Insertion of an Aspartic Acid Moiety into Cyclic Pseudopeptides: Synthesis and Biological Characterization of Potent Antagonists for the Human Tachykinin NK-2 Receptor

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Received April 27, 2004

A new series of monocyclic pseudopeptide tachykinin NK-2 receptor antagonists has been derived from the lead compound MEN11558. A synthesis for these molecules sharing the same intermediate was designed and performed. The replacement of the succinic moiety with an aspartic acid and the functionalization of its amino group with a wide variety of substituents led to very potent and selective NK-2 antagonists. Best results were obtained through the insertion in position 12 of an amino group with *R* configuration, linked by a short spacer to a saturated nitrogen heterocycle (morpholine, piperidine, or piperazine). The study led to compounds **54** and **57**, endowed with high in vivo potency at very low doses and long duration of action in animal models of bronchoconstriction. In particular **54** and **57** completely inhibited NK-2 agonist induced bronchoconstriction in guinea pig after intratracheal administration at subnanomolar doses (ED_{50} = 0.27 nmol/kg and 0.15 nmol/kg, respectively).

Introduction

The tachykinins substance P (SP), neurokinin A (NKA), and neurokinin B (NKB) form a family of peptide neurotransmitters that are widely distributed in the mammalian peripheral and central nervous system and produce their biological actions by activating three distinct receptor types, respectively termed NK-1, NK-2, and NK-3.[1] NKA exerts its biological effects mainly by activation of the tachykinin NK-2 receptor. The human NK-2 receptor has been identified and validated as a suitable target for the development of novel drugs to be used for the treatment of a number of diseases in the respiratory, gastrointestinal, and genitourinary tract and in the CNS.[2] In particular, human NK-2 receptor antagonists are considered potential candidates for the treatment of asthma, bronchial hyperreactivity, irritable bowel syndrome, cystitis, or depression.[3] In our previous studies[4] MEN11558, a monocyclic pseudopeptide, was identified as a potential new lead for a class of antagonists with simpler structures with respect to nepadutant (MEN11420, Figure 1),[5] a bicyclic hexapeptide currently in phase II clinical trials. It was clear from previous structural studies[4] that MEN11558 maintains its biological activity thanks to the fact that the Trp-Phe fragment is adjusted in a type I β-turn by the appropriate cyclic linker. The correct *R* configuration of the carbon atom in position 8 (Figure 2), bearing the benzyl substituent on the diamine moiety, was also defined in previously reported investigations.[4] In the current contribution we present the results of the replacement of the succinic portion (positions 12 and 13 of the structure of Figure 2) with an aspartic moiety, which allowed us to obtain new potent antagonists with enhanced physicochemical properties.

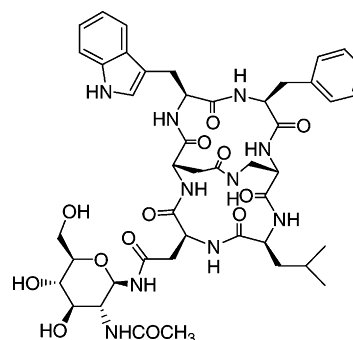

Figure 1. The structure of nepadutant (MEN 11420).

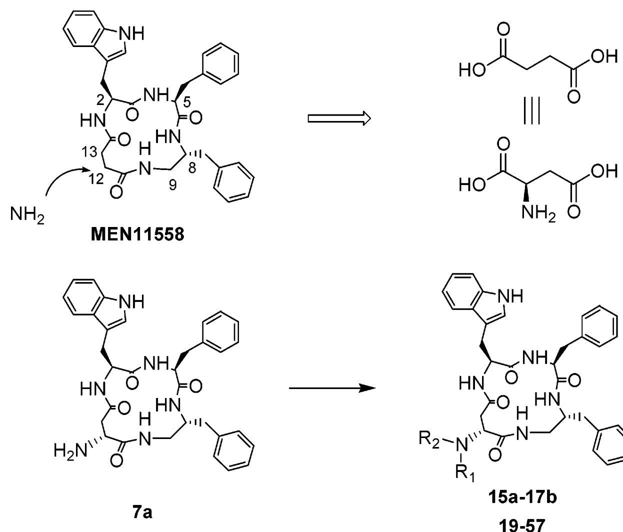

Figure 2. Insertion and further modifications of an amino moiety on the initial lead MEN11558.

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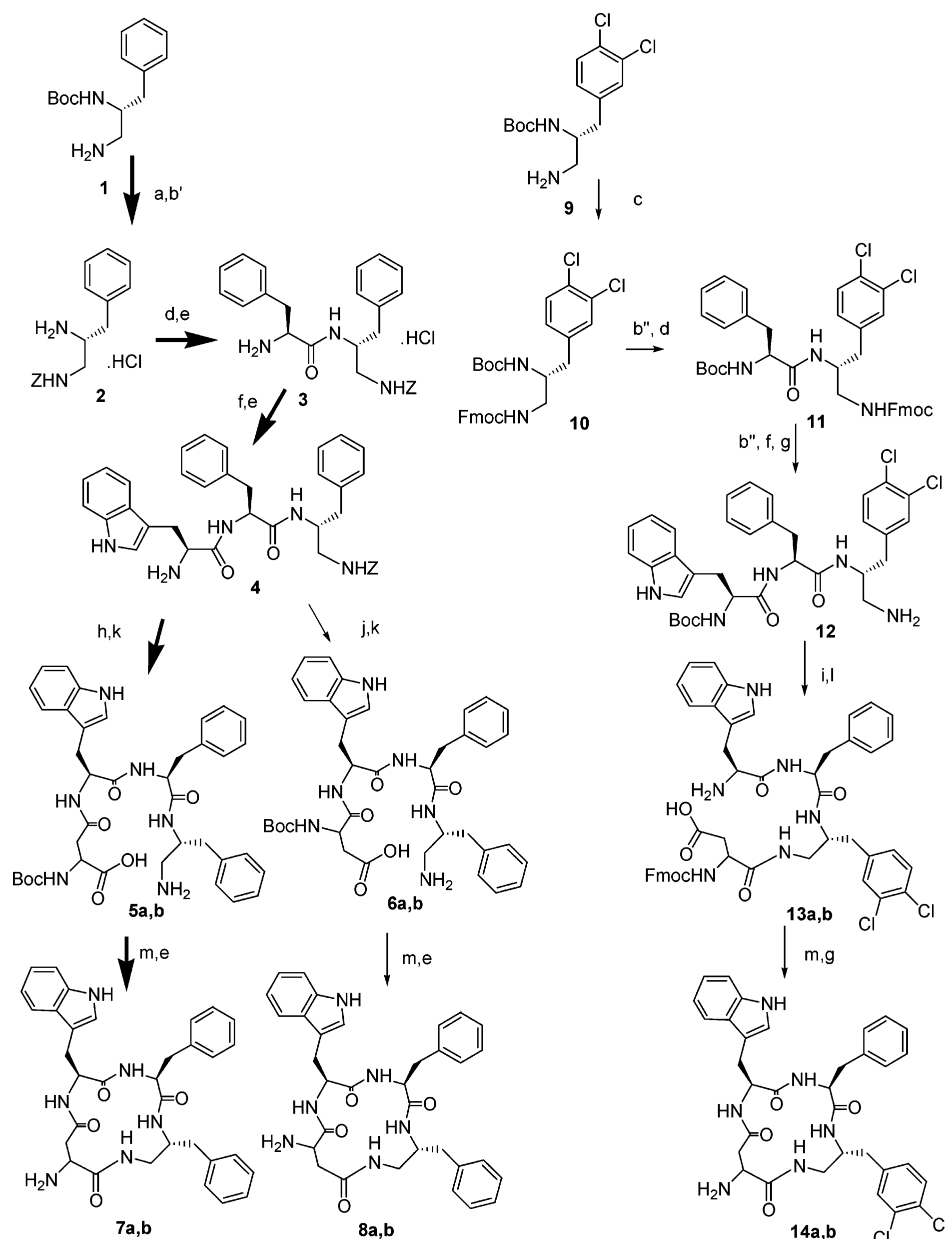
10.1021/jm040832y CCC: $27.50 © 2004 American Chemical Society
Published on Web 12/07/2004

Scheme 1[a]



[a] Reagents: (a) Benzyl chloroformate, NEt_3, THF, 0 °C (75−80%); (b′) HCl in dioxane, MeOH, room temperature (95%); (b′′) CF_3COOH, CH_2Cl_2, 0 °C → room temperature (80%); (c) FmocOSu, THF (70−75%); (d) Boc-Phe-OH, HOBt, EDC·HCl, NEt_3, CH_3CN (or DMF), 0 °C → room temperature (85%); (e) HCl in dioxane, MeOH, 5 °C → room temperature (90−95%); (f) Boc-Trp-OH, HOBt, EDC·HCl, NEt_3, DMF, 0 °C → room temperature (90%); (g) Et_2NH, DMF, room temperature; (h) Boc-Asp(OH)-OBzl (D or L),[b] HOBt, EDC·HCl, NEt_3, DMF, room temperature (70%); (i) Fmoc-Asp-(OtBu)-OH (D or L),[b] HOBt, EDC·HCl, DMF (>95%); (j) Boc-Asp(OBzl)-OH (D or L),[b] HOBt, EDC·HCl, NEt_3, DMF, room temperature (70%); (k) H_2, Pd/C, DMF, H_2O, room temperature (93%); (l) ethanedithiol, CF_3COOH, CH_2Cl_2, 0 °C → room temperature (80%); (m) HOBt, EDC·HCl, DMF, room temperature (80%, 62%). [b] The D or L amino acid derivative is used to obtain compounds of the **a** or **b** series, respectively.

There were several advantages in selecting the aspartic acid from the chiral pool for our purposes: it is easily available in both enantiomers; with respect to MEN11558, it guarantees the same number of terms in the resulting cycle while introducing a new functionalization; the use of differentially protected derivatives (at the α- and β-carboxy groups) allows the insertion of amino groups in both positions 12 and 13 of the original molecule; primary or substituted amines were expected to increase water solubility of the final products. Furthermore, the QSAR model recently described[6] by our group suggested that modifications in that part of the lead molecule should have been well tolerated by the receptor. The *R* configuration of the amino acid was initially chosen to minimize the risk of recognition by hydrolytic enzymes (peptidases), but during our investigation we found that the derivatives of (*R*)-aspartic acid were also more effective than the derivatives of (*S*)-aspartic acid.

In the first part of the present work a small group of unsubstituted or substituted derivatives was syntesized to check the potential of the new class and to confirm the validity of the new lead **7a** in comparison to its epimer **7b** and its regioisomers **8a** and **8b** (Scheme 1). It was immediately clear that introducing a hydrophilic group in the succinic linker to enhance physicochemical

properties did not negatively affect the binding affinity, while some of the functionalizations led to a substantial increment of the NK-2 receptor antagonist activity. In a further development of the SAR study a larger space of substituents was considered to improve the pharmacological and physicochemical profile of these compounds.

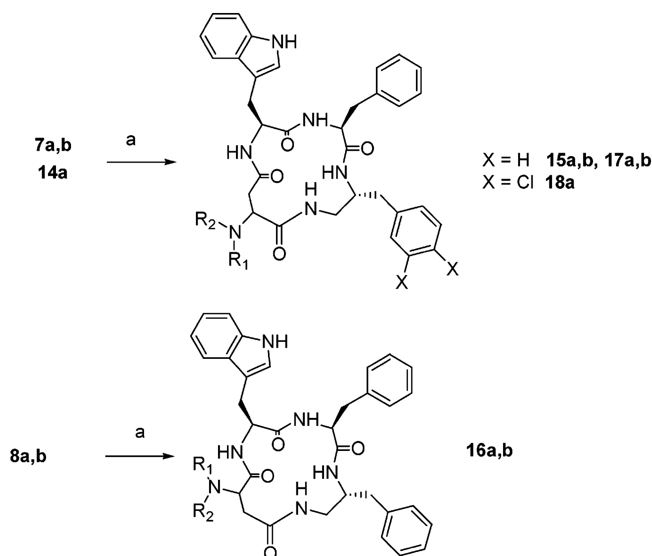
This study eventually led to selection of **54** and **57**, whose in vitro and in vivo pharmacological profile is discussed below.

Chemistry. The synthesis of the new lead compound **7a** and that of its analogues bearing the amino group in a different configuration (**7b**) or in a different position (on C13, **8a**,**b**), together with the preparation of dichlorobenzyl derivatives **14a**,**b**, are depicted in Scheme 1.

The starting monoprotected diamine **1**[7] was opportunely transformed to a derivative suitably protected on the distal amino group, **2**, and subsequently coupled with Boc protected D-phenylalanine. Deprotection of Boc under standard conditions gave amine **3**. The corresponding monoprotected diamine **9** was subjected to a similar procedure, using a Fmoc protecting strategy. During the synthesis all the intermediates containing a free amino group in the presence of a Fmoc protected amine were isolated or conveniently stored to avoid partial deprotection. As presented in Scheme 1, **9** was initially transformed to the diprotected derivative **10**, and then deprotection of Boc under standard conditions and immediate coupling with Boc-L-phenylalanine were performed to give **11**, once again as a diprotected intermediate. The second coupling step to introduce Boc-L-tryptophan was followed in the first case (left) by the Boc deprotection to obtain **4** while in the synthesis of the dichloro derivatives (right) it was preceded by the Boc-deprotection and followed by the Fmoc deprotection to **12**. The last synthetic strategy was chosen due to the impossibility to perform any subsequent catalytic hydrogenation to remove benzyloxycarbonyl or benzyl protective groups without the concomitant partial loss of chloride from the aromatic ring of the diamine portion. Following the synthetic pathway depicted in the left part of Scheme 1, **4** was subsequently acylated with diprotected aspartic acid, the regioselectivity being defined by the position of the free carboxylic group. The reaction of the two enantiomers of Boc-Asp-(OH)-OBzl with **4** under standard activating conditions and the double deprotection of the benzyl ester and the benzylcarbamate by hydrogenolysis gave **5a** (from the D-Asp derivative) and **5b** (from the L-Asp derivative), respectively. Under the same conditions the enantiomers of Boc-Asp-(OBzl)-OH gave with comparable yields the regioisomers **6a** (from the D-Asp derivative) and **6b** (from the L-Asp derivative). Intermediate **12** (right) was on the contrary acylated under standard conditions only with the two enantiopure Fmoc-Asp-(OtBu)-OH and subsequently doubly deprotected to **13a**,**b** with TFA and ethanedithiol. The final cyclization step of the obtained zwitterions was performed under controlled high dilution conditions to minimize the parasite intermolecular reaction to oligomeric compounds, the threshold for the concentration of acyclic reactant being 0.01 M in DMF.

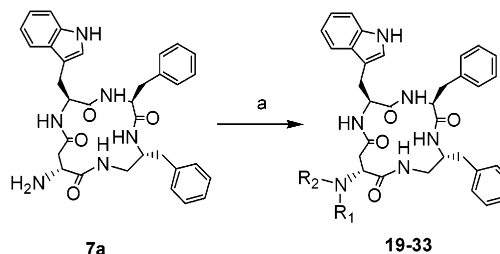
The amino group of **7a**,**b**, **14a**, **8a**,**b** was mono- or dialkylated through reductive amination as shown in Scheme 2, using sodium cyanoborohydride and acetic acid to obtain products **15a**−**18a**.

Scheme 2[a]



[a] Reagents: (a) appropriate carbonyl compound, $Na(CN)BH_3$, AcOH, MeOH, room temperature.

Scheme 3[a]



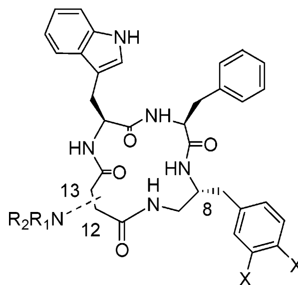
[a] Reagents: (a) appropriate carbonyl compound, $Na(CN)BH_3$, AcOH, MeOH, room temperature.

To enlarge the structure−activity relationship study about the substitution on the amino group, other alkylations employing aldehydes or ketones containing optionally protected additional functional groups were performed on the lead compound **7a** (Scheme 3), giving compounds listed in Table 2.

A wide range of carbohydrates (Scheme 4 and Table 3) was introduced in order to further increase the water solubility of the final products and to evaluate, in this series, the effect of the insertion of a sugar moiety which, in the case of the previously described bicyclic peptide nepadutant, gave a compound with excellent pharmacological properties. Three general methods were used for the polyhydroxylated substituents: method A, to attach a linker to the amino group in position 12 of the lead **7a** via an acylation, then create the linkage with the new hydrophilic functional group through another acylation step (see **34, 35**); method B, to directly attach the selected sugar to the amine in position 12 through its aldehydic function in a reductive medium (see **36**−**40**); method C, to directly acylate the amino functionality of **7a** with an oxidized sugar (**41**).

The last part of the present synthetic work regards target compounds **48**−**57**, obtained through the acylation of **7a** with amino acids coming from a nucleophilic substitution on bromoacetic acid. As described in Scheme 5, the amino nucleophile was added to the bromoacetic

Table 1. Structures of Compounds **7**, **8**, and **14−18** and Affinities for the Tachykinin NK-2 Receptor Evaluated in Binding Experiments (pK_i Values) and Functional in Vitro Experiments (pK_B Values)



entry	NR_1R_2 position	R_1	R_2	X	NK-2 activity[e] pK_i[a] (hNK-2)	pK_B[b] (RPA)	pK_B[c] (RUB)
MEN11558[d]				H	8.7 ± 0.1	7.6 ± 0.1	8.3 ± 0.1
MEN11690[d]				Cl	10.0 ± 0.1	8.1 ± 0.1	8.1 ± 0.1
7a	12-(*R*)	H	H	H	8.7 ± 0.1	7.9 ± 0.2	
7b	12-(*S*)	H	H	H	8.5 ± 0.2	7.5 ± 0.2	
8a	13-(*R*)	H	H	H	7.8 ± 0.1	7.2 ± 0.2	
8b	13-(*S*)	H	H	H	8.5 ± 0.2	7.7 ± 0.2	
14a	12-(*R*)	H	H	Cl	9.8 ± 0.2	8.1 ± 0.2	
14b	12-(*S*)	H	H	Cl	9.7 ± 0.1	8.6 ± 0.2	8.9 ± 0.2
15a	12-(*R*)	H	4-tetrahydropyranyl	H	10.2 ± 0.1	9.2 ± 0.2	9.1 ± 0.2
15b	12-(*S*)	H	4-tetrahydropyranyl	H	9.0 ± 0.2	8.6 ± 0.2	
16a	13-(*R*)	H	4-tetrahydropyranyl	H	8.1 ± 0.1		7.4 ± 0.2
16b	13-(*S*)	H	4-tetrahydropyranyl	H	8.2 ± 0.2		7.8 ± 0.2
17a	12-(*R*)	$-CH_2CH_2OCH_2CH_2-$		H	9.8 ± 0.2	8.9 ± 0.2	9.2 ± 0.2
17b	12-(*S*)	$-CH_2CH_2OCH_2CH_2-$		H	9.1 ± 0.2	8.5 ± 0.3	8.9 ± 0.2
18a	12-(*R*)	$-CH_2CH_2OCH_2CH_2-$		Cl	9.6 ± 0.1	8.4 ± 0.2	8.0 ± 0.2

[a] pK_i = −log K_i. Affinities for the human NK-2 receptor estimated against [^{125}I]neurokinin A in radioligand binding experiments. [b] pK_B = −log K_B. Antagonist affinities of compounds for rabbit NK-2 receptor estimated against neurokinin A in the rabbit isolated pulmonary artery (RPA). [c] Antagonist affinities of compounds for rat NK-2 receptor estimated against [*β*-Ala^8]NKA(4−10) in the rat isolated urinary bladder (RUB). [d] Data from ref 4. [e] Each value is the mean ± SEM of 4−8 determinations.

Table 2. In Vitro Activity of Compounds **19−33** Evaluated in Binding Experiments (pK_i Values) and Functional Experiments (pK_B Values)

entry	R_1	R_2	NK-2 activity[e] pK_i[a] (hNK-2)	pK_B[b] (RUB)
MEN11558[c]			8.7 ± 0.1	8.3 ± 0.1
7a[c]			8.7 ± 0.1	7.9 ± 0.2[d]
17a[c]	$-CH_2CH_2OCH_2CH_2-$		9.8 ± 0.2	9.2 ± 0.2
19	$-(CH_2)_5-$		8.8 ± 0.1	8.2[d] ± 0.2
20	$-CH_2CH_2CH(NH_2)CH_2CH_2-$		8.3 ± 0.1	8.5 ± 0.1
21	$-CH_2CH_2NHCH_2CH_2-$		8.8 ± 0.1	7.7[d] ± 0.2
22	$-CH_2CH_2N(CH_3)CH_2CH_2-$		9.4 ± 0.1	9.2 ± 0.1
23	$-CH_2CH_2N(COCH_3)CH_2CH_2-$		9.9 ± 0.1	9.6 ± 0.2
24	$-CH_2CH_2N(SO_2CH_3)CH_2CH_2-$		8.5 ± 0.1	9.3 ± 0.2
25	$-CH_2CH_2CH$(1-piperidin)CH_2CH_2-		9.7 ± 0.2	8.9 ± 0.2
26	$-CH_2CH_2CH$(4-morpholin)CH_2CH_2-		9.7 ± 0.1	8.9 ± 0.1
15a[c]	H	4-tetrahydropyranyl	10.2 ± 0.1	9.1 ± 0.2
27	H	4-tetrahydrothiopyranyl	10.0 ± 0.1	7.8 ± 0.1
28	H	4-tetrahydrothiopyranyl-1-oxide	10.6 ± 0.1	9.1 ± 0.2
29	H	4-tetrahydrothiopyranyl-1,1-dioxide	9.8 ± 0.1	9.3 ± 0.2
30	CH_3	4-tetrahydropyranyl	9.8 ± 0.2	8.8 ± 0.2
31	H	4-piperidinyl-1-methanesulfonyl	10.2 ± 0.1	9.5 ± 0.2
32	H	4-piperidinyl-1-sulfonic acid amide	10.3 ± 0.1	9.5 ± 0.2
33	H	4-piperidinyl-1-methyl	9.7 ± 0.1	9.0 ± 0.2

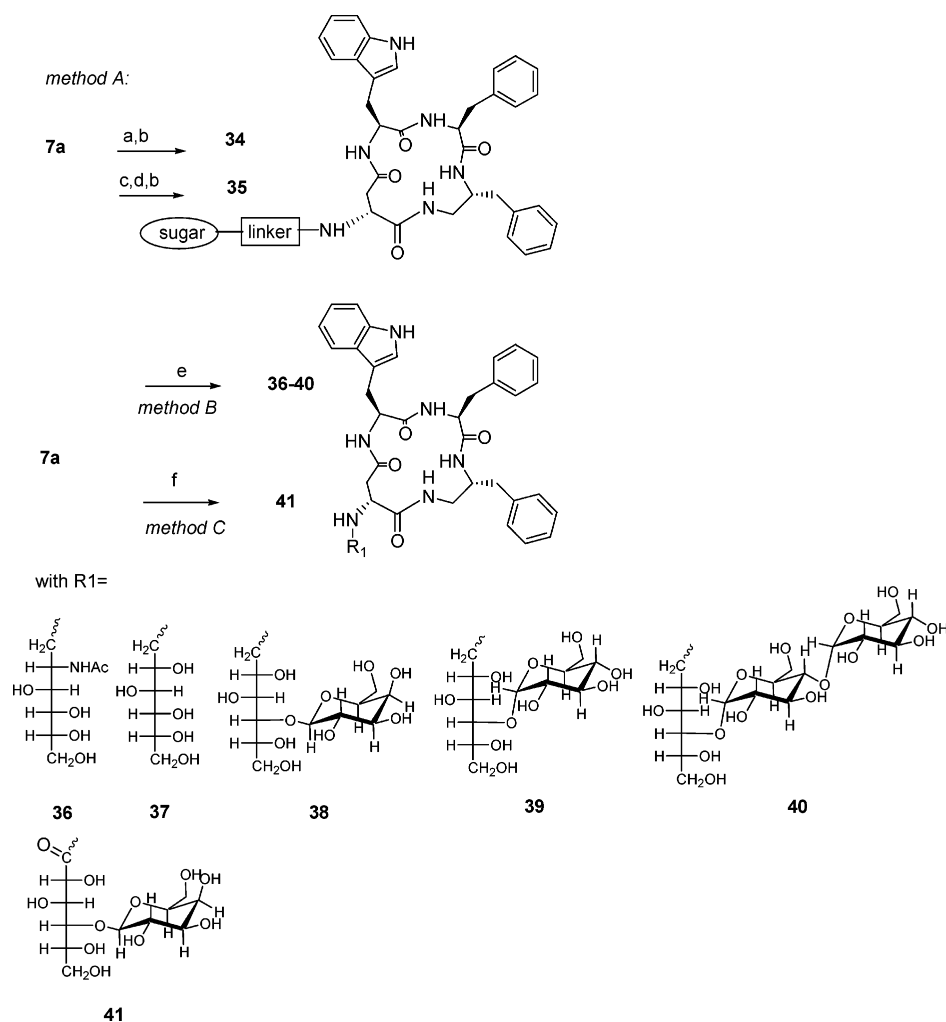
[a] See corresponding footnote in Table 1. [b] See footnote *c* in Table 1. [c] Data from Table 1. [d] pK_B measured on RPA, see footnote *b* in Table 1. [e] Each value is the mean ± SEM of 4−8 determinations.

tert-butyl ester in the presence of triethylamine in THF, and the resulting amino ester was hydrolyzed under acid catalysis to give the corresponding amino acid. In the particular case of **54** the aminosulfonylpiperazine nucleophile was obtained by reaction of the freshly prepared sulfamoyl chloride[8] with *N*-benzyl protected piperazine in triethylamine and THF and further hydrogenation catalyzed by Pd/C in methanol. Finally the coupling reactions with **7a** were performed through activation of the carboxylic function with HOBt, EDC·HCl, usually in DMF as a solvent.

Results and Discussions

The binding affinity at the human tachykinin NK-2 receptor together with the functional activity on isolated tissues was measured for all the derivatives according

Scheme 4[a]



[a] Reagents: (a) succinic anhydride, CH_3CN, DMF; (b) starting sugar moiety (see Table 3), HOBt, EDC·HCl, DMF; (c) Ac-Asp-(O*t*Bu)-OH, HOBt, EDC.HCl, DMF; (d) TFA, CH_2Cl_2; (e) starting sugar moiety (see Table 3), CH_3COOH, $NaCNBH_3$, MeOH; (f) lactobionic acid, PyBOP, diisopropylethylamine, DMF.

Table 3. In Vitro Activity of Compounds **34**−**41** Evaluated in Binding Experiments (pK_i Values) and Functional Experiments (pK_B Values)

				NK-2 activity[g]	
entry	spacer	starting sugar moiety	method	pK_i[a] (hNK-2)	pK_B[b] (RUB)
34	succinic acid	2-acetamido-2-deoxy-β-D-glucopyranosyl amine	A	8.8 ± 0.1	7.6 ± 0.2
35	*N*-acetyl-aspartic acid	2-acetamido-2-deoxy-β-D-glucopyranosyl amine	A	7.6 ± 0.2	7.7 ± 0.2
36		2-acetamido-2-deoxy-β-D-glucose	B	8.7 ± 0.1	8.1 ± 0.2
37		D-(+)-glucose	B	9.4 ± 0.1	8.8 ± 0.1
38		D-(+)-lactose[c]	B	8.9 ± 0.1	8.2 ± 0.2
39		D-(+)-maltose[d]	B	9.2 ± 0.1	8.5 ± 0.2
40		maltotriose[e]	B	9.3 ± 0.1	8.5 ± 0.2
41		lactobionic acid[f]	C (A)	8.4 ± 0.2	8.7 ± 0.1

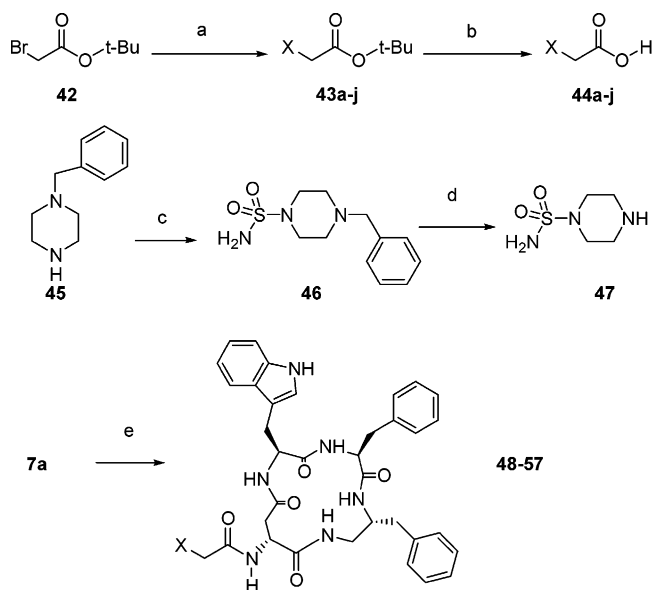
[a] See corresponding footnote in Table 1. [b] See footnote *c* in Table 1. [c] 4-*O*-β-D-galactopyranosyl-D-glucose. [d] 4-*O*-α-D-glucopyranosyl-D-glucose. [e] *O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-D-glucose. [f] 4-*O*-β-D-galactopyranosyl-D-gluconic acid. [g] Each value is the mean ± SEM of 4−8 determinations.

to experimental methodologies reported in the Experimental Section. Results are listed in Tables 1−4.

Regarding functional assays, after initial testing of the early compounds in the rabbit pulmonary artery (RPA) test, the more reliable and convenient RUB (rat urinary bladder) test was chosen as a functional model for the NK-2 receptor, once results had demonstrated the equivalence between data obtained in the RPA vs data obtained in the RUB (see Table 1).

In Table 1 the in vitro pharmacological profiles of the two reference compounds MEN11558 and MEN11690[4] are compared with the amino substituted derivatives. The introduction of the amino moiety in the *R* configuration in position 12 of MEN11558 gave product **7a**, which luckily showed comparable activity with respect to the starting lead molecule while bearing an amino group suitable to further functionalization. To confirm the opportunity of the selection of **7a** as a new lead, the

Scheme 5[a]



[a] Reagents: (a) amine (see corresponding X group in Table 4), NEt_3, THF, 65 °C (>98%); (b) TFA, CH_2Cl_2, room temperature (>98%); (c) sulfamoyl chloride, THF, 0 °C → room temperature (26%); (d) H_2, Pd/C 10%, MeOH, H_2O (76%); (e) **44a**–**j**, HOBt, ECD·HCl, DMF, room temperature (20–50%).

Table 4. In Vitro Activity of Compounds **48**–**57** Evaluated in Binding Experiments (pK_i Values) and Functional Experiments (pK_B Values)

Entry	X	NK-2 activity[c]	
		pK_i[(a)](hNK-2)	pK_B[(b)] (RUB)
48		9.5±0.1	8.5±0.1
49		9.9±0.1	9.1±0.2
50		9.5±0.1	8.9±0.2
51		8.8±0.1	8.5±0.2
52		9.3±0.1	9.2±0.2
53		9.5±0.1	8.7±0.2
54		**10.0**±0.1	**9.5**±0.1
55		9.4±0.1	9.2±0.2
56		9.6±0.1	9.0±0.2
57		**9.8**±0.1	**9.7**±0.1

[a] See corresponding footnote in Table 1. [b] See footnote *c* in Table 1. [c] Each value is the mean ± SEM of 4–8 determinations.

regio- and stereoisomers **7b** and **8a**,**b** were synthesized and tested: these all showed a worse or at most comparable value of pK_i for the binding at the human NK-2 receptor and pK_B in the rabbit pulmonary artery (RPA) functional test. The insertion of two chlorine atoms on the benzyl substituent in position 8 was suggested by the encouraging results shown by the reference compound MEN11690.[4] As expected the corresponding dichloro derivative **14a** and also its epimer **14b** showed interesting values in both binding and functional tests. To further check the potential of **7a** as new lead in comparison with the other candidates, two types of substitution on the amino group were realized: the first, consisting of the introduction of a 4-tetrahydropyranyl pendant on the primary amine of the four isomers, gave rise to products **15a**,**b** and **16a**,**b**; the second, consisting of the transformation of the free amine pendant in a morpholine, was performed on the precursors **7a**,**b** and on the chlorinated analogue **14a** to give the corresponding **17a**,**b** and **18a**. In the first four-component library the regiochemistry and absolute configuration of **7a** were confirmed as the best (compare **15a** with **15b** and **16a**,**b**), in the second three-component group the *R* isomer was once again the best (**17a** vs **17b**) and, interestingly, the dihalogenated product **18a** seemed to lose the advantage over the nonhalogenated compounds shown by the unsubstituted precursor (**14a**). In conclusion, considering that the dichlorinated series would have been more expensive, heavier in terms of molecular weight, and consequently less favorable for some of the physicochemical properties (e.g. solubility), and also that the in vitro activity shown by the first derivative **18a** was not better than that of the corresponding unsubstituted product **17a**, it was reasonable to elect compound **7a** as the new lead for further derivatization.

Results on an enlarged group of both tertiary and secondary amino derivatives are presented in Table 2. Starting from the consideration that **17a** showed good activity, simple modifications on the terminal cyclic moiety were performed: the substitution of the oxygen atom of the morpholine with CH_2 (**19**), $CH-NH_2$ (**20**), NH (**21**), and NSO_2CH_3 (**24**) turned out in evident loss of affinity, while the introduction in the same position of $N-(CH_3)$ (**22**) and $N-(COCH_3)$ (**23**) gave rise to products with comparable activity with respect to **17a**. The insertion of an extra amino functionality in a distal heterocycle, as in **25** and **26**, was well tolerated by the receptor and is potentially advantageous for the hydrophilicity of the resulting compounds. A brief structure–activity study was undertaken also on the tetrahydropyranyl derivative **15a** presented in the first part of this discussion. The attempt to substitute the oxygen atom of the heterocycle with sulfur (**27**) seemed to emphasize the differences in terms of activity between the two considered species giving rise to a product with high affinity for the human receptor but with a pK_B of more than 100-fold smaller in rat tissues. This gap was easily recovered passing from the sulfur derivative to the sulfoxide (**28**) and sulfone (**29**) functionalities in the same position. The substitution of the remaining hydrogen on the amino functionality of **15a** with a methyl group does not seem to improve further the biological results.

Thanks to the information obtained with this first sample set of molecules, N-substituted piperidines were used in alternative to the tetrahydropyran of **15a** and tested. The two sulfonyl derivatives (**31** and **32**) turned out to be among the most active compounds. These levels of affinity result extremely interesting even if compared with the other known selective antagonists for this receptor.[9]

Another set of products was prepared and tested, on the same target, to verify the opportunity of the introduction of a sugar moiety for receptor affinity and, in the case of a positive result, in terms of ADME features. This approach was dictated by our previous

investigations around one of the bicyclic lead structures (MEN 10627),[10] onto which the introduction of an *N*-acetyl-glucosamine pendant in place of the residue of a cysteine gave rise to nepadutant (MEN11420), a compound with improved pharmacokinetic and physicochemical properties, presently under clinical trials.[5,11] In general sugar scaffolds are quite interesting hydrophilic groups because of the abundance of hydroxylic functions that insist on a small structure in a precise, naturally defined stereochemistry. Results on this subset are reported in Table 3. Even at a first glance it is clear that the mean value of binding and functional activity for this series is not so exciting with respect to the other investigated manipulations, although some of these derivatives maintain an interesting pharmacological pattern. Our first attempt (see **34** and **35**) was to insert an ω-carboxylated linker able to support the aminosugar (2-acetamido-2-deoxy-β-D-glucopyranosyl amine)[12] characteristic of our reference compound (nepadutant), and mimicking at the same time the distance between the peptide skeleton and the sugar moiety in the above-mentioned molecule. With this purpose in mind, succinic acid and *N*-acetyl-aspartic acid were introduced and further functionalized with the aforementioned aminosugar as described in Scheme 3. Both **34** and **35** not being satisfactory for their pharmacological profile, the elongation chain was suppressed and other derivatives were obtained by direct reductive amination on the aldehydic function of diverse monosaccharides as easily as with di-and trisaccharides: **36** with *N*-acetyl-D-glucose, **37** with D-glucose, **38** with D-lactose, **39** with D-maltose, **40** with D-maltotriose. This unusual synthetic methodology allowed the amino functionality to be maintained in conjunction with the insertion of highly hydrophilic moieties giving rise to compounds with subnanomolar affinity at the human NK-2 receptor and favorable activity in functional tests. On the other hand the loss of the basic functionality resulting from the introduction of the D-lactobionic fragment (see **41**) provoked a sensible decrease in the binding ability.

Some deductions arise by the analysis of these results: (a) the presence of an amino group in this part of the molecule does seem to increase the potency against the NK-2 receptor; (b) among the considered nitrogen-containing heterocycles, morpholine, piperidine, and acylated piperazine emerged as the best.

Further work has been done with these thoughts in mind to probe the better position for the basic nitrogen, and a new small subset was obtained as depicted in Scheme 5. The binding and functional properties of the new compounds are listed in Table 4. The common feature of these derivatives is the presence of the amide functionality in place of the original amine in the aspartic portion and the concomitant reinsertion of a basic nitrogen in a farther position with respect to the other compounds, usually spaced out by a single methylene. As evident from Table 4, all compounds, except the *N*-methyl-piperazine derivative **51**, showed subnanomolar affinity to the receptor, with $pK_i > 9.0$, indicating that this strategy is as easy as it is satisfactory. Many basic moieties selected among the most active of the previous series were inserted in the pendant giving rise to very active products. In particular derivatives with 4-substituted piperidines (**49** and **52**) showed

Table 5. In Vitro Activity of Selected Compounds for the Tachykinin NK-1, NK-2, and NK-3 Receptors Evaluated in Functional Experiments (pK_B Values) in Comparison with the Reference Compound MEN11558

entry	NK-1 pK_B (GPI)[a,e]	NK-2 pK_B (RUB)[b,e]	NK-3 pK_B (GPI)[c,e]
MEN11558[d]	5.7 ± 0.1	7.6 ± 0.1	6.1 ± 0.1
15a	6.1 ± 0.1	9.1 ± 0.2	6.4 ± 0.1
17a	6.2 ± 0.1	9.2 ± 0.2	6.1 ± 0.1
54	<5.5	9.5 ± 0.1	5.9 ± 0.2
55	6.8 ± 0.1	9.2 ± 0.2	5.8 ± 0.1
57	7.5 ± 0.2	9.7 ± 0.1	6.4 ± 0.2

[a] $pK_B = -\log K_B$. Antagonist affinities of compounds for guinea pig NK-1 receptor estimated against substance P methyl ester (SPOMe) in the isolated guinea pig ileum (GPI). [b] See footnote *c* in Table 1. [c] Antagonist affinities of compounds for guinea pig NK-3 receptor estimated against senktide in the isolated guinea pig ileum (GPI). [d] Data from ref 4. [e] Each value is the mean ± SEM of 4−8 determinations.

subnanomolar affinity for the human receptor together with a good activity in the functional test on the isolated rat tissue. The synthesis of the thiomorpholine sulfoxide **53** took its origin from the three results previously obtained on the differently oxidized thio derivatives depicted in Table 2 and suggesting an advantage for the monooxidized **28**. However, even maintaining a good in vitro profile, **53** resulted less active than its above-mentioned precursor.

Finally, the insertion of differently substituted amino groups in position 12 on our reference compound MEN11558 does not seem to alter the selectivity profile respect to the other tachykinin receptors, shown by the latter compound. Table 5 compares the results of functional experiments at the NK-1, NK-2, and NK-3 receptors for a number of "key" compounds: **15a** and **17a**, the lead compounds of the two groups based on the insertion of a 4-tetrahydropyranyl pendant (**15a**) or a morpholine (**17a**), respectively; compound **55**, a more advanced compound bearing the 4-piperidylpiperidine moiety; and two of the most active compounds of the series, **54** and **57**. All compounds reported in Table 5 show an improved selectivity when compared to that of MEN11558, with at least 100-fold higher affinity at the NK-2 receptor versus the other two tachykinin receptors.

Compound **54**, bearing an aminosulfonyl piperazine, and compound **57**, with a 4-morpholine-piperidine pendant, were among the most active, in in vitro tests, we have ever encountered during our work, and these compounds were chosen for deeper pharmacological investigations. In addition to the affinity shown at the human tachykinin NK-2 receptor at subnanomolar concentrations ($pK_i = 9.8$ for **54**, $pK_i = 10.0$ for **57**), they showed very high selectivity in binding tests at the human tachykinin NK-1 and NK-3 receptors, with affinity at least 1000-fold lower for NK-1 ($pK_i = 6.7$ for **54**, $pK_i = 6.9$ for **57**) and 10000-fold lower for NK-3 ($pK_i = 5.1$ for **54**, $pK_i = 5.9$ for **57**) than for the NK-2 receptor.

Both compounds **54** and **57** showed high in vivo potency at very low doses, and long duration of action in animal models of bronchoconstriction. In particular, after intratracheal (i.t.) administration in anesthetized guinea pigs the two compounds were active at subnanomolar concentrations. At a dose of 10 nmol/kg i.t., **54** and **57** strongly inhibited (about 80%) bronchocon-

striction induced by the selective NK-2 agonist [β-Ala[8]]-NKA(4−10) (1 nmol/kg iv) for at least 4 h, with ED_{50} = 0.27 nmol/kg for **54** and ED_{50} = 0.15 nmol/kg for **57**. In comparison, previous leads **15a** (bearing a 4-tetrahydropyranyl pendant, see Table 1) and **17a** (with a morpholine substituent, see Tables 1 and 2) produced similar effects, as duration of action and maximal inhibition of bronchocostriction, only after i.t. administration of 100 nmol/kg, a 10 times higher dose compared to **54** and **57**.

Conclusions

Starting from the previously described lead MEN11558, we have designed and realized a new series of potent and selective NK-2 antagonists. Results presented in this work show that the identification of a versatile position in the cyclic moiety of the reference compound (C-12) and the juxtaposition there of a functionalizable exocyclic amine in that precise position led to a wide range of compounds with high affinity. A careful examination of structure−activity relationships and an optimization process were conducted through the investigation on many types of substitution on the primary amine of the newly identified lead molecule **7a**. This iterative process finally gave rise to two interesting candidates, **54** and **57**, which showed the ability to act as potential drugs for the therapy of respiratory diseases, such as asthma, with excellent performances after intratracheal administration.

Experimental Section

General. All reagents and solvents were used without further purification or drying. Amino acids and coupling reagents were purchased from Bachem AG (Bubendorf, Switzerland) and Novabiochem (Läufelingen, Switzerland). All other reagents were purchased from Sigma-Aldrich (Milan, Italy) unless otherwise specified. Commercial grade anhydrous solvents were purchased from J. T. Baker (Deventer, Holland). All reactions were performed under an atmosphere of nitrogen, unless otherwise specified. ^{1}H NMR spectra were acquired on a Varian 200 MHz or a Bruker 500 MHz instrument and recorded in parts per million (ppm) δ values, relative to $CHCl_3$ (δ 7.27) or DMSO (δ 2.50) as the internal standards. The data were transferred to an Apple Macintosh computer and processed using the program SwaN-MR.[13] Mass spectra were obtained with a Finnigan LCQ ion trap mass spectrometer, operated in positive-ion electrospray ionization. The samples (about 10 mg/mL) were dissolved in acetonitrile/ammonium acetate (10 mM) 1:1 v/v and introduced by direct infusion at 5 mL/min through the built-in syringe pump. The samples were analyzed by full-scan MS and product ion MS/MS of the protonated quasi-molecular ions, at 30% relative collision energy, using helium as the collision gas. Exact mass measurements were performed using a microTOF orthogonal acceleration time-of-flight mass spectrometer (Bruker Daltonics, Billerica, MA), using positive-ion electrospray ionization. The samples, dissolved in 50:50:0.1 water/acetonitrile/formic acid, were directly infused into the instrument at 4 μL/min via a syringe pump. Mass calibration was performed internally using Agilent APCI tuning solution. HPLC were recorded on a HP-1100 instrument using the following methods. Method A: Symmetry column RP-8 (3.6 × 150 mm) H_2O + 0.1% TFA/MeCN + 0.1% TFA 80/20 → 20/80 in 20′, flow rate 1 mL/min, λ = 215, 254 nm. Method B: Luna column RP-8(2) (4.6 × 250 mm) H_2O + 1% TFA/MeCN + 0.1% TFA 80/20 → 20/80 in 20′ + 6′ isocratic, flow rate 1 mL/min, λ = 215, 254 nm. Method C: Zorbax column SB-18, 3.5 mm, 100 Å (4.6 × 50 mm), H_2O + 0.1% TFA/MeCN + 0.1% TFA, 95/5 → 5/95 in 6.5′ + 1′ isocratic, flow rate 3 mL/min, λ = 220, 270 nm.

Methods. Binding Experiments. Radioligand binding experiments were performed at human NK-1, NK-2, and NK-3 tachykinin receptors stably transfected on membranes of U-373MG (NK-1) or CHO-K1 (NK-2 or NK-3) cells, as reported previously.[5,14,15] All compounds were tested for their ability to displace the following radioligands from the three tachykinin receptors (in brackets): [^{3}H][Sar[9]]SP sulfone, 1.2 nM (NK-1); [^{125}I]neurokinin A, 0.15 nM (NK-2); [^{125}I][MePhe[7]]NKB, 50 pM (NK-3). All radioligands were from Amersham Biosciences (Buckinghamshire, U.K.). Nonspecific binding was determined in the presence of unlabeled [Sar[9]]SP sulfone 1 μM (at NK-1 receptor), or of unlabeled neurokinin A 1 μM (at NK-2 receptor), or of unlabeled [MePhe[7]]NKB 1 μM (at NK-3 receptor), and ranged between 5% and 10% of total binding at each receptor. Affinity of test compounds for the tachykinin receptors determined in these competition experiments was expressed in terms of pK_i ($-\log K_i$).

Organ Bath Experiments. The experiments were performed on the following isolated smooth muscle preparations: rabbit pulmonary artery circular muscle strips deprived of the endothelium (RPA), guinea pig ileum longitudinal muscle myenteric plexus strips (GPI), and strips of detrusor muscle of rat urinary bladder (RUB). All of the experiments were performed in oxygenated (96% O_2 and 4% CO_2) Krebs−Henseleit solution having the following composition: NaCl, 119 mM; $NaHCO_3$, 25 mM; KH_2PO_4, 1.2 mM; $MgSO_4$, 1.5 mM; $CaCl_2$, 2.5 mM; KCl, 4.7 mM; and glucose 11 mM.

RPA, GPI, and RUB preparations were set up according to the methods previously described:[16] briefly, RPA strips were connected to isometric force transducers (load 10 mN), whereas GPI (load 3 mN) and RUB (load 0.5 g) strips were prepared for isotonic recording of mechanical activity. Activity of test compounds at tachykinin NK-1 receptors was evaluated in the GPI (in the presence of atropine and chlorpheniramine 1 μM, indomethacin 3 μM) by using substance P methyl ester (SPOMe) as tachykinin NK-1 receptor-selective agonist. Activity of test compounds at tachykinin NK-2 receptors was assessed against neurokinin A (in the RPA) or [β-Ala[8]]NKA-(4−10) (in the RUB) as the agonists. The activity of test compounds at tachykinin NK-3 receptors was evaluated in the GPI (in the absence of premedication) by using senktide as the tachykinin NK-3 selective agonist. Cumulative concentration−response curves to the agonists were obtained in all preparations, each concentration being added when the effect of the preceding one had reached a steady state. The antagonist affinity of all test compounds (incubation period of 15 min) was expressed as pK_B (negative logarithm of K_B, the antagonist dissociation constant), which was estimated as the mean of the individual values obtained with the equation

$$pK_B = \log[\text{dose ratio } -1] - \log[\text{antagonist concentration}]$$

In Vivo Experiments. The activity of test compounds, after intratracheal administration, was checked on bronchoconstriction produced by intravenous (iv) administration of the selective tachykinin NK-2 receptor agonist, [β-Ala[8]]NKA(4−10), in anesthetized guinea pigs as previously described.[17]

Male Dunkin Hartley guinea pigs (Charles River, Italy), weighing 350−400 g, were anesthetized with urethane (1.5 g/kg sc), and the body temperature was kept constant at 36 °C. The left jugular vein was cannulated for drug administration, and the animals were mechanically ventilated at a rate of 60 strokes/min. The basal insufflation pressure and bronchoconstriction were measured by connecting a pressure transducer to a sidearm of tracheal cannula and recorded by a MacLab (ADInstruments, Australia) data acquisition system. A polyethylene catheter (PE10, Clay Adams) was stably inserted into the tracheal cannula and its tip positioned closely to the bronchi bifurcation to allow intratracheal administration of compounds.

After two reproducible control responses to the agonist (1 nmol/kg iv) the antagonist was administered intratracheally 5 and 30 min before the challenges with the agonist that was repeated every 30 min up to 4 h from antagonist administration.

The bronchoconstriction was calculated as amplitude (mmHg) of the response over the basal value of insufflation pressure. The effect of the antagonist at various times after treatment was expressed as % inhibition of the control response. The ED_{50} were calculated (Prism 3 software, Graphpad, San Diego, CA) considering the maximum value of inhibition reached at each dose during the experimental time.

Synthetic Method for the Obtainment of Compounds 7a,b, 8a,b, and 14a,b. The synthesis of (2-amino-1-(*R*)-benzyl-ethyl)-carbamic acid *tert*-butyl ester **1** was conducted as previously described,[7] and that of [2-amino-1-(*R*)-(3,4-dichloro-benzyl)-ethyl]-carbamic acid *tert*-butyl ester **9** was conducted in a similar way with the simple substitution of the catalytic hydrogenation step with a reduction with $LiAlH_4$ according to a known procedure.[4]

(2-(*R*)-Amino-3-phenyl-propyl)-carbamic Acid Benzyl Ester Hydrochloride (2). Benzyl chloroformate (0.7 mL, 3.7 mmol) was dropped slowly into a solution of **1** (0.925 g, 3.7 mmol) and triethylamine (1.25 mL, 8.8 mmol) in anhydrous THF (30 mL) and the mixture stirred at 0 °C for 1 h. The resulting suspension was filtered and the residue washed with THF. The collected filtrates were evaporated to dryness, then treated with ethyl acetate, and extracted with $NaHCO_3$ and brine. The organic phase was evaporated to a solid, which was treated with diethyl ether. The suspended solid was filtrated and dried under vacuum to give the diprotected intermediate (1.09 g, 77% yield). TLC (ethyl acetate/petroleum ether 40–60° 1/3): R_f = 0.40. Mp: 147–148 °C. MS (*m*/*z*): 385 (MH^+), 402 (MNH_4^+), 346, 329, 285, 177. ^1H NMR (200 MHz, $CDCl_3$): δ 7.4–7.1 (m, 10H), 5.3–5.1 (m, 1H), 5.11 (s, 2H), 4.9–4.6 (m, 1H), 4.05–3.75 (m, 1H), 3.43–3.05 (m, 2H), 2.95–2.62 (m, 2H), 1.37 (s, 9H).

Three grams (7.8 mmol) of that solid were subsequently suspended in methanol (55 mL) and treated with a 4 M solution of HCl in dioxane (22.5 mL, 90 mmol). After 1 h the solvent was evaporated under reduced pressure and the solid residue treated with diethyl ether in an ice bath for 10 min and then filtered off by suction to give 2.49 g (7.8 mmol, 99% yield) of **2** as a white powder (overall yield from **1** 76%). HPLC (A): 6.76′, 98% purity. ^1H NMR (500 MHz, DMSO-d_6): δ 7.99 (br s, 3H), 7.62–7.42 (br m, 1H), 7.41–7.14 (m, 10H), 5.02 (s, 2H), 3.46–3.33 (m, 1H), 3.27–3.08 (m, 2H), 3.05–2.72 (m, 2H).

[2-(*R*)-(2-(*S*)-Amino-3-phenyl-propionylamino)-3-phenyl-propyl]-carbamic Acid Benzyl Ester Hydrochloride (3). Boc-L-phenylalanine (20.6 g, 77.6 mmol) was combined with HOBt (15.8 g, 117 mmol) in acetonitrile (280 mL) with mechanical stirring. The solution was cooled to 0 °C, and EDC·HCl (17.9 g, 93.3 mmol) was added. After 30 min a solution of **2** (25 g, 77.9 mmol) and triethylamine (11 mL, 79 mmol) in acetonitrile (70 mL) was added dropwise, and the mixture was warmed to room temperature and stirred for a further 3 h. The formed precipitate was filtered, washed with acetonitrile (2 × 75 mL) and *tert*-butyl methyl ether (3 × 100 mL), and dried to give the crude diprotected dipeptide (35.3 g, 66.8 mmol, 85% yield). MS (*m*/*z*): 532 (MH^+), 476, 432. HPLC: (A) 18.06′, >95% purity; (B) 21.80′, >95% purity. ^1H NMR (200 MHz, DMSO-d_6): δ 7.94 (d, J = 8.6 Hz, 1H), 7.54–6.96 (m, 16H), 6.72 (d, J = 8.21 Hz, 1H), 5.05 (s, 2H), 4.18–3.85 (m, 2H), 3.28–2.93 (m, 2H), 2.90–2.40 (m, 4H), 1.30 (s, 9H).

The diprotected derivative (35.3 g, 66.4 mmol) was suspended in methanol (350 mL) and cooled to 5 °C with an ice bath. HCl 7 M in dioxane (150 mL, 1.05 mol) was added dropwise through a dropping funnel. The reaction mixture was left to warm to room temperature. After 8 h a mixture of methanol and dioxane (7:3 v/v, 50 mL) was added to make the solution completely clear, and after 1 h of stirring the solvent was evaporated under reduced pressure and the resulting viscous oil was crystallized from 100 mL of *tert*-butyl methyl ether. The obtained solid **3** was filtered by suction and dried under high vacuum (30 g, 64 mmol, 96% yield). Overall yield from **2**: 82%. HPLC: (B) 13.49′. MS (*m*/*z*) (ES+): 432.2 (MH^+). ^1H NMR (200 MHz, DMSO-d_6): δ 8.57 (d, J = 7.9 Hz, 1H), 8.1 (br s, 3H), 7.48–6.86 (m, 16H), 5.00 (br s, 2H), 4.12–3.78 (m, 2H), 3.24–2.4 (m, 6H).

(2-(*R*)-{2-(*S*)-[2-(*S*)-Amino-3-(1*H*-indol-3-yl)-propionylamino]-3-phenyl-propionylamino}-3-phenyl-propyl)-carbamic Acid Benzyl Ester Hydrochloride (4). A solution of Boc-L-tryptophan (19.5 g, 64 mmol), HOBt (27 g, 199 mmol), and EDC·HCl (15 g, 78.2 mmol) in 300 mL of DMF was prepared and stirred for 15 min. After that time a suspension of **3** (30 g, 64 mmol) and triethylamine (10 mL, 72 mmol) in 150 mL of DMF was added and the resulting mixture stirred for 2 h at room temperature. The solvent was then evaporated under reduced pressure and the crude residue treated with $NaHSO_4$ (5% aqueous solution, 500 mL) and filtered. This procedure was repeated four times, and the resulting solid was further washed with water (3 × 500 mL). After stripping with toluene and with absolute ethanol the solid was dried under vacuum for 4 h. The obtained Boc-protected derivative (42 g, 58.5 mmol, 91% yield) was used without further purification for the next deprotecting step. HPLC: (B) 22.53′, (A) 19.23′. MS (*m*/*z*) (ES+): 718.0 (MH^+). ^1H NMR (200 MHz, DMSO-d_6): δ 10.77 (s, 1H), 7.99 (d, J = 7.77 Hz, 1H), 7.18 (d, J = 7.77 Hz, 1H), 7.65–6.69 (m, 22H), 5.01 (br s, 2H), 4.60–4.32 (m, 1H), 4.24–3.78 (m, 2H), 3.00–2.35 (m, 8H), 1.25 (s, 9H).

A suspension of that solid (58.5 mmol) in methanol (700 mL) was cooled to 5 °C and HCl 7 N in dioxane (55 mL, 385 mmol) and subsequently 100 mL (400 mmol) of HCl 4 N in dioxane were dropped slowly, the internal temperature being kept under 10 °C during the addition. The reaction mixture was then warmed to room temperature, stirred for 5.5 h, and then evaporated down under reduced pressure. The resulting solid was treated with *tert*-butyl methyl ether (200 mL), filtered by suction, and dried under vacuum to give **4** (35.8 g, 55 mmol, 92% yield). Overall yield from **3**: 84%. HPLC: (B) 15.7′, >99% purity. MS (*m*/*z*) (ES+): 618.2 (MH^+). ^1H NMR (200 MHz, DMSO-d_6): δ 11.02 (m, 1H), 8.88 (d, J = 8.04 Hz, 1H), 8.24 (d, J = 8.5 Hz, 1H), 8.04 (br s, 3H), 7.73 (d, J = 7.3 Hz, 1H), 7.50–6.89 (m, 20H), 5.03 (s, 2H), 4.58–4.39 (m, 1H), 4.15–3.87 (m, 2H), 3.35–2.55 (m, 8H).

General Procedure for the Coupling of 4 with Monoprotected Aspartic Acid. *N*-[1-(*S*)-[1-(*S*)-(2-Amino-1-(*R*)-benzyl-ethylcarbamoyl)-2-phenyl-ethylcarbamoyl]-2-(1*H*-indol-3-yl)-ethyl]-2-(*R*)-*tert*-butoxycarbonylamino-succinamic Acid (5a). Boc-(D)-Asp-OBzl (18 g, 55.6 mmol) was dissolved in 1 L of DMF with magnetic stirring. HOBt (27 g, 200 mmol) and subsequently 15 g (78.2 mmol) of EDC·HCl were added to that solution. After 15 min a suspension of **4** (35.8 g, 54.8 mmol) and triethylamine (12.7 mL, 91.2 mmol) in DMF (150 mL) was added dropwise. After the addition of 15 mL (108 mmol) of triethylamine, the reaction mixture was left at room temperature for 4 h, and then the solvent was evaporated under reduced pressure. The residue was treated with portions of aqueous $KHSO_4$ (5%, 2 × 500 mL), water (2 × 500 mL), $NaHCO_3$ (5%, 2 × 500 mL), and water (2 × 500 mL). The resulting solid was washed with hot ethanol (96%) until the alcoholic solution was approximately colorless, filtered, and dried under high vacuum for 4 h obtaining 35 g (38 mmol, 69% yield) of the benzyl ester as a solid, directly used for the next synthetic step. HPLC: (B) 23.80′; (A) 19.94′, >99% purity. MS (*m*/*z*) (ES+): 923.0 (MH^+). ^1H NMR (200 MHz, DMSO-d_6): δ 10.77 (br s, 1H), 8.12 (d, J = 7.6 Hz, 1H), 7.94 (d, J = 8.1 Hz, 1H), 7.78 (d, J = 8.4 Hz, 1H), 7.80 (d, J = 7.6 Hz, 1H), 7.40–6.80 (m, 26H), 5.05–4.94 (m, 4H), 4.57–4.21 (m, 3H), 4.12–3.86 (m, 1H), 3.18–2.3 (m, 10H), 1.34 (s, 9H).

The product (17.5 g, 18.9 mmol) was subsequently treated with hydrogen in the presence of Pd/C (2 g, 10% Pd) in DMF (550 mL) and water (50 mL) during 6 h. The solution was filtered and evaporated under reduced pressure. The obtained solid was washed repeatedly with *tert*-butyl methyl ether and dried under vacuum to give **5a** as a whitish solid (12.3 g, 93% yield). HPLC: (B) 14.2′, >98% purity. MS (*m*/*z*) (ES+): 699.3 (MH^+). ^1H NMR (200 MHz, DMSO-d_6): δ 10.79 (d, J = 1.9 Hz, 1H), 8.75 (d, J = 9.2 Hz, 1H), 8.53 (d, J = 6.0 Hz, 1H), 8.61–7.91 (br s, 3H), 7.55–6.82 (m, 16H), 6.24 (d, J = 6.0 Hz, 1H), 4.50–3.99 (m, 5H), 3.40–2.60 (m, 8H), 2.03 (dd, J = 12.4, 11.6 Hz, 1H), 1.40 (s, 9H).

With the same methodology and with comparable yields, the following products were obtained: **5b** (using Boc-(L)-Asp-OBzl), **6a** (using Boc-(D)-Asp(OH)-OBzl), and **6b** (using Boc-(D)-Asp-(OH)-OBzl).

General Procedure for the Obtainment of the Cyclized Pseudopeptides Bearing the Free Amino Group (7ab, 8ab). 12-(*R*)-Amino-5-(*S*),8-(*R*)-dibenzyl-2-(*S*)-(1*H*-indol-3-ylmethyl)-1,4,7,10-tetraaza-cyclotetradecane-3,6,11,14-tetraone (7a). Product **5a** (12.2 g, 17.45 mmol) was dissolved in DMF (1000 mL) and maintained at room temperature with stirring. HOBt (9.3 g, 68.8 mmol) and, after 10 min, EDC·HCl (5.6 g, 29.2 mmol) were added in portions. After 2.5 h the mixture was evaporated down under reduced pressure and the remaining residue was dissolved in ethyl acetate (1250 mL) and extracted with $KHSO_4$ (5%, 2 × 200 mL), $NaHCO_3$ (5%, 2 × 200 mL), and finally water (2 × 250 mL). The organic phase, dried over anhydrous sodium sulfate (40 g) with magnetic stirring, was then filtered and evaporated to dryness. The resulting solid was washed twice with *tert*-butyl methyl ether (60 mL). After recrystallization from ethyl acetate (155 mL) the obtained solid (7.3 g, 62% yield) was directly deprotected.

Seven grams of the solid (10.28 mmol) were suspended in 200 mL of methanol, and the temperature of the solution was maintained around 5°C during the addition of 25 mL of HCl 4 M in dioxane. After that operation the mixture was left to reach room temperature, and after 20 h the solvent was removed under reduced pressure and the residue, after treatments with ethyl acetate (50 mL) and *tert*-butyl methyl ether (50 mL), was filtered and maintained under vacuum for 2 h, giving the hydrochloride of **7a** as a white solid (5.8 g, 9.4 mmol, 92% yield). HPLC: (B) 12.5′, 97.7% purity. MS (ES+): 581 (MH^+).

The corresponding free amine was extracted with ethyl acetate from a saturated solution of $NaHCO_3$, washed with brine, and purified by flash column chromatography using solutions of methanol in ethyl acetate with increasing polarity. HPLC: (C) 3.42′, 99% purity. MS (*m/z*) (ES+): 581 (MH^+), 603 (MNa^+), 619 (MK^+), 564, 378, 281, 231. ^1H NMR (500 MHz, DMSO-d_6): δ 10.80 (d, J = 1.35 Hz, 1H), 8.63 (d, J = 5.2 Hz, 1H), 7.82 (bs, 1H), 7.68 (bs, 1H), 7.43 (d, J = 7.8 Hz, 1H), 7.33 (d, J = 8.1 Hz, 1H), 7.29−7.22 (m, 6H), 7.21−7.16 (m, 4H), 7.07 (t, J = 7.03 Hz, 2H), 6.98 (t, J = 7.6 Hz, 1H), 6.73 (d, J = 9.1 Hz, 1H), 4.24 (m, 1H), 4.14 (m, 1H), 4.04 (m, 1H), 3.53 (bs, 1H), 3.51 (t, J = 5.2 Hz, 1H), 3.38 (dd, J = 14.3, 3.8 Hz, 1H) 3.09 (d, J = 12.0 Hz, 1H), 2.95 (dd, J = 14.4, 4.5 Hz, 1H), 2.81−2.69 (m, 5H), 2.20 (dd, J = 14.3, 6.1 Hz, 1H).

In a similar way the following products were obtained starting from the appropriate intermediates: **7b** (from **5b**), **8a** (from **6a**), **8b** (from **6b**). Purifications in these cases were conducted through preparative HPLC.

[1-(*R*)-(3,4-Dichloro-benzyl)-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-ethyl]-carbamic Acid *tert*-Butyl Ester (10). The starting amine **9** (2.79 g, 8.75 mmol) was dissolved in anhydrous THF (75 mL). Addition of triethylamine (1.25 mL, 8.8 mmol) and of *N*-(9-fluorenylmethoxycarbonyloxy)succinimide (2.95 g, 8.75 mmol) were conducted slowly at room temperature, and the resulting mixture was stirred for 1 h. The solvent was evaporated under reduced pressure, and the residue, solubilized in ethyl acetate (1 L), was treated with 10% citric acid, brine, 5% aqueous $NaHCO_3$, and brine. The organic layer was evaporated to dryness and the solid treated with diethyl ether, obtaining 3.29 g (6.55 mmol, 70% yield) of the diprotected intermediate. HPLC: (A) 23.31′, 91% purity. MS (*m/z*) (TS+): 541 (MH^+, isotopic pattern for dichloro compound). ^1H NMR (200 MHz, DMSO-d_6): δ 7.88 (d, J = 7.0 Hz, 2H), 7.69 (d, J = 7.1 Hz, 2H), 7.56−7.23 (m, 7H), 7.16 (dd, J = 1.7, 8.2 Hz, 1H), 6.7 (d, J = 8.6 Hz, 1H), 4.42−4.11 (m, 3H), 3.78−3.52 (m, 1H), 3.14−2.90 (m, 2H), 2.81−2.36 (m, 2H), 1.27 (s, 9H).

[2-(*R*)-(2-(*S*)-*tert*-Butoxycarbonylamino-3-phenyl-propionylamino)-3-(3,4-dichlorophenyl)-propyl]-carbamic Acid 9*H*-Fluoren-9-ylmethyl Ester (11). 10 (1.62 g, 3 mmol) was suspended in dichloromethane (50 mL), cooled to 0 °C, and treated with CF_3COOH (13 mL), then warmed to room temperature, and stirred for further 30 min. After the evaporation of the solvent the crude product was treated three times with diethyl ether, to give after settling 1.33 g (2.4 mmol, 80% yield) of the deprotected derivative, which was immediately used for the coupling reaction. HPLC: (A) 13.1′, 95% purity. MS (*m/z*) (ES+): 441 (MH^+, isotopic pattern for dichloro compound). Boc-L-phenylalanine (0.49 g, 1.85 mmol) was combined with HOBt (0.66 g, 4.9 mmol) and EDC·HCl (0.46 g, 2.4 mmol) in DMF (9 mL). The solution was cooled to 0 °C. After 30 min a solution of the aforementioned intermediate (1.03 g, 1.85 mmol) in DMF (8 mL) and subsequently triethylamine (0.6 mL, 4.8 mmol) was added dropwise, and the mixture was warmed to room temperature and stirred for a further 4 h. The solvent was removed under reduced pressure, and the residue was dissolved in ethyl acetate (400 mL) and washed with brine, 5% aqueous $NaHCO_3$, and brine. The organic phase gave, after drying and evaporation, a solid compound (1.13 g, 1.64 mmol, 82% yield), which was directly subjected to the deprotection conditions. HPLC: (A) 23.34′, >99% purity. MS (*m/z*) (ES+): 687 (MH^+, isotopic pattern for dichloro compound). ^1H NMR (200 MHz, DMSO-d_6): δ 8.05−7.82 (m, 3H), 7.78−7.59 (m, 2H), 7.55−7.00 (m, 13H), 6.66 (d, J = 8.1 Hz, 1H), 4.42−4.17 (m, 3H), 4.15−3.87 (m, 2H), 3.27−2.95 (m, 2H), 2.90−2.37 (m, 4H), 1.28 (s, 9H).

[1-(*S*)-{1-(*S*)-[2-Amino-1-(*R*)-(3,4-dichloro-benzyl)-ethylcarbamoyl]-2-phenyl-ethylcarbamoyl}-2-(1*H*-indol-3-yl)-ethyl]-carbamic Acid *tert*-Butyl Ester (12). 11 (1.13 g, 1.64 mmol) was suspended in dichloromethane (32 mL), cooled to 0 °C, treated with trifluoroacetic acid (7.5 mL), then warmed to room temperature, and stirred for further 30 min. After the evaporation of the solvent the crude residue was treated three times with diethyl ether, to give, after filtration, the deprotected intermediate (1.5 mmol, 91% yield), readily used for the next coupling step.

A solution of the aforemetioned intermediate (1.5 mmol) in 10 mL of DMF was added at room temperature to a solution of Boc-L-tryptophan (0.457 g, 1.5 mmol), HOBt (0.625 g, 4.6 mmol), and EDC·HCl (0.385 g, 2.0 mmol) in DMF (10 mL). Then DIPEA (0.33 mL, 1.84 mmol) was dropped slowly into the resulting mixture. After 2 h the reaction was complete and the solvent was evaporated under reduced pressure. The residue, dissolved in ethyl acetate, was treated with citric acid (10%), $NaHCO_3$ solution (5%), and brine and then was dried over anhydrous sodium sulfate and evaporated. The resulting solid was subsequently washed, with stirring, with ethyl acetate (20 mL), collected by filtration, and dried to obtain the diprotected derivative (1.14 g, 1.3 mmol, 87% yield). This solid was dissolved in DMF (30 mL), treated with Et_2NH (3 mL), and stirred for 2 h. After evaporation of the solvent under high vacuum the residue was pulverized in diethyl ether and filtered to give **12** as a crystalline white solid (0.666 g, 1.02 mmol, 68% yield). HPLC: (A) 13.75′, 96.4% purity. MS (*m/z*) (ES+): 652 (MH^+, isotopic pattern for dichloro compound). ^1H NMR (500 MHz, DMSO-d_6): δ 10.80 (s, 1H), 7.91 (d, J = 8.6 Hz, 1H), 7.80 (d, J = 8.1 Hz, 1H), 7.55−7.46 (m, 3H), 7.31 (d, J = 8.1 Hz, 1H), 7.22−7.15 (m, 4H), 7.11−6.92 (m, 5H), 6.75 (d, J = 8.2 Hz, 1H), 4.56−4.41 (m, 1H), 4.17−4.04 (m, 1H), 3.90−3.80 (m, 1H), 2.97−2.51 (m, 8H), 1.28 (s, 9H).

General Procedure for the Coupling of 12 with the Appropriate Diprotected Aspartic Acid for the Obtainment of 13a,b and the Obtainment of the Cyclized Pseudopeptides (14a,b) Bearing the Free Amino Group. 12-(*R*)-Amino-5-(*S*)-benzyl-8-(*R*)-(3,4-dichloro-benzyl)-2-(*S*)-(1H-indol-3-ylmethyl)-1,4,7,10-tetraaza-cyclotetradecane-3,6,11,14-tetraone (14a). Fmoc-(D)-Asp-(OtBu)-OH (0.13 g, 0.316 mmol) was combined with HOBt (0.128 g, 0.95 mmol) and EDC·HCl (0.073 g, 0.38 mmol) in DMF (3 mL). After 30 min was slowly added, with stirring, a solution of **12** (0.841 g, 1.29 mmol) in DMF (6 mL). The reaction mixture was stirred at room temperature for 2 h, then the solution was concentrated to dryness, and the residue was treated with citric acid (5%), then filtered, and washed with water. The solid was suspended in $NaHCO_3$ (5%), then newly filtered, and washed

with water. The dried solid (0.278 g, 0.3 mmol, quantitative yield) was used without further purification for the double deprotecting step. This intermediate (0.3 mmol) was suspended in dichloromethane (4 mL), cooled to 0 °C, and treated with ethanedithiol (0.025 mL, 0.3 mmol) and trifluoroacetic acid (2.5 mL). The reaction mixture was then warmed to room temperature and maintained under magnetic stirring for 1 h. The residue obtained after evaporation of the solvent was washed repeatedly with diethyl ether to give **13a** as a solid (0.233 g). HPLC: (A) 15.07′, 78.9% purity. MS (*m/z*) (ES+): 889 (MH^+, isotopic pattern for dichloro compound). ^1H NMR (200 MHz, DMSO-d_6): δ 10.97 (s, 1H), 8.80 (d, *J* = 7.8 Hz, 1H), 8.35−6.85 (m, 24H), 4.60−3.85 (m, 7H), 3.28−2.35 (m, 10H).

In the same way **13b** was obtained with comparable yield. Both compounds were used for the following synthesis of **14a** and **14b** without further purification.

Compound **13a** (0.23 g, 0.2 mmol) was solubilized in DMF (20 mL). To this solution HOBt (99.5 mg, 1 mmol) and EDC. HCl (89 mg, 0.46 mmol) were added and the mixture was stirred for 2 h. After that time the reaction was complete and the solvent was evaporated. The residue was treated with citric acid (5%) then filtered and washed with water, $NaHCO_3$ (5%) and water. The obtained solid (0.210 g, 0.188 mmol, calcd. yield 93%) showed an HPLC purity of about 78% and was used for the deprotecting step without purification.

The cyclic compound (0.188 mol) was solubilized in DMF (10 mL), treated with Et_2NH (1 mL), and maintained under stirring for 2 h. After evaporation of the solvent under high vacuum, the residue was triturated in diethyl ether and filtered to give a white solid, which was further purified via a preparative HPLC (Deltapak RP18 column (300 × 19 mm), flow rate 15 mL/min, H_2O + 0.1% TFA/CH_3CN + 0.1% TFA, gradient 75/25 > 15/85 in 60′) obtaining 47 mg (0.06 mmol, 35% yield) of lyophilized **14a** (total yield 32%). HPLC: (A) 11.67′, >99% purity. MS (*m/z*) (ES+): 649 (MH^+, isotopic pattern for dichloro compound). ^1H NMR (500 MHz, DMSO-d_6): δ 10.88 (m, *J* = 1.8 Hz, 1H), 8.86 (d, *J* = 5.1 Hz, 1H), 8.59 (d, *J* = 5.9 Hz, 1H), 8.17 (s, 3H), 7.54 (d, *J* = 1.9 Hz, 1H), 7.50 (d, *J* = 8.2 Hz, 1H), 7.46 (d, *J* = 7.9 Hz, 1H), 7.39 (m, 1H), 7.35 (d, *J* = 8.1 Hz, 1H), 7.26 (t, *J* = 7.2 Hz, 1H), 7.22 (dd, *J* = 8.3, 1.9 Hz, 1H), 7.18 (tt, *J* = 7.4, 1.33 Hz, 1H), 7.13 (d, *J* = 7.1 Hz, 1H), 7.08 (ddd, *J* = 8.1, 7.0, 1.2 Hz, 1H), 7.06 (d, *J* = 2.4 Hz, 1H), 6.99 (ddd, *J* = 8.0, 7.0, 1.0 Hz, 1H), 6.78 (d, *J* = 9.0 Hz, 1H), 4.28 (m, 1H), 4.16−4.11 (m, 2H), 4.05 (m, 1H), 3.70 (dd, *J* = 12.6, 8.9 Hz, 1H), 3.11 (dd, *J* = 15.0, 3.8 Hz, 1H), 3.06 (dt, *J* = 13.6, 4.0 Hz, 1H), 2.89−2.72 (m, 5H).

With the same method the product **14b** was obtained from **13b**.

General Procedure for the Obtainment of Products 15−33: Reductive Amination on the Primary Amino Group of Compounds 7a,b, 8a,b, 14a. 5-(*S*),8-(*R*)-Dibenzyl-2-(*S*)-(1*H*-indol-3-ylmethyl)-12-(*R*)-(tetrahydro-pyran-4-ylamino)-1,4,7,10-tetraaza-cyclotetradecane-3,6,11,14-tetraone Trifluoroacetate (15a). To a solution of **7a** (0.044 g, 0.076 mmol) in methanol (5 mL), with magnetic stirring, were added at room temperature acetic acid (0.1 mL, 1.6 mmol), a solution of tetrahydro-4*H*-pyran-4-one (0.018 g, 0.18 mmol) in methanol (1 mL), and finally sodium cyanoborohydride (0.012 g, 0.2 mmol). After a further 30 min of stirring and 8 h at rest the solution was acidified to pH 1−2 with HCl (1 N in water). After dilution with water (20 mL), methanol was evaporated under reduced pressure and the resulting solution treated with $NaHCO_3$ to alkaline pH and then extracted with ethyl acetate. The organic phase was washed with brine, anhydrified with Na_2SO_4, then filtered, and evaporated to dryness. Purification by preparative HPLC (Deltapak RP18 column (300 × 19 mm), flow rate 15 mL/min, H_2O + 0.1% TFA/CH_3CN + 0.1% TFA, gradient 75/25 → 15/85 in 120′) gave **15a** as trifluoroacetate (0.025 g, 0.032 mmol, 37% yield). HPLC: (B) 10.40′, >99% purity. MS (*m/z*) (ES+): 665.4 (MH^+). ^1H NMR (500 MHz, DMSO-d_6): δ 10.89 (d, *J* = 2.3 Hz, 1H), 8.99−8.92 (m, 4H), 7.48 (d, *J* = 7.9 Hz, 1H), 7.45 (bs, 1H), 7.35 (d, *J* = 8.1 Hz, 1H), 7.27−7.23 (m, 6H), 7.20−7.15 (m, 2H), 7.12−7.07 (m, 4H), 7.00 (t, *J* = 8 Hz, 1H), 6.86 (d, *J* = 8.5 Hz, 1H), 4.33 (m, 1H), 4.25 (m, 1H), 4.06 (m, 1H), 4.00 (m, 1H), 3.90 (m, 2H), 3.73 (m, 1H), 3.29−3.13 (m, 1H), 2.95−2.72 (m, 5H), 2.42 (t, *J* = 11.6 Hz, 1H), 1.97 (m, 2H), 1.57 (m, 2H).

In a similar way, products **15b**−**18a** were obtained using the appropriate carbonyl compound and the desired starting cyclic amine. Target compounds **19**−**33** were obtained starting from the same amine **7a**.

***N*-(3-(*R*)-Acetylamino-4-(*R*),5-(*R*)-dihydroxy-6-(*R*)-hydroxymethyl-tetrahydro-pyran-2-(*R*)-yl)-*N*′-[5-(*S*),8-(*R*)-dibenzyl-2-(*S*)-(1*H*-indol-3-ylmethyl)-3,6,11,14-tetraoxo-1,4,7,10-tetraaza-cyclotetradec-12-(*R*)-yl]-succinamide (34).** To a solution of **7a** (0.2 g, 0.34 mmol) as free base in CH_3CN (3 mL) was added succinic anhydride (0.06 g, 0.6 mmol). DMF was subsequently added till the solution was completely clear (0.5 mL), and the reaction mixture was stirred for 2 h at room temperature. The solvent was evaporated under reduced pressure, and the residue was treated with diethyl ether to obtain, after filtration, a white solid (200 mg), which was used directly for further transformations. The obtained solid (0.2 g, 0.29 mmol calcd) was dissolved in DMF (7 mL), and HOBt (0.13 g, 0.99 mmol), EDC·HCl (0.067 g, 0.35 mmol), and, after 10 min, 2-acetamido-2-deoxy-β-D-glucopyranosyl amine (0.067 g, 0.29 mmol) were added at room temperature. After 2 h of stirring, the solution was evaporated to dryness and the residue was treated with 5% $KHSO_4$, filtrated, and finally washed with water, 5% $NaHCO_3$, and water. The solid was extracted with ethyl acetate, and the organic phase was evaporated and purified by preparative HPLC (Vydac column (19 × 250 mm), flow rate 20 mL/min, H_2O + 0.1% TFA/CH_3CN + 0.1% TFA, gradient 77/23 → 47/53 in 60′) giving **34** (72 mg, 0.072 mmol, 24% yield) as a white lyophile. HPLC: (B) 11.3′, >99% purity. MS (*m/z*) (ES+): 883.2 (MH^+). ^1H NMR (500 MHz, DMSO-d_6): δ 10.81 (d, *J* = 1.7 Hz, 1H), 8.78 (d, *J* = 4.8 Hz, 1H), 8.11 (m, 2H), 8.01 (bs, 1H), 7.79 (d, *J* = 8.7 Hz, 1H), 7.48 (bs, 1H), 7.43 (d, *J* = 7.9 Hz, 1H), 7.33 (d, *J* = 8.1 Hz, 1H), 7.28−7.14 (m, 8H), 7.09−7.04 (m, 2H), 6.97 (t, *J* = 7.6 Hz, 1H), 6.81 (d, *J* = 9.0 Hz, 1H), 4.95 (bs, 2H), 4.80 (t, 1H, *J* = 9.3 Hz, 1H), 4.55 (m, 1H), 4.48 (bs, 1H), 4.19−4.14 (m, 2H), 4.00 (bs, 1H), 3.59−3.49 (m, 3H), 3.42−3.23 (m, 5H), 3.07 (m, 2H), 2.92 (dd, *J* = 13.8, 4.4 Hz, 1H), 2.85−2.71 (m, 5H), 2.55 (t, *J* = 5.3 Hz, 1H), 2.38−2.23 (m, 5H), 1.81 (s, 3H).

2-(*S*)-Acetylamino-N^4-(3-(*R*)-acetylamino-4-(*R*),5-(*R*)-dihydroxy-6-(*R*)-hydroxymethyl-tetrahydro-pyran-2-(*R*)-yl)-N^1-[5-(*S*),8-(*R*)-dibenzyl-2-(*S*)-(1*H*-indol-3-ylmethyl)-3,6,11,14-tetraoxo-1,4,7,10-tetraaza-cyclotetradec-12-(*R*)-yl]-succinamide (35). To a solution of Ac-Asp(OtBu)-OH (0.04 g, 0.17 mmol) in DMF (5 mL) were added, at room temperature, HOBt (0.07 g, 0.52 mmol), EDC·HCl (0.033 g, 0.17 mmol), and, after 10 min, **7a** (0.1 g, 0.17 mmol). Stirring of the solution was maintained overnight, then the solvent was evaporated under reduced pressure, and the residue, dissolved in CH_2Cl_2, was washed subsequently with 5% $KHSO_4$, 5% $NaHCO_3$, and water. The organic layer was anhydrified over $MgSO_4$, the solvent was completely removed under reduced pressure, and the crude ester (100 mg) was used without any purification for the deprotecting step. It was directly dissolved in 20 mL of CH_2Cl_2 and treated with TFA (10 mL) for 90 min. The solvent was then removed and the residue treated three times with ethyl acetate and finally with diethyl ether to obtain a white solid (70 mg, approximately 0.09 mmol), which showed a mass spectrum consistent with the deprotected carboxylic acid. The crude product was dissolved in DMF (5 mL), and HOBt (0.039 g, 0.29 mmol), EDC·HCl (0.018 g, 0.09 mmol), and, after 10 min, 2-acetamido-2-deoxy-β-D-glucopyranosyl amine (0.021 g, 0.09 mmol) were added at room temperature. After 12 h at room temperature the mixture was directly purified by preparative HPLC (Symmetry RP-18 column (19 × 300 mm), flow rate 15 mL/min, H_2O + 0.1% TFA/CH_3CN + 0.1% TFA, gradient 70/30 → 30/70 in 60′) to obtain **35** as a lyophile (0.05 g, 0.05 mmol, 30% yield). HPLC: (B) 11.10′, >99% purity. MS (*m/z*) (ES+): 962.4 (MH^+). ^1H NMR (500 MHz, DMSO-d_6): δ 10.79 (d, *J* = 1.9 Hz, 1H), 8.86 (d, *J* = 5.1 Hz, 1H), 8.22 (d, *J* = 9.0 Hz, 1H), 8.17 (d, *J* = 7.0 Hz, 1H),

7.99 (d, J = 8.7 Hz, 1H), 7.78 (d, J = 8.8 Hz, 1H), 7.63 (bs, 1H), 7.57 (bs, 1H), 7.42 (d, J = 8.1 Hz, 1H), 7.33 (d, J = 8.1 Hz, 1H), 7.29−7.15 (m, 10H), 7.06 (t, J = 7.5 Hz, 1H), 7.01−6.96 (m, 2H), 6.69 (d, J = 9.1 Hz, 1H), 4.80 (t, J = 9.4 Hz, 1H), 4.54 (m, 1H), 4.41 (q, J = 6.5 Hz, 1H), 4.23 (m, 1H), 4.15 (m, 1H), 4.02 (m, 1H), 3.64 (d, J = 11.3 Hz, 1H), 3.55 (m, 1H), 3.35 (m, 1H), 3.12−2.99 (m, 4H), 2.79−2.71 (m, 5H), 2.56 (dd, J = 15.7, 5.7 Hz, 1H), 2.43 (dd, J = 15.7, 6.9 Hz, 1H), 2.29 (dd, J = 14.9, 6.1 Hz, 1H), 1.83 (s, 3H).

General Procedure To Obtain Products 36−40: Reductive Amination on the Carbonylic Function of the Starting Sugar Moiety. *N*-(1-(*S*)-{[5-(*S*),8-(*R*)-Dibenzyl-2-(*R*)-(1*H*-indol-3-ylmethyl)-3,6,11,14-tetraoxo-1,4,7,10-tetraaza-cyclotetradec-12-(*R*)-ylamino]-methyl}-2-(*R*),3-(*S*),4-(*R*),5-tetrahydroxy-pentyl)-acetamide (36). To a solution of **7a** (0.041 g, 0.07 mmol) in anhydrous methanol (5 mL) were added acetic acid (0.08 mL, 1.3 mmol), 2-acetamido-2-deoxy-β-D-glucopyranosyl amine (0.018 g, 0.08 mmol), and Na(CN)-BH_3 (0.02 g, 0.3 mmol). After 48 h, the conversion being not more than 25%, further 2-acetamido-2-deoxy-β-D-glucopyranosyl amine (0.018 g, 0.08 mmol) and Na(CN)BH_3 (0.02 g, 0.3 mmol) were added and the reaction mixture was maintained under magnetic stirring at room temperature totally for 7 days. The solution was then acidified to pH 1−2 with HCl 1 N, diluted with water, and concentrated to eliminate methanol, then $NaHCO_3$ was added, and extractions with ethyl acetate were repeated three times. The organic phase, after treatments with brine and $MgSO_4$, was concentrated to dryness, and the residue was purified by preparative HPLC (Symmetry RP-18 column (19 × 300 mm), flow rate 15 mL/min, H_2O + 0.1% TFA/CH_3CN + 0.1% TFA, gradient 90/10 → 60/30 in 60′) to give **36** (0.018, 0.018 mmol, 27% yield) as trifluoroacetate. HPLC: (B) 11.70′, 98% purity. MS (*m/z*) (ES+): 786.3 (MH^+). ^1H NMR (500 MHz, DMSO-d_6): δ 10.87 (bs, 1H), 8.91 (bs, 2H), 8.78 (bs, 1H), 7.76 (bs, 1H), 7.47 (d, J = 7.6 Hz, 1H), 7.44 (bs, 1H), 7.35 (d, J = 8.1 Hz, 1H), 7.26−6.97 (m, 17H), 6.85 (bs, 1H), 5.02 (bs, 1H), 4.56 (bs, 1H), 4.45 (bs, 1H), 4.30 (bs, 1H), 4.20 (bs, 1H), 4.11 (bs, 1H), 4.04 (bs, 2H), 3.80 (bs, 1H), 3.72 (bs, 1H), 3.57 (d, J = 8.3 Hz, 1H), 3.41 (m, 3H), 3.20−3.11 (m, 3H), 2.94−2.72 (m, 6H), 1.86 (s, 3H).

In a similar way starting from **7a** and the correct starting sugar (Table 3) products **37**−**40** were obtained (25−40% yields).

2-(*S*),3-(*R*),5-(*R*),6-Tetrahydroxy-4-(*R*)-(O-α-D-glucopyranosyl)-hexanoic Acid [5-(*S*),8-(*R*)-Dibenzyl-2-(*S*)-(1-*H*-indol-3-ylmethyl)-3,6,11,14-tetraoxo-1,4,7,10-tetraaza-cyclotetradec-12-(*R*)-yl]-amide (41). 7a (0.049 g, 0.084 mmol), PyBOP (0.133 g, 0.25 mmol), 4-*O*-β-D-galactopyranosyl-D-gluconic acid (0.09 g, 0.25 mmol), and diisopropyl ethyl amine (0.13 mL, 0.07 mmol) were dissolved in DMF (1 mL) and stirred for 4 days at room temperature. The crude mixture was then directly passed through preparative HPLC (Vydac C-18 column (19 × 250 mm), flow rate 24 mL/min, H_2O + 0.1% TFA/CH_3CN + 0.1% TFA, gradient 90/10 → 60/40 in 120′) for purification of **41**, which was obtained as a lyophile (0.026 g, 0.032 mmol, 40% yield). HPLC: (C) 3.06′, >99% purity. MS (*m/z*) (ES+): 921.4 (MH^+). ^1H NMR (500 MHz, DMSO-d_6): δ 10.79 (m, J = 2.1 Hz, 1H), 8.81 (d, J = 4.8 Hz, 1H), 7.96 (d, J = 8.9 Hz, 1H), 7.68 (d, J = 6.5 Hz, 1H), 7.55 (t, J = 5.6 Hz, 1H), 7.42 (d, J = 7.9 Hz, 1H), 7.32 (d, J = 8.1 Hz, 1H), 7.29−7.15 (m, 10H), 7.06 (t, J = 7.9 Hz, 1H), 6.99−6.96 (m, 2H), 6.75 (d, J = 9.1 Hz, 1H), 5.32 (bs, 1H), 5.09 (bs, 1H), 4.78 (bs, 1H), 4.67 (m, 1H), 4.55 (bs, 1H), 4.29−4.22 (m, 3H), 4.14 (m, 1H), 4.05−4.00 (m, 2H), 3.71 (m, 2H), 3.60−3.58 (m, 2H), 3.53−3.50 (m, 5H), 3.15 (dd, J = 12.3, 6.3 Hz, 1H), 3.04 (dd, J = 14.5, 4.7 Hz, 1H), 2.80−2.70 (m, 5H), 2.29 (dd, J = 14.5, 7.0 Hz).

General Procedure for the Obtainment of Products 49−57 via Acylation of 7a with Carboxylic Acids 44a−j. Morpholin-4-yl-acetic Acid *tert*-Butyl Ester (43a). *tert*-Butyl bromoacetate (11.19 g, 57 mmol) was dissolved in THF (70 mL) and the solution treated by dropwise addition with an equimolar mixture of morpholine (5 g, 57 mmol) and triethylamine (8 mL, 57 mmol) at such a rate as to maintain a gentle reflux of the solvent. At the end of the addition of the amine, the reaction mixture was refluxed for 2 h. After this time the mixture was cooled to room temperature and then to 0 °C. The suspension was then filtered off by suction and the filtrate evaporated down to dryness. The semisolid residue was then taken up in ethyl acetate (100 mL) and the extract washed with water (100 mL) and then dried over sodium sulfate. Removal of the solvent under reduced pressure gave **43a** (12.11 g, 57 mmol, >98% yield) as a clear yellow brown liquid. This material was used for the next synthetic step without further purification. TLC (ethyl acetate/MeOH/Et_3N 94/5/1): R_f = 0.71. MS (*m/z*) (ES+): 202.1 (MH^+). ^1H NMR (200 MHz, $CDCl_3$): δ 3.66−3.42 (m, 4H), 2.96 (2, 2H), 2.50−2.25 (m, 4H), 1.32 (s, 9H).

Morpholin-4-yl-acetic Acid (44a). The morpholine ester **43a** (2.5 g, 12 mmol) was dissolved in dichloromethane (35 mL), the solution treated with TFA (15 mL), and the mixture stirred for 5 h at room temperature. After this time the solution was evaporated down to dryness and the product crystallized out by treatment of the residue with diisopropyl ether. The off-white crystalline solid **44a** (2.47 g, 12 mmol, 99% yield), filtered off by suction and dried over $CaCl_2$ overnight, was obtained as a nonstoichiometric complex with trifluoroacetic acid and used for the next synthetic step. ^1H NMR (200 MHz, DMSO-d_6): δ 12.65−11.66 (br s, 1H), 4.06 (s, 2H), 3.84−3.73 (m, 4H), 3.30−3.27 (m, 4H).

***N*-[5-(*S*),8-(*R*)-Dibenzyl-2-(*S*)-(1*H*-indol-3-ylmethyl)-3,6,-11,14-tetraoxo-1,4,7,10-tetraaza-cyclotetradec-12-(*R*)-yl]-2-morpholin-4-yl-acetamide Trifluoroacetate (48).** The amino acid **44a** (0.021 g, 0.1 mmol) was dissolved in DMF (5 mL), and the solution was treated with HOBt (0.06 g, 0.45 mmol) and EDC·HCl (0.02 g, 0.1 mmol). After 10 min of stirring the solution was completely clear, **7a** (0.06 g, 0.1 mmol) was added, and the resulting mixture was stirred for 4 h. After that time the solvent was removed under reduced pressure and the residue was purified by preparative HPLC (Symmetry RP-18 column (19 × 300 mm), flow rate: 15 mL/min, H_2O + 0.1% TFA/CH_3CN + 0.1% TFA, gradient 70/30 → 30/70 in 60′) to give **48** (0.015 g, 0.02 mmol, 20%) as lyophile. HPLC: (B) 12.2′, 97.7 purity. MS (*m/z*) (ES+): 708.5 (MH^+). ^1H NMR (500 MHz, DMSO-d_6): δ 10.71 (bs, 1H), 8.62 (bs, 1H), 8.60 (d, J = 5.2 Hz, 1H), 8.04 (bs, 1H), 7.46 (d, J = 7.8 Hz, 1H), 7.34 (d, J = 8.1 Hz, 1H), 7.28−7.24 (m, 7H), 7.19−7.16 (m, 4H), 7.08 (t, J = 7.2 Hz, 1H), 7.07 (m, 1H), 6.99 (t, J = 7.1 Hz, 1H), 6.79 (d, J = 8.8 Hz, 1H), 4.64 (m, 1H), 4.23 (m, 1H), 4.18 (m, 1H), 4.05 (m, 1H), 3.79 (bs, 4H), 3.36 (dd, J = 14.2, 4.1 Hz, 1H), 3.12 (bs, 3H), 2.97 (dd, J = 14.2, 4.1 Hz, 1H), 2.87−2.74 (m, 5H), 2.34 (dd, J = 14.1, 8.3 Hz, 1H).

In a similar way products **49**−**57** were obtained.

Procedure for the Obtainment of the Amine Portion 47 (Precursor of 54). 4-Benzyl-piperazine-1-sulfonic Acid Amide (46). To a solution of benzyl piperazine (14.1 g, 80 mmol) in THF (100 mL) cooled at 0 °C was added dropwise a solution of sulfamoyl chloride (9.25 g, 80 mmol) in THF (250 mL) during a period of 1 h. The reaction mixture was then stirred at room temperature for 4 h and left without stirring overnight. The insoluble solid was then filtered by suction, washed with THF, and treated with a saturated solution of $NaHCO_3$. Extracts with ethyl acetate were collected, washed with brine, and dried over sodium sulfate. The organic phase was then evaporated to dryness under reduced pressure and the resulting solid treated with diethyl ether, filtered, washed, and dried to give 5.24 g (20.8 mmol, 26% yield) of **46**, which was directly used for the deprotecting treatment. TLC (ethyl acetate): R_f = 0.54. HPLC: (A) 3.2′. MS (*m/z*) (ES+): 256.1 (MH^+). ^1H NMR (200 MHz, DMSO-d_6): δ 7.40−7.17 (m, 5H), 6.74 (br s, 2H), 3.48 (s, 2H), 3.04−2.87 (m, 4H), 2.48−2.37 (m, 4H).

Piperazine-1-sulfonic Acid Amide (47). 4-Benzyl-piperazine-1-sulfonic acid amide **46** (1.35 g, 5.3 mmol) was dissolved in methanol (25 mL), and the solution was treated with a suspension of Pd/C 10% (0.15 g) in methanol/H_2O 1/1 v/v (5 mL). The resulting suspension was shaken under H_2 atmosphere at normal pressure for 3 h. During that time 120

mL of H_2 was absorbed. The solution was then filtered off by suction and evaporated to dryness, obtaining a white solid, which was treated with chloroform (20 mL). The unsoluble solid was filtered off, washed again with chloroform (10 mL), and dried to give **47** (0.67 g, 4.05 mmol, 76% yield). TLC (chloroform/MeOH 1:1): R_f = 0.18. MS (*m/z*) (ES+): 166.0 (MH^+). ^{1}H NMR (200 MHz, DMSO-d_6): δ 6.72 (br s, 2H), 2.20 (br s, 2H), 2.86−2.6 (m, 8H).

Acknowledgment. The authors thank Dr. Andrea Raffaelli for performing exact mass measurements.

Supporting Information Available: Table with HPLC purity of all products according to two different methods (retention time, purity). ^{1}H NMR of the remaining final products. LRMS of all final products (MH^+ and significative fragments). HRMS for key compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM040832Y