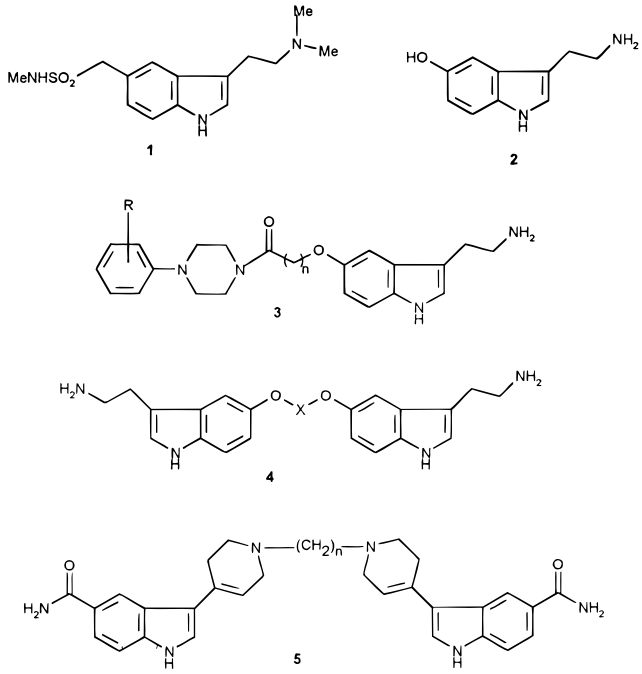


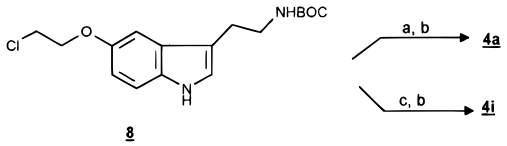
Figure 1.

Several other studies have also previously reported that dimerization can result in an increase of potency/selectivity and, interestingly enough, can also improve resistance to degradation in the case of peptide agonists or antagonists.[13] In the field of monoamine neurotransmitters, however, only a few examples of bivalent ligands have been documented to date. These include hexaprenalin[14] (a β-adrenergic receptor agonist), dimers derived from dopamine as potent nonselective D_1 and D_2 receptor agonists,[15] and bivalent indoles derived from 5-CT of formula **5** (Figure 1) exhibiting serotonergic binding activity with 5-HT$_{1D}$ versus 5-HT$_{1A}$ selectivity dependent on the spacer length.[16]

We have recently prepared and evaluated a new series of 5-arylpiperazide derivatives of serotonin **3** which have been characterized as potent and selective 5-HT$_{1B/1D}$ receptor agonists.[17,18] These results, taken together with data recently reported by Glennon and co-workers,[19] suggest that 5-HT$_{1B/1D}$ receptors possess a deep binding pocket in the binding domain recognizing the substituent attached in position 5 of the tryptamine residue as in compounds **3** (Figure 1). This region of bulk tolerance seems to discriminate between 5-HT$_{1B/1D}$ and 5-HT$_{1A}$ receptors and, therefore, represents an interesting opportunity to introduce a linker between two pharmacophoric tryptamine residues in order to design selective and potent 5-HT$_{1B/1D}$ bivalent ligands.

In this paper, we wish to report the synthesis, binding properties, and intrinsic activity at 5-HT$_{1B/1D}$ and 5-HT$_{1A}$ receptors of the first examples of serotonin dimers of formula **4** in which two serotonin moieties are linked together through their 5-hydroxyl residue.

Scheme 2[a]



[a] Reagents and conditions: (a) **6** (2 equiv), NaH (2.2 equiv), DMF, KI (0.1 equiv), 25 °C, 0.5 h, then **8** (1 equiv), 80 °C, overnight, 54%; (b) TFA (excess), toluene, 25 °C, 1.5 h, 49−91%; (c) piperazine (0.5 equiv), DMF, K_2CO_3 (2.5 equiv), KI (0.1 equiv), 90 °C, 48 h, 64%.

Chemistry

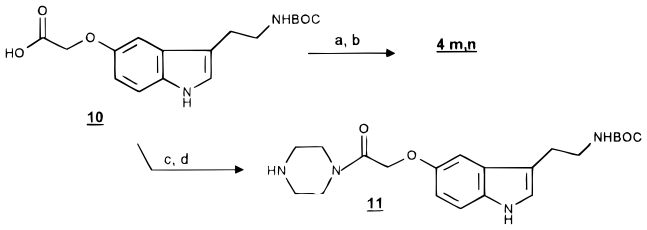
The serotonin dimers **4b**−**g**,**j** (Table 1) have all been prepared by condensation of the previously described[17] *N*-BOC serotonin derivative **6** with a bifunctional electrophile (Cl−X−Cl, in which X corresponds to the definition of X in Table 1) in refluxing methyl ethyl ketone in the presence of K_2CO_3 and KI or in DMF in the presence of Cs_2CO_3 (Scheme 1) followed by removal of the BOC protecting group using trifluoroacetic acid in toluene at room temperature.

Compounds **4a**,**i** have been obtained from the known[18] chloroethoxy derivative of *N*-BOC tryptamine **8** according to Scheme 2. The bis-amides **4m**,**n** have been synthesized by condensation of the free diamines **9m** (1,3-diaminopropane) and **9n** (4,4′-bis-piperidine) with the 5-*O*-carboxymethyl derivative of *N*-BOC serotonin **10**[17] according to Scheme 3. The unsymmetrical piperazide derivative of bis-serotonin **4h** has been obtained by the condensation of the piperazide intermediate **11** (prepared from the acid **10** according to Scheme 3) with the chloroethoxy derivative of tryptamine **8** (Scheme 2) in the presence of K_2CO_3 and KI in DMF (91% yield) followed by removing the *N*-BOC protecting group with TFA.

The amide **4k** and the urea **4l** have been obtained by classical methods from the intermediary amine **12**, according to Scheme 4. The intermediate amine **12** is easily prepared in two steps from *N*-BOC serotonin **6** which is first condensed with bromoacetonitrile in the presence of potassium carbonate in methyl ethyl ketone followed by reduction of the intermediate nitrile using Pd on charcoal under an atmospheric pressure of hydrogen. Finally the bis-sulfonamide **4o** has been obtained in three steps from *N*-BOC serotonin **6** by a route very similar to that depicted in Scheme 5.

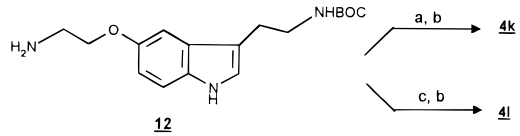
All compounds were purified by column chromatography on silica gel. Subsequent treatment of the free

Scheme 3[a]



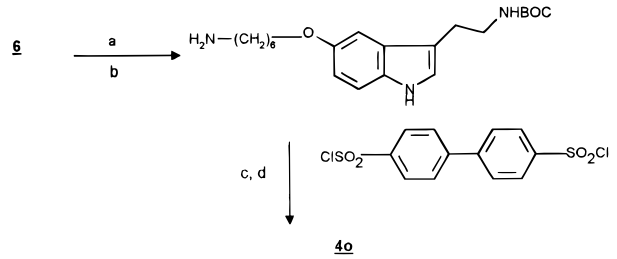
[a] Reagents and conditions: (a) ClCOOEt (1.9 equiv), NMM (1.5 equiv), CH_2Cl_2, −15 °C, 0.5 h, then 1,3-diaminopropane (**9m**) (0.5 equiv), −15 to 25 °C, 1 h, 64% or bis-piperidine **9n** (0.5 equiv), CH_2Cl_2/MeOH (1/1), PyBOP (1 equiv), iPr_2NEt (2.75 equiv), 25°C, 6 h, 44%; (b) TFA (excess), toluene, 25 °C, 1.5 h, 57% and 29%; (c) ClCOOEt (1.3 equiv), NMM (1.5 equiv), CH_2Cl_2, −15 °C, 0.5 h, then *N*-benzylpiperazine (2.5 equiv), −15 to 25 °C, 1 h, 90%; (d) H_2, Pd/C, MeOH, 4 days, 77%.

Scheme 4[a]



[a] Reagents and conditions: (a) **10** (1 equiv), ClCOOEt (1.3 equiv), NMM (1.5 equiv), CH_2Cl_2, −15 °C, 0.5 h, then **12** (1 equiv) −15 to 25 °C, 1 h; (b) TFA (excess), toluene, 25 °C, 1.5 h, 64−91%; (c) triphosgene (1/6 equiv), CH_2Cl_2, Et_3N (1 equiv), 0−25 °C, 1.5 h, 79%.

Scheme 5[a]



[a] Reagents and conditions: (a) $Br(CH_2)_5CN$ (1.8 equiv), MEK, K_2CO_3 (2.5 equiv), KI (0.1 equiv), reflux, 28 h, 94%; (b) H_2, Raney Ni (cat.), THF, NH_4OH, 25 °C, 36 h, 97%; (c) DCM, Et_3N, 25 °C, 1 h, 80%; (d) TFA (excess), toluene, 25 °C, 73%.

amines with HCl in dichloromethane afforded the hydrochloride salts of the bis-serotonin derivatives **4** suitable for biological evaluation.

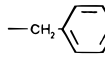
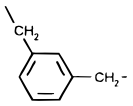
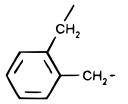
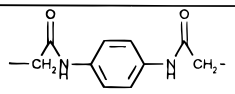
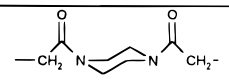
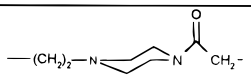
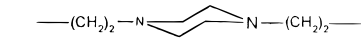
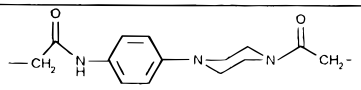
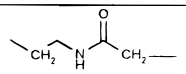
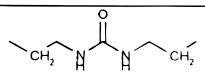
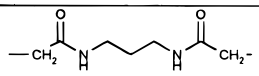
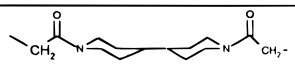
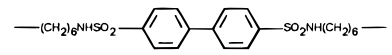
Biological Results and Discussion

The pharmacological properties of the serotonin dimers **4a**−**o** reported in this paper have been compared to serotonin itself and to sumatriptan at the cloned human 5-HT_{1B}, 5-HT_{1D}, and 5-HT_{1A} receptors, and their intrinsic activity has been assessed as their ability to inhibit the forskolin-stimulated c-AMP formation mediated by human 5-HT_{1B} receptors in CHO-K1 cells. The results obtained are summarized in Table 1. In all cases reported, serotonin dimers **4a**−**o** bind to human cloned 5-HT_{1B} and 5-HT_{1D} receptors with a higher affinity than serotonin itself. Moreover, except for compounds **4a**,**e**, all dimers bind to both receptor subtypes with subnanomolar affinities. None of these dimers is able to discriminate between 5-HT_{1B} and 5-HT_{1D} receptor subtypes, but interestingly enough, large discrepancies can be observed when comparing binding values between 5-$HT_{1B/1D}$ and 5-HT_{1A} receptors. In that respect, the affinities of most of the serotonin dimers for 5-HT_{1A} are either very similar to serotonin itself or even less pronounced (see **4g** for example). Thus, dimerization of serotonin, as in compounds **4a**−**o**, leads to binding selectivity between 5-$HT_{1B/1D}$ and 5-HT_{1A} receptors. This increase in selectivity when compared to serotonin is mainly due to an increased affinity for the 5-$HT_{1B/1D}$ sites with either no change or a decrease at 5-HT_{1A} sites. As for interaction with other 5-HT receptor subtypes, six representative examples of the serotonin dimers (compounds **4b**−**g**) were found to have almost no affinity ($IC_{50} > 10^{-6}$ M) for 5-HT_2, 5-HT_3, and 5-HT_4 receptor subtypes. Considering the intrinsic activity of the dimers **4a**−**o** at human 5-HT_{1B} receptors (previously 5-$HT_{1D\beta}$ receptors), it can be concluded that all of them appear as potent agonists with EC_{50} values lower than what has been found for serotonin. EC_{50} values of compounds **4g**,**j** were also measured at cloned human 5-HT_{1A} receptors (inhibition of forskolin-stimulated c-AMP formation[20]) and found to be respectively 1000 and 280 nM (compared to 15 nM for serotonin). Thus, compounds **4g**,**j**, which appear as partial agonists at 5-HT_{1A} receptors, show a large selectivity pattern when comparing their agonist efficacies at human cloned 5-HT_{1B} and 5-HT_{1A} receptors. The superior agonist potency of such types of dimers compared to that of sumatriptan has been confirmed in the rabbit isolated saphenous vein contraction model.[21] This functional model, traditionally used to characterize 5-$HT_{1B/1D}$ agonists, is particularly relevant in view of the recent identification of a 5-$HT_{1D\beta}$ receptor gene in rabbit saphenous vein having an amino acid sequence 93% homologous to the human 5-HT_{1B} receptor.[22] Thus, compounds **4f** ($pD_2 = 7.8$), **4g** ($pD_2 = 7.6$), and **4j** ($pD_2 = 7.6$) were far more potent in contracting the rabbit saphenous vein than sumatriptan ($pD_2 = 5.8$), reaching maximum effects that do not statistically differ from that observed with 5-HT.

Altogether, these results demonstrate that dimerization of serotonin through the 5-OH position represents a useful way to design potent ($K_i < 1$ nM; $EC_{50} \sim 1$ nM) and selective 5-$HT_{1B/1D}$ agonists. Comparing these data (K_i and EC_{50} values) to those obtained for sumatriptan in the same experimental conditions (Table 1) further demonstrates the potential of serotonin dimers **4a**−**o** as new useful 5-$HT_{1B/1D}$ receptor agonists.

In order to cancel out the effect of the spacer on the high potency (at both the binding affinity and intrinsic activity) of serotonin dimers reported in Table 1, some 5-O-substituted serotonin derivatives attached only to the spacer, as found in compounds **4g**,**h**,**j**, have been prepared and evaluated in the same models as previously described. The results of this investigation (reported in Table 2) show a dramatic influence of the second serotonin pharmacophore especially when comparing the results obtained with compounds **13** and **4g** or **4h**. However, recent investigations from our laboratory had previously demonstrated the importance of an aromatic ring as the substituent in position 4 of the piperazine ring[17] in such types of derivatives. Therefore, compounds **4g**,**h** have also been compared to the arylpiperazide derivative **14** (Table 2). Here again, the same conclusion can be reached; serotonin dimers are more potent 5-$HT_{1B/1D}$ ligands than the monomeric derivatives (compare **4g**,**h** with **14**). Introduction of a second serotonin pharmacophore to **14**, as in compound **4j,** leads again to an increase in binding affinity at 5-$HT_{1B/1D}$ receptor subtypes. This comparative study

Table 1. Comparison of Dimers **4a**−**o** with Sumatriptan and 5-HT at Human 5-HT$_{1B}$, 5-HT$_{1D}$, and 5-HT$_{1A}$ Receptors

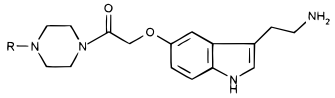
		Ki(nM)[a]			$EC_{50(nM)}$[a]
Compound	**X**	**5-HT$_{1D}$**	**5-HT$_{1B}$**	**5-HT$_{1A}$**	**5-HT$_{1B}$**
4a	—(CH$_2$-CH$_2$)—	1.0	2.5	14.3	4.0
4b	—(CH$_2$)$_6$—	0.46	0.5	1.8	0.6
4c		0.11	0.10	1.6	3.0
4d		0.35	0.32	1	0.85
4e		1.5	1.7	4.3	2.07
4f		0.11	0.27	4.06	0.3
4g		0.42	0.36	20	0.28
4h		0.16	0.11	9.5	0.22
4i		0.35	0.26	9.5	0.19
4j		0.09	0.14	9.8	0.4
4k		0.18	0.16	2.2	0.44
4l		0.4	0.2	2.2	1.6
4m		0.3	0.3	8.6	0.09
4n		0.27	0.22	4.0	0.26
4o		0.45	0.38	1.66	0.10
5-HT		5.06	6.76	2.48	4.82
Sumatriptan		8.5	23.1	440	77

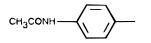
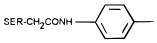
[a] K_i and EC_{50} values are given as the mean of two or three independent determinations, each performed in duplicate, typically with individual values within ±10−20% of the mean.

stresses the importance of having two pharmacophoric units (serotonin) in the same molecule to optimize binding and intrinsic activity at 5-HT$_{1B/1D}$ sites.

Comparison of the results reported in this paper with those previously described[16] for conformationally restricted 5-CT dimers of formula **5** (Figure 1) show that in both cases, the bivalent ligand approach has allowed the identification of more potent 5-HT$_{1B/1D}$ ligands in term of binding affinity (when compared to their respective monomer) and selectivity (especially vs 5-HT$_{1A}$ receptors). However, in the previous study,[16] the spacer length has been shown to be crucial for optimal binding and selectivity: Several dimers bind poorly to 5-HT$_{1D}$ sites and demonstrate a selectivity pattern in favor of 5-HT$_{1A}$ receptors. Results reported here differ drastically on that point of view since all dimers **4a**−**o** (see Table 1) are better 5-HT$_{1B/1D}$ receptor ligands than the monomer (serotonin) and all of them show an increased selectivity ratio in favor of 5-HT$_{1B/1D}$ receptors. The observation that all serotonin dimers (with the exception

Table 2. Comparison of Binding Affinities (K_i Values, nM) at Human 5-$HT_{1B/1D}$ and 5-HT_{1A} Receptors of Monomers **13** and **14** and Dimers **4g**−**i**



Compound	R[a]	5-HT_{1D}[b]	5-HT_{1B}[b]	5-HT_{1A}[b]
13	H	> 100	> 100	> 100
4g	SER-CH_2CO-	0.42	0.36	20
4h	SER-CH_2CH_2-	0.16	0.11	9.5
14		1.7	3.3	134
4j		0.09	0.14	9.8

[a] SER stands for 5-O-substituted serotonin (cf. Table 1). [b] K_i values are given as the mean of two or three independant determinations, each performed in duplicate, typically with individual values within ±10−20% of the mean.

Table 3. Physical Properties of Compounds Listed in Table 1

no.	mp[a] (°C)	formula[b]	anal.[c]
4a	270	$C_{22}H_{26}N_4O_2 \cdot 2HCl \cdot 1H_2O$	C,H,N
4b	235	$C_{26}H_{34}N_4O_2 \cdot 2HCl \cdot 1H_2O$	C,H,N,Cl
4c	280	$C_{28}H_{30}N_4O_2 \cdot 2HCl \cdot 0.8H_2O$	C,H,N
4d	222	$C_{28}H_{30}N_4O_2 \cdot 2HCl \cdot 0.5H_2O$	C,H,N,Cl
4e	186	$C_{28}H_{30}N_4O_2 \cdot 2HCl \cdot 0.5H_2O$	C,H,N,Cl
4f	250	$C_{30}H_{32}N_6O_4 \cdot 2.4HCl \cdot 2.9H_2O$	C,H,N,Cl
4g	236	$C_{28}H_{34}N_6O_4 \cdot 2HCl \cdot 3.4H_2O$	C,H,N
4h	187	$C_{28}H_{36}N_6O_3 \cdot 3.4HCl \cdot 1.3H_2O$	C,H,N,Cl
4i	182	$C_{28}H_{38}N_6O_2 \cdot 4HCl \cdot 2.1H_2O$	C,H,N
4j	195	$C_{34}H_{39}N_7O_4 \cdot 3.5HCl \cdot 1.6H_2O$	C,H,N,Cl
4k	168	$C_{24}H_{29}N_5O_3 \cdot 2HCl \cdot 1.1H_2O$	C,H,N
4l	140	$C_{25}H_{32}N_6O_3 \cdot 2HCl \cdot 1H_2O$	C,H,N,Cl
4m	260	$C_{27}H_{34}N_6O_4 \cdot 2HCl \cdot 1H_2O$	C,H,N,Cl
4n	193	$C_{34}H_{44}N_6O_4 \cdot 2.1HCl \cdot 3.4H_2O$	C,H,N,Cl
4o	140	$C_{44}H_{56}N_6O_6S_2 \cdot 2HCl \cdot 1.5H_2O \cdot 0.3EtOH$	C,H,N,Cl

[a] All compounds were crystallized from CH_2Cl_2/Et_2O or $CHCl_3/Et_2O$. [b] Satisfactory ^1H NMR were obtained for all compounds. [c] The analyses are within ±0.4% of the theoretical values (compound **4d** did not agree with calculated values for Cl possibly due to the accuracy of the method used).

of **4a**,**e**) bind to 5-$HT_{1B/1D}$ with nearly the same affinity (K_i values between 0.1 and 0.5 nM) shows that the compounds of our series appear to lack any dependence between affinity and spacer length or flexibility. This is particularly intriguing when very different linkers are compared in these dimers as, for example, with compounds **4k** (short linker) and **4o** (extended linker). It is noteworthy that agonist potency of dimers **4a**−**o** is also spacer independent since all of them appear as potent 5-$HT_{1B/1D}$ agonists with similar EC_{50} values (comparison with dimers of formula **5** can not be made at that level since intrinsic activity of these compounds has not been reported). Therefore, it can be speculated that such dimers are able to activate 5-$HT_{1B/1D}$ receptors by interactions at least at one agonist binding site, interactions which are probably very similar for all of them and probably closely related to the interaction mode between serotonin and the receptor.

Several theories have been proposed to rationalize bivalent ligand activity and selectivity. Among them, two potential bridging mechanisms have been proposed[11] from the above-mentioned extensive studies of bivalent ligands in the opioid receptors field. First, if the spacer is of sufficient length, it may be possible for both pharmacophores in a bivalent ligand to occupy distinct but very similar or identical neighboring recognition sites; a second possible mechanism involves the bridging of the second pharmacophore of a bivalent ligand to an adjacent accessory site which is unique to the receptor system. These hypotheses can easily explain selectivity of a bivalent ligand for a particular receptor subtype, but under such circumstances, it is reasonable that the bridging of neighboring sites by a divalent ligand would be dependent on the nature (length or flexibility) of the spacer. Such a situation has been reported in many cases as, for example, in dimers of opioid alkaloid antagonists,[11] dimers of peptides derived from enkephalins,[10] and dimers of conformationally restricted 5-CT derivatives.[16] However, this hypothesis is unlikely to apply to the new series of serotonin dimers reported in this paper. The large diversity of spacer lengths reported here associated with a very similar binding profile is not in favor of simultaneous binding of the serotonin dimers **4a**−**o** at neighboring receptor sites. However, as pointed out previously by Portoghese,[11] enhanced potency in binding of bivalent ligands can proceed through a univalently bound state, the unbound recognition unit being in the locus of neighboring binding sites. This is equivalent to a high local concentration of the free recognition unit in the vicinity of the neighboring sites because it is tethered to the bound pharmacophore. Assuming such a hypothesis can be taken into consideration to explain enhanced affinity for serotonin dimers **4a**−**o** at 5-$HT_{1B/1D}$ receptors; it does, however, not explain the 5-$HT_{1B/1D}$ vs 5-HT_{1A} enhanced selectivity found for these dimers, especially when compared to serotonin. We and others have recently shown[17,19] that 5-$HT_{1B/1D}$ receptors tolerate a large variety of substituents at the tryptamine 5-position; for example, compounds of general formula **3** (Figure 1) with *n* values varying from 1 to 5 bind with very good affinity (nanomolar range) to 5-$HT_{1B/1D}$ receptor subtypes and show some interesting binding selectivity vs 5-HT_{1A} receptors. These observations suggest that a deep binding pocket is available in the binding domain of 5-$HT_{1B/1D}$ receptors but not in the binding domain of 5-HT_{1A} receptors. This deep binding pocket in 5-$HT_{1B/1D}$ receptors is able to accommodate a large diversity of 5-O-substituents on serotonin and can explain the selectivity found with the serotonin dimers described in this study.

In summary, the enhanced potency observed with dimers **4a**−**o** at 5-$HT_{1B/1D}$ binding sites can be explained by the dimeric structural feature of these derivatives, but their enhanced selectivity (vs 5-HT_{1A}) can better be explained by the choice of the position on the serotonin residue where the spacer has been attached. The increased selectivity observed for all dimers **4a**−**o** compared to serotonin is due to the discriminative effect of 5-O-substituents between 5-$HT_{1B/1D}$ and 5-HT_{1A} binding sites. This hypothetical explanation can accommodate the discrepancies observed between the serotonin dimers reported here and the above-mentioned 5-CT-derived types of dimers **5** (in which selectivity is very dependent on the spacer length) since the latter ones result from a dimerization at the level of the basic nitrogen atom, and therefore, the spacers of these derivatives can probably not occupy the deep binding pocket we assume to be important for the selectivity observed with compounds **4a**−**o** or compounds of formula **3**.[17]

Conclusions

The first synthesis of serotonin dimers and their biological properties related to serotonin receptors has been described. Binding affinity and intrinsic activity of these dimers (compounds **4a**−**o**) at cloned human 5-HT$_{1B/1D}$ and 5-HT$_{1A}$ receptor subtypes (and for some of them at 5-HT$_2$, 5-HT$_3$, and 5-HT$_4$ subtypes) show that dimerization of serotonin through the 5-OH residue allows the identification of a new series of potent and selective 5-HT$_{1B/1D}$ agonists, for example, compounds **4g**,**j**, which are much more potent agonists than sumatriptan. Results analysis based on comparisons with serotonin supports the hypothesis that the marked increase in potency of the serotonin dimers **4a**−**o** can be attributed to the presence of two serotonin pharmacophores in the same molecule, while the enhanced selectivity for 5-HT$_{1B/1D}$ receptor subtypes may be due to the choice of the position of the spacer attachment to serotonin. Further work is in progress to extend these hypotheses to the design of new bivalent ligands as potent and selective 5-HT$_{1B/1D}$ agonists of therapeutic interest.

Experimental Section

Melting points were recorded on a electrothermal 9200 apparatus and are uncorrected. ^{1}H NMR spectra were obtained on a Brüker AC200 (200 MHz) instrument. IR spectra were obtained on a Nicolet FT510P spectrometer. Mass spectra were recorded on a Nermag R10-10B spectrometer. Purification by chromatography refers to flash chromatography on silica gel (0.04−0.063 mm supplied by S.D.S.) with the eluent indicated applied at a pressure of 0.5 atm. Elemental analyses for carbon, hydrogen, and nitrogen were determined with a Fisons EA 1108/CHN instrument.

General Procedures for the Preparation of Compounds 4b−g,j. General Method A: 2-[5-[[2-[[[3-(2-Aminoethyl)-1*H*-indol-5-yl]oxy]methyl]benzyl]oxy]-1*H*-indol-3-yl]ethylamine (4e). This procedure was used for the preparation of **4b**,**e**−**g**,**j.** A mixture of *N*-BOC serotonin **6** (1.0 g, 3.62 mmol), α,α′-dichloro-*o*-xylene (317 mg, 1.81 mmol), and Cs_2CO_3 (2.2 g, 7.24 mmol) in DMF (6.3 mL) was stirred at 70 °C overnight. The mixture was diluted with EtOAc and filtered through Celite, and the filtrate was washed with H_2O and saturated aqueous NaCl, dried (Na_2SO_4), and concentrated. The crude product was purified by chromatography (CH_2Cl_2/acetone, 10/1) to give the bis-O-alkylated product (820 mg, 69%). This intermediate (780 mg, 1.19 mmol) was treated in toluene (45 mL) and, at 25 °C, by excess TFA (11 mL). After 1 h, the mixture was evaporated to dryness and coevaporated (3×) with toluene to remove excess TFA. The crude residue was purified by chromatography (CH_2Cl_2/MeOH/NH_4OH, 78/19/3) to give **4e** (388 mg, 72%) which, upon treatment with HCl in Et_2O, gave the hydrochloride salt: mp 186 °C; ^{1}H NMR (DMSO-d_6) δ 2.99 (br s, 8H, CH_2), 5.26 (s, 4H, CH_2O), 6.84 (dd, 2H, J = 2.2, 8.8 Hz, 6,6′-CH), 7.19−7.28 (m, 8H, Ar), 7.36 (dd, 2H, J = 3.4, 5.4 Hz, Ar), 7.59 (dd, 2H, J = 3.6, 5 Hz, Ar), 8.08 (br s, 6H, NH_3^+), 10.85 (s, 2H, NH). Anal. ($C_{28}H_{32}Cl_2N_4O_2\cdot 0.5H_2O$) C, H, N, Cl.

General Method B: 2-[5-[[3-[[[3-(2-Aminoethyl)-1*H*-indol-5-yl]oxy]methyl]benzyl]oxy]-1*H*-indol-3-yl]ethylamine (4d). This procedure was used for the preparation of **4c**,**d**. A mixture of *N*-BOC serotonin **6** (1.0 g, 362 mmol), α,α′-dichloro-*m*-xylene (317 mg, 1.81 mmol), K_2CO_3 (1.25 g, 9.05 mmol), and KI (60 mg, 0.36 mmol) in MEK (25 mL) was refluxed for 16 h. The mixture was diluted with CH_2Cl_2, washed with H_2O, dried (Na_2SO_4), and concentrated. The crude product was purified by chromatography (CH_2Cl_2/acetone, 10/1) to give the bis-O-alkylated product (639 mg, 54%). This intermediate (620 mg, 0.94 mmol) was deprotected under the conditions described for general method A to give **4d** (320 mg, 75%) isolated as the hydrochloride salt: mp 222 °C; ^{1}H NMR (DMSO-d_6) δ 2.99 (br s, 8H, CH_2), 5.12 (s, 4H, CH_2O), 6.81 (dd, 2H, J = 2.0, 8.8 Hz, 6,6′-CH), 7.19−7.27 (m, 6H, Ar), 7.42 (s, 3H, Ar), 7.60 (s, 1H, Ar), 8.11 (br s, 6H, NH_3^+), 10.85 (s, 2H, NH). Anal. ($C_{28}H_{32}Cl_2N_4O_2\cdot 0.5H_2O$) C, H, N.

General Procedures for the Preparation of Compounds 4m,n. 2-[[3-(2-Aminoethyl)-1*H*-indol-5-yl]oxy]-*N*-[3-[[2-[[3-(2-aminoethyl)-1*H*-indol-5-yl]oxy]acetyl]amino]-propyl]acetamide (4m). A mixture of intermediate **10** (1.2 g, 3.58 mmol) and *N*-methylmorpholine (0.59 mL, 5.37 mmol) in CH_2Cl_2 (60 mL) was treated, at −15 °C and under nitrogen, by ethyl chloroformate (0.44 mL, 4.65 mmol). The reaction mixture was stirred at −15 °C for 0.5 h, and then 1,3-diaminopropane (0.15 mL, 1.79 mmol) was added. The reaction mixture was allowed to stir from −15 °C to room temperature for 1 h and then diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ and H_2O, dried (Na_2SO_4), and concentrated. The crude product was purified by chromatography (CH_2Cl_2/MeOH/NH_4OH, 95/4.5/0.5) to give the *N*-BOC-protected compound (815 mg, 64%). This intermediate was deprotected under the conditions described for general method A to give **4m** (332 mg, 57%) isolated as the hydrochloride salt: mp 260 °C; ^{1}H NMR (DMSO-d_6) δ 1.62 (q, 2H, J = 6.4 Hz, CH_2), 3.00−3.37 (m, 12H, CH_2), 4.48 (s, 4H, CH_2O), 6.85 (dd, 2H, J = 2.2, 8.7 Hz, 6,6′-CH), 7.14 (d, 2H, J = 2.1 Hz, 2,2′-CH), 7.21 (d, 2H, J = 2.2 Hz, 4,4′-CH), 7.28 (d, 2H, J = 8.7 Hz, 7,7′-CH), 8.08 (br s, 6H, NH_3^+), 8.26 (t, 2H, J = 5.8 Hz, NHCO), 10.90 (d, 2H, J = 2.1 Hz, NH). Anal. ($C_{27}H_{36}Cl_2N_6O_4\cdot 1H_2O$) C, H, N, Cl.

Preparation of 2-[5-[2-[*N*-(*tert*-Butoxycarbonyl)amino]-ethoxy]-1*H*-indol-3-yl]ethylamine (12). A mixture of *N*-BOC serotonin **6** (18.0 g, 65.1 mmol), K_2CO_3 (22.5 g, 162.7 mmol), and KI (1.1 g, 6.51 mmol) in MEK (240 mL) was treated at room temperature with bromoacetonitrile (5.4 mL, 78.1 mmol). The mixture was refluxed for 31 h and then diluted with EtOAc, washed with NaOH (2 N), H_2O, and saturated NaCl, dried (Na_2SO_4), and concentrated. The crude product was purified by chromatography (CH_2Cl_2/acetone, 20/1) to give the pure intermediate (13.9 g, 68%). This intermediate (5.0 g, 15.8 mmol), in solution in anhydrous THF and under nitrogen, was treated dropwise, at 0 °C, by $LiAlH_4$ (1 M in THF) (39.6 mL, 39.5 mmol). The reaction mixture was allowed to stir from 0 °C to room temperature for 1 h, and then excess $LiAlH_4$ was removed by treatment with "wet" Na_2SO_4. The mixture was filtered through Celite, and the filtrate was concentrated. The resulting oil was purified by chromatography (CH_2Cl_2/MeOH/NH_4OH, 90/9.5/0.5) to give **12** (3.8 g, 74%): ^{1}H NMR (DMSO-d_6) δ 1.37 (s, 9H, tBu), 2.73 (t, 2H, J = 7.8 Hz, CH_2), 2.86 (t, 2H, J = 5.7 Hz, CH_2), 3.11−3.20 (m, 2H, CH_2), 3.90 (t, 2H, J = 5.7 Hz, CH_2O), 6.71 (dd, 1H, J = 2.3, 8.7 Hz, 6-CH), 6.88 (t, 1H, J = 8.0 Hz, NHBOC), 6.98 (d, 1H, J = 2 Hz, 2-CH), 7.06 (d, 1H, J = 2.3 Hz, 4-CH), 7.19 (d, 1H, J = 8.7 Hz, 7-CH), 10.65 (d, 1H, J = 2 Hz, 2-CH); MS m/z 320 (MH^+).

Preparation of 2-[[3-(2-Aminoethyl)-1*H*-indol-5-yl]oxy]-*N*-[2-[[3-(2-aminoethyl)-1*H*-indol-5-yl]oxy]ethyl]acetamide (4k). Compound **4k** was prepared from intermediate **10** (300 mg, 0.897 mmol) and intermediate **12** (286 mg, 0.897 mmol) following the conditions described for the preparation of compound **4m**. The desired product was purified by chromatography (CH_2Cl_2/MeOH/NH_4OH, 75/20/5) to give pure **4k** (355 mg, 91% for both steps) isolated as the hydrochloride salt: mp 168 °C; ^{1}H NMR (DMSO-d_6) δ 3.01 (br s, 8H, CH_2), 3.50−3.60 (m, 2H, CH_2), 4.07 (t, 2H, J = 6.0 Hz, CH_2), 4.52 (s, 2H, CH_2O), 6.75 (dd, 1H, J = 2.2, 8.7 Hz, Ar), 6.86 (dd, 1H, J = 2.3, 8.8 Hz, Ar), 7.13 (d, 1H, J = 2.2 Hz, Ar), 7.17 (d, 1H, J = 2.3 Hz, Ar), 7.19−7.31 (m, 4H, Ar), 8.1 (br s, 6H, NH_3^+), 8.37 (t, 1H, J = 5.6 Hz, NHCO), 10.86 (d, 1H, J = 1.9 Hz, NH), 10.92 (d, 1H, J = 1.8 Hz, NH). Anal. ($C_{24}H_{31}Cl_2N_5O_3\cdot 1.1H_2O$) C, H, N.

Preparation of 1,3-Bis[2-[[3-(2-aminoethyl)-1*H*-indol-5-yl]oxy]ethyl]urea (4l). A solution of intermediate **12** (650 mg, 2.03 mmol) and Et_3N (0.28 mL, 2.03 mmol) in anhydrous CH_2Cl_2 (11 mL) was treated, dropwise at 0 °C and under nitrogen, with a solution of triphosgene (100 mg, 0.338 mmol) in CH_2Cl_2 (2 mL). The mixture was stirred from 0 °C to room temperature for 1.5 h and then diluted with CH_2Cl_2, washed with H_2O and saturated NaCl, dried (Na_2SO_4), and concentrated. The crude product was purified by chromatography (CH_2Cl_2/MeOH/NH_4OH, 95/4.5/0.5) to give the *N*-BOC-pro-

tected compound (634 mg, 94%). This intermediate was deprotected under the conditions described for general method A to give **4l** (338 mg, 64%) isolated as the hydrochloride salt: mp 140 °C; ^{1}H NMR (DMSO-d_6) δ 2.98 (br s, 8H, CH_2), 3.40 (t, 4H, J = 5.0 Hz, CH_2), 3.93−3.99 (m, 4H, CH_2), 6.74 (dd, 2H, J = 2.1, 8.7 Hz, 6,6′-CH), 7.10 (d, 2H, J = 2.0 Hz, 2,2′-CH), 7.18 (d, 2H, J = 2.1 Hz, 4,4′-CH), 7.24 (d, 2H, J = 8.7 Hz, 7,7′-CH), 8.08 (br s, 6H, NH_3^+), 10.84 (d, 2H, J = 2.0 Hz, NH). Anal. ($C_{25}H_{34}Cl_2N_6O_3 \cdot 1H_2O$) C, H, N, Cl.

Preparation of 4,4′-Bis[[[[6-[[3-(2-aminoethyl)-1*H*-indol-5-yl]oxy]hexyl]amino]sulfonyl]-1,1′-biphenyl (4o). A solution of intermediate **13** (1.0 g, 2.66 mmol) and Et_3N (0.56 mL, 3.99 mmol) in anhydrous CH_2Cl_2 (15 mL) was treated at 0 °C with 4,4′-biphenyldisulfonyl chloride (470 mg, 1.33 mmol). The mixture was stirred from 0 °C to room temperature for 1 h, diluted with CH_2Cl_2, washed with H_2O and saturated NaCl, dried (Na_2SO_4), and concentrated. The crude oil was purified by chromatography (CH_2Cl_2/MeOH, 30/1) to give the *N*-BOC-protected product (1.1 g, 80%). This intermediate (600 mg, 0.583 mmol) was deprotected under the conditions described for general method A to give **4o** (396 mg, 73%) isolated as the hydrochloride salt: mp 140 °C; ^{1}H NMR (DMSO-d_6) δ 1.33 (br s, 12H, CH_2), 1.60−1.70 (m, 4H, CH_2), 2.78 (m, 4H, CH_2), 2.90−3.00 (m, 8H, CH_2), 3.91 (t, 4H, J = 6.3 Hz, CH_2O), 6.69 (dd, 2H, J = 2.2, 8.6 Hz, 6,6′-CH), 7.04 (d, 2H, J = 2.0 Hz, 2,2′-CH), 7.16 (d, 2H, J = 2.2 Hz, 4,4′-CH), 7.22 (d, 2H, J = 8.6 Hz, 7,7′-CH), 7.73−7.97 (m, 14H, Ar, NH_3^+), 10.80 (d, 2H, J = 2.0 Hz, NH). Anal. ($C_{44}H_{56}N_6O_6S_2 \cdot 2HCl \cdot 1.5H_2O \cdot 0.3EtOH$) C, H, N, Cl.

Preparation of 2-[5-[2-[[3-(2-Aminoethyl)-1*H*-indol-5-yl]oxy]ethoxy]-1*H*-indol-3-yl]ethylamine (4a). A solution of compound **5** (1.5 g, 5.43 mmol) in dry DMF (15 mL) was treated at room temperature by NaH (60% in oil) (217 mg, 5.43 mmol) for 1 h. Compound **7** (1.8 g, 5.43 mmol) was added, and the mixture was heated at 80 °C overnight. The reaction mixture was diluted with EtOAc, washed with H_2O and saturated NaCl, dried (Na_2SO_4), and concentrated. The crude product was purified by chromatography (CH_2Cl_2/MeOH/NH_4-OH, 97.75/2/0.25 to 96.5/3/0.5) to give the *N*-BOC-protected product (1.7 g, 54%). This intermediate (8.61 mg, 1.49 mmol) was then deprotected to give **4a** (275 mg, 49%) isolated as the hydrocholoride salt: mp 270 °C; ^{1}H NMR (DMSO-d_6) δ 3.00 (br s, 8H, CH_2), 4.34 (s, 4H, CH_2), 6.77 (dd, 2H, J = 2.1, 8.7 Hz, 6,6′-CH), 7.18−7.29 (m, 6H, Ar), 8.14 (br s, 6H, NH_3^+), 10.86 (d, 2H, J = 2.0 Hz, NH). Anal. ($C_{22}H_{28}Cl_2N_4O_2 \cdot 1H_2O$) C, H, N, Cl.

Preparation of 2-[5-[2-[4-[2-[[3-(2-Aminoethyl)-1*H*-indol-5-yl]oxy]ethyl]piperazin-1-yl]ethoxy]-1*H*-indol-3-yl]-ethylamine (4i). A mixture of compound **7** (1.5 g, 4.43 mmol), K_2CO_3 (1.8 g, 13.2 mmol), KI (73 mg, 0.44 mmol), and piperazine (191 mg, 2.21 mmol) in DMF (4 mL) was heated at 90 °C for 48 h. The reaction mixture was then diluted with EtOAC, washed with H_2O and NaCl, dried (Na_2SO_4), and concentrated. The crude product was purified by chromatography (CH_2Cl_2/MeOH/NH_4OH, 95/4.5/0.5) to give the *N*-BOC-protected product (986 mg, 64%) which, after deprotection, afforded **4i** (638 mg, 91%) isolated as the hydrochloride salt: mp 182 °C; ^{1}H NMR (DMSO-d_6) δ 3.02 (br s, 8H, CH_2), 3.65−3.86 (m, 12H, CH_2), 4.47 (br s, 4H, CH_2O), 6.85 (dd, 2H, J = 2.1, 8.6 Hz, 6,6′-CH), 7.22−7.32 (m, 6H, Ar), 8.21 (br s, 6H, NH_3^+), 10.95 (d, 2H, J = 2.0 Hz, NH). Anal. ($C_{28}H_{42}Cl_4N_6O_2 \cdot 2.1H_2O$) C, H, N.

Preparation of 2-[[3-(2-Aminoethyl)-1*H*-indol-5-yl]oxy]-1-piperazin-1-ylethanone (11). A mixture of compound **10** (2.0 g, 5.98 mmol) and *N*-methylmorpholine (0.98 mL, 8.97 mmol) in CH_2Cl_2 (100 mL) was treated, at −15 °C and under nitrogen, with ethyl chloroformate (0.74 mL, 7.77 mmol). The reaction mixture was stirred at −15 °C for 0.5 h, and then benzylpiperazine (2.6 mL, 15 mmol) was added. The reaction mixture was allowed to stir from −15 °C to room temperature for 1 h and then diluted with CH_2Cl_2, washed with $NaHCO_3$ and H_2O, dried ($NaSO_4$), and concentrated. The crude product was purified by chromatography (CH_2Cl_2/MeOH/NH_4OH, 95/4.5/0.5) to give the pure intermediate (2.66 g, 90%). This product was hydrogenated over Pd/C (10%) (∼250 mg, 0.27 mmol) in MeOH (160 mL), under 1 atm of H_2, at room temperature for 4 days. The mixture was filtered through Celite, and the filtrate was concentrated. The crude product was purified by chromatography (CH_2Cl_2/MeOH/NH_4OH, 90/9.5/0.5) to give **11** (1.68 g, 77%).

Preparation of 2-[[3-(2-Aminoethyl)-1*H*-indol-5-yl]oxy]-1-[4-[2-[[3-(2-aminoethyl)-1*H*-indol-5-yl]oxy]ethyl]piperazin-1-yl]ethanone (4h). A mixture of compound **11** (600 mg, 1.49 mmol), compound **8** (505 mg, 1.49 mmol), K_2CO_3 (618 mg, 4.47 mmol), and KI (25 mg, 0.15 mmol) in DMF (2 mL) was heated at 90 °C for 48 h. The mixture was diluted with EtOAC, washed with H_2O and saturated NaCl, dried (Na_2SO_4), and concentrated. The crude product was purified by chromatography (CH_2Cl_2/MeOH/NH_4OH, 95/4.5/0.5) to give the *N*-BOC-protected product (959 mg, 91%) which after deprotection afforded compound **4h** (295 mg, 44%) isolated as the hydrochloride salt: mp 187 °C; ^{1}H NMR (DMSO-d_6) δ 3.01 (br s, 8H, CH_2), 3.20−3.48 (m, 8H, CH_2), 4.20−4.47 (m, 4H, CH_2), 4.87 (s, 2H, OCH_2CO), 6.76−6.85 (m, 2H, 6,6′-CH), 7.19−7.31 (m, 6H, Ar), 8.17 (br s, 6H, NH_3^+), 10.86 (d, 1H, J = 2.0 Hz, NH), 10.91 (d, 1H, J = 2.0 Hz, NH), 11.65 (br s, 1H, NH^+). Anal. ($C_{28}H_{36}N_6O_3 \cdot 3.4HCl \cdot 1.3H_2O$) C, H, N, Cl.

Acknowledgment. The authors wish to thank Elisabeth Dupeyron for preparing the manuscript.

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JM960552L