Regio- and Stereoselective Cycloadditions of Cyclic Nitrones to Maleic Diamide Forced in a Peptide: Synthesis of Potent Ligands of Human NK-2 Receptor

Silvia Valenza, Franca M. Cordero, and Alberto Brandi*

Dipartimento di Chimica Organica "U. Schiff" and Centro CNR dei Composti Eterociclici, Università di Firenze, Via G. Capponi 9, I-50121 Firenze, Italy

Antonio Guidi, Maria Altamura,* Alessandro Giolitti, Francesca Giuntini,† Franco Pasqui, Anna Rita Renzetti, and Carlo Alberto Maggi

Menarini Ricerche S.p.A., Via dei Sette Santi 3, I-50131 Firenze, Italy

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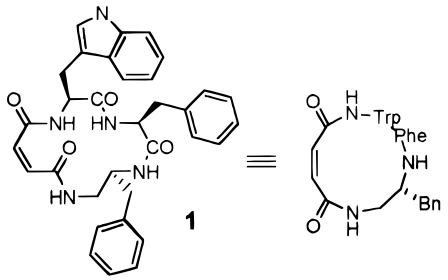
The regioselectivity and the stereoselectivity induced by relatively small peptidomimetic maleic diamide **1** in cycloaddition reactions with cyclic nitrones **2**−**5** was studied. The high regio- and stereoselectivity observed, sensibly increased by nonpolar solvents, was the effect of a double-asymmetric induction produced by the nitrone substituent on the pseudopeptidic tether. A new class of potent human tachykinin NK-2 receptor ligands was synthesized.

Introduction

The tachykinins form a family of peptide neurotransmitters that share a common carboxy terminal sequence, Phe-Xxx-Gly-Leu-Met-NH_2: neurokinin A (NKA, Xxx = Val) is the natural tachykinin with highest affinity for the NK-2 receptor whose activation in mammals is thought to play a role in regulation of airways, gut, and urinary tract motility.[1]

Among the most potent and selective antagonists of NKA at the NK-2 receptor,[2,3] we have focused our attention on the bicyclic peptide MEN 10627, cyclo(-Met-Asp-Trp-Phe-Dap-Leu-)cyclo(2β-5β),[2d,4] and starting from experimental evidence showing that the fragment Trp-Phe in MEN 10627 is crucial for binding with high affinity to the human NK-2 receptor,[5] we were successful in deriving the monocyclic analogue **1** (Chart 1) as a novel high-affinity lead structure. In fact, **1** is only 30 times less potent than MEN 10627 in its ability to bind NK-2 receptor, and it is suitable for further chemical modification because of its olefinic moiety.

Chart 1. The Dipolarophile



As part of a program devoted to the pharmacological investigation of compounds deriving from addition reactions onto the double bond of **1**, we concentrated on nitrones cycloadditions.

It is believed that the chiral environment in proximity of the peptide backbone of relatively small pseudopeptidic or peptidomimetic molecules can behave as an enzyme-like system displaying molecular recognition processes.[6] Actually, experimental results coming from the reduction in size from an enzyme to a small peptide, catalytically used to perform a kinetic resolution of secondary alcohols, have been published only recently by Miller and others.[7] Regarding cycloaddition reactions, to our knowledge examples of facial diastereoselectivity induced by relatively small peptidomimetic molecules have not been reported. We tested, then, this possibility in a challenging problem, that is the regiochemical discrimination in a 1,3-dipolar cycloaddition of a nitrone to an unsymmetrically substituted maleic amide. Concerted nitrone cycloadditions are among the most powerful tools for stereospecific

† On leave from Dipartimento di Chimica Organica "Ugo Schiff", Università di Firenze.

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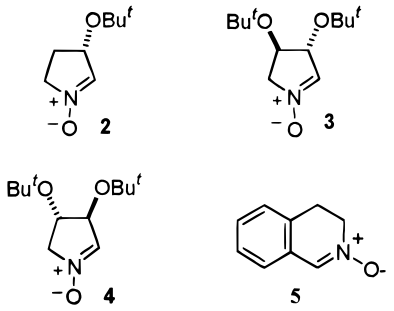
Chart 2. The Nitrones



creation of new chiral centers in organic molecules.[8] Generally, three new contiguous stereogenic centers can be formed in a highly stereospecific manner by the attack of the dipole to olefinic double bonds.

Discussion

To study the regioselectivity and the stereoselectivity of the cycloaddition to an unsymmetrical substituted maleic amide it was necessary to use a nitrone characterized by a high diastereofacial selectivity, to put in evidence the role of the dipolarophile. Cyclic enantiopure hydroxylated nitrones **2**−**4**[9] derived from L-malic acid,[9a] D- and L-tartaric acids,[9b] respectively (Chart 2), were appropriate to the scope, as they are known to approach the dipolarophile in an *exo* fashion selectively anti to the alkoxy group vicinal to the nitrone moiety.[10]

A computational study of compound **1** shows (Figure 1) that the conformations within 5 kcal/mol from the most stable conformer, identified by conformational analysis (simulated annealing, in Sybyl 6.5.3, Tripos, Inc.), exhibit torsion values for φ and ψ compatible with a range of reverse turns. In Figure 1 is reported the most stable conformer, with geometry optimized in MOPAC using the AM1 Hamiltonian, whose φ and ι values are $\varphi\ (i+1) = -96.7°$, $\psi\ (i+1) = -48.4°$, $\varphi\ (i+2) = -122.2°$, $\psi\ (i+2) = 37.7°$. The shape of the pseudopeptide **1** suggests that the indole group and the benzyl group near the maleate moiety, residing on different faces of the dipolarophile, can influence the selectivity in cycloaddition reactions.

The results of the cycloadditions of maleic amide **1** with nitrones **2**−**4**, and with the achiral nitrone **5** for comparison, are reported in Table 1.

The cycloadditions occurred when 0.2 M solutions of reagents in DMSO were heated at 85 °C for several hours. The yields were quantitative or very high in all cases. The occurrence of thermodynamic processes in the cycloadditions at this temperature, common with this kind of dipolarophiles,[11] was excluded by checking the stability of the adducts and the absence of any cross-cycloadduct when compound **6a** was heated at 90 °C in DMSO for long time in the presence of 3-buten-1-ol as a second dipolarophile.

Nitrones **2** (entries 1 and 2) and **3** (entries 3 and 4) gave only two cycloadducts **6a/6b** and **7a/7b** in the same

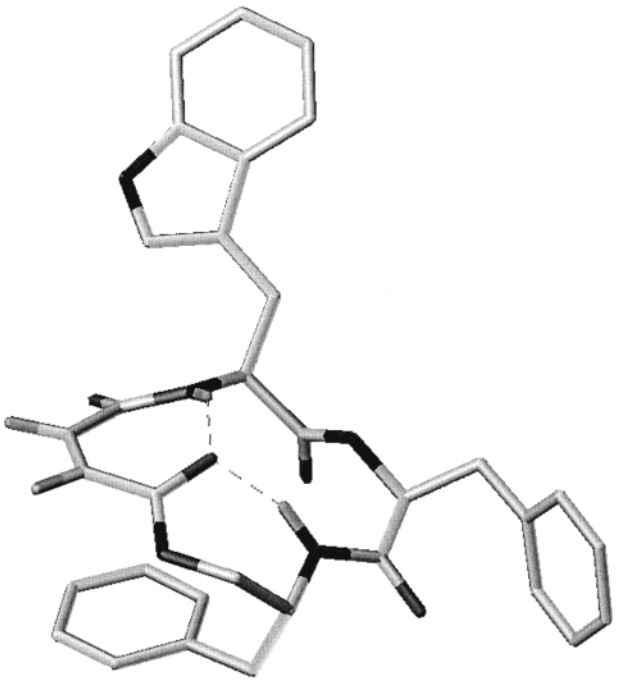


Figure 1. Structure of compound **1** derived by computational study.

4:1 ratio. The reactions run in toluene at the same temperature, where the compounds were only partially soluble, gave good yields of the same adducts only after a longer time, but strikingly afforded compound **6a** with a much higher 25:1 ratio over **6b**.[12] Since the formation of an equilibrium should be excluded, this result has to be explained as a different conformation of the dipolarophile in the two solvents.

The assignment of the structure to the two isomers was possible through the interpretation of a complete array of mono- and bidimensional NMR spectra (Figure 2). The regiochemistry of the cycloadducts was determined from ROESY correlation peaks of the two amidic N−H (clearly distinguishable by their multiplicity) on maleic residue with ex-olefinic protons H_2 and H_3. Correlation peaks of NH_a, H_2 [δ 8.23 (dd, J = 7.6, 5.5 Hz, H_a), 4.86 (d, J = 9.7 Hz, H_2)] and NH_b, H_3 [δ 8.95 (d, J = 3.8 Hz, H_b), 3.78−3.71 (m, H_3)] were observed for **6a**, and these data are congruent for the nitrone oxygen attached to the carbon nearest to the benzyl-diamine residue of the peptide. 2D ROESY correlation peaks of H_4 with H_2 and H_3 suggested that these three protons are on the same side of the molecule and confirm the exo-anti mode of approach of the nitrone. The shape and width of the multiplet for H_{3a} showed a $^3J < 3$ Hz typical for a trans relationship with the two vicinal protons H_4 and H_3. Correlation peaks NH_b, H_2 [δ 8.19 (d, J = 3.9 Hz, H_b), 4.70 (d, J = 9.0 Hz, H_2)] and NH_a, H_3 [δ 8.67 (dd, 7.9, J = 5.2 Hz, H_a), 3.70−3.67 (m, H_3)] were observed for compound **6b**, whereas the isoxazolidine moiety of the molecule exhibited the same correlation peaks as for compound **6a**. These data confirmed that in **6b** the oxygen was linked to the carbon nearest to the Trp residue, and therefore, the nitrone assumed the exo-anti mode of approach in the other regioisomeric arrangement on the opposite face. The same argument applied for structure assignment to diastereoisomers **7a**, **7b**.

The major diastereoisomer **6a** and **7a** derived, then, from favored transition states in which the nitrones

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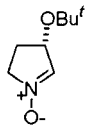
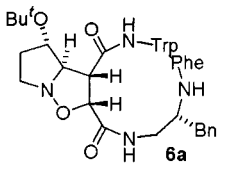
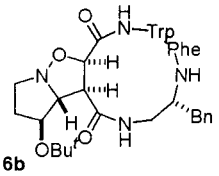
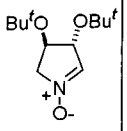
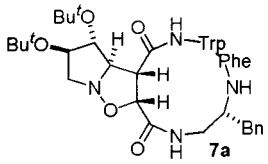
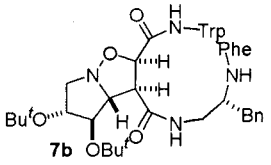
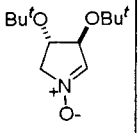
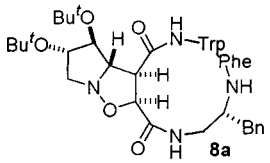
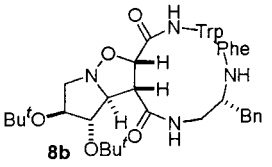
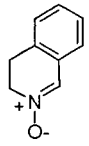
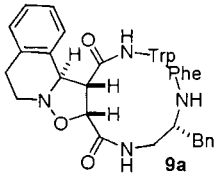
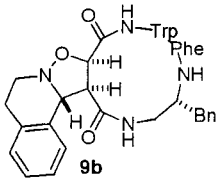
Table 1. Cycloadditions of Nitrones 2−4 and 5 to Maleic Amide 1

Entry	Nitrone	Reaction Conditions	Cycloadducts
Entry 1	 **2**	DMSO (8 h, 99%)	 **6a**  **6b** 4 : 1
Entry 2	**2**	Toluene (27 h, 86 %)	25 : 1
Entry 3	 **3**	DMSO (5 h, 99 %)	 **7a**  **7b** 4 : 1
Entry 4	**3**	Toluene (11 h, 100 %[a])	5 : 1
Entry 5	 **4**	DMSO (5 h, 95 %)	 **8a**  **8b** **8a** : **8b** : **8c** : **8d** 5 : 32 : 2 : 1
Entry 6	**4**	Toluene (18 h, 100 %[a])	9 : 4 : 1 : 2
Entry 7	 **5**	DMSO (5 h, 93 %)	 **9a**  **9b** **9a** : **9b** : **9c** 7 : 3 : 1
Entry 8	**5**	Toluene (11 h, 100 %[a])	30 : **4** : **1**

[a] Quantitative yield of the cycloadducts mixture measured after workup without separation.

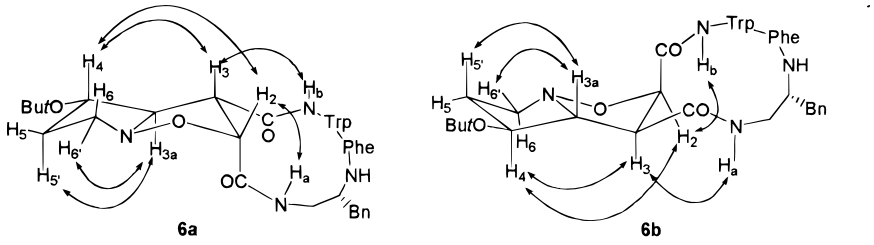


Figure 2. Structural assignment from ROESY correlation peaks

approached the dipolarophile in an exo mode on the face opposite to the indole residue and on their face opposite to the C-3 alkoxy substituent (Figure 3). The matching minimization of steric interactions at the transition state was responsible for the observed regio- and stereoselectivity, with the indole residue of **1** exerting the role of face discriminator. Due to the distance from the reaction site, the indole is not able to completely shield the approach on the other face of the dipolarophile, but this approach is only possible with the opposite regiochemistry, as the nitrone always attack opposite to the C-3 alkoxy substituent. The diastereomeric ratio observed

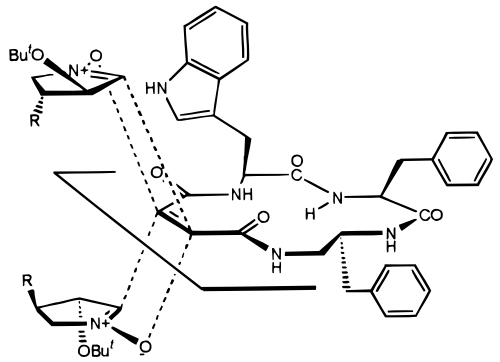


Figure 3. Transition-state trajectories for cycloaddition of **2** and **4** with **1**.

was indeed a measure of the selectivity induced by the indole on discriminating the diastereofaces of the maleic diamide. On changing the reaction solvent from a polar to a nonpolar one, this selectivity increased sensibly (25:1 in toluene vs 4:1 in DMSO) likely as a result of a conformational change of the pseudopeptide. In the nonpolar solvent a strengthening of the interchain hydrogen bonds must locate the indole group closer to the reacting site, increasing its role as face discriminator. *The overall result achieved, then, consisted of a control of the regioselectivity of the cycloaddition to a maleic diamide dictated by the stereochemistry of the used nitrone.*

The reaction with the enantiomeric nitrone **4** added essential confirmations to the interpretation of the results. Nitrone **4** gave with **1** in the same conditions in DMSO (Table 1, entries 5 and 6) a mixture of four cycloadducts **8a**,**b**,**c**,**d** in a 5:32:2:1 ratio, respectively. The major isomer could be isolated and fully characterized and was assigned the structure **8b** via the previous NMR analysis. Clearly, nitrone **4** can approach the most favored face of **1,** opposite to the indole moiety only in the other regioisomeric array. Although the abundance of the major isomer reflected those of entries 1 and 3 (Table 1), the formation of four isomers in this case demonstrates that nitrone **4** and **1** constituted a mismatching pairs. This was also confirmed in the reaction in toluene (entry 6, Table 1), where a much lower selectivity was observed, with a preference for isomer **8a** apparently for the same conformational change caused by the strengthening of hydrogen bonds in **1**.

The preferred transition-state trajectories for this interaction are reported in Figure 4.

The cycloaddition of achiral nitrone **5** gave in the same conditions in DMSO only three cycloadducts **9a**,**b**,**c** (entry 7, Table 1) in 7:3:1 ratio, respectively. In toluene (entry 8, Table 1) the selectivity increased sensibly (30:4:1), in complete analogy with nitrone **2**. The previous results allowed an easy assignment of the structure of the adducts **9a** and **9b** and proved, also for the cyclic nitrone **5**, the preference for an exo approach anti to the indole group of **1** and the attack of the oxygen of the nitrone on the carbon more distant from the triptophan residue, as in **9a**.

The isolated and fully characterized adducts **6a**,**b**, **7a**,**b**, **8b**, and **9a**,**b** were tested as human tachykinin NK-2 receptor ligands. The results for inhibition of binding of [^{125}I]-NKA to the human tachykinin NK-2 receptor are reported in Table 2 as pK_i. It is noteworthy that all the cycloadducts showed pK_i values higher than their olefinic precursor **1**. In addition, affinity shown by

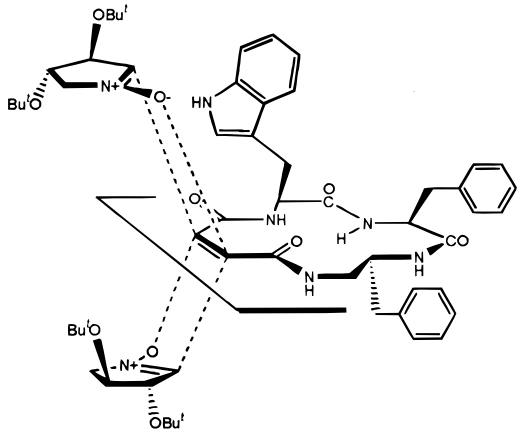


Figure 4. Transition-state trajectories for cycloaddition of **4** with **1**.

Table 2. Inhibition of [^{125}I]NKA Binding by NKA and Human NK-2 Receptor Ligands

	pK_D[a]	pKi[a]
NKA	8.6 ± 0.7	
MEN 10627		9.2 ± 0.10
1		8.0 ± 0.10
6a		9.0 ± 0.22
6b		8.7 ± 0.01
7a		9.0 ± 0.03
7b		9.3 ± 0.15
8b		9.6 ± 0.15
9a		8.7 ± 0.20
9b		8.1 ± 0.13

[a] Mean pK_i and pK_D values ± sem (n = 2−4). pK_i and pK_D are −log of equilibrium dissociation constants for the radioligand (K_D) and for the competing compounds (K_i), respectively. K_D and K_i values were calculated by using EBDA/LIGAND, a nonlinear iterative fitting program.[13]

a number of them (**6a**, **7a**, **7b**, **8b**) was very similar or superior to that of the bicyclic hexapeptide MEN 10627. The correlation between structure and receptor affinity of the compound series suggests some considerations, with the basic assumption, usual when comparing homogeneous series of derivatives, that the binding mode of the common structural feature is conserved. The affinity changes in the regioisomers pairs seem to indicate the importance of an oxygen atom in a proper orientation (cf. **6b** vs **6a**, **7b** vs **7a**), while a good steric allowance exists below and above the main ring plane (cf. **7b** vs **8b**). Finally, the presence of a fourth aromatic group on the peptide molecule does not seem to have any beneficial effect on the affinity for the NK-2 receptor, as is shown by the lower affinity values of both stereoisomers **9a** and **9b**, bearing the tetrahydroisoquinoline moiety condensed with the isoxazolidine.

Conclusions

A new class of potent human tachykinin NK-2 receptor ligands was synthesized and the study added information about the structural features necessary to increase the binding affinity toward the NK-2 receptor, within the class of ligands based on a Trp-Phe structural determinant.

The indole group on the cyclic pseudopeptidic compound **1** was able to discriminate the face selectivity in a cyclic maleic derivative, inducing consequently an high to excellent control of the regioselectivity of the cyclo-

addition of nitrones to a maleic diamide. Although this effect could be exerted by other chiral auxiliaries in a cyclic arrangement, the excellent results obtained are absorbable to the presence of the small peptide, whose role is particularly evident in the enhancement of the selectivity on changing the solvent nature. This effect on the diastereoselectivity of nitrone cycloadditions is, to our knowledge, unprecedented and, as a general concept, can find useful applications in asymmetric syntheses.

Experimental Section

Proton NMR spectra were recorded on a 500 MHz instrument in deuterated DMSO and carbon-13 NMR spectra were recorded on a 200 MHz instrument in $CDCl_3$. HPLC analyses were performed with a LUNA C8 5 μm (2) column (25 cm × 4.6 mm); eluents: (A) H_2O (Milli Q, TFA 0.1%), (B) CH_3CN (0.1% TFA); gradient: B from 20% to 80%in 20 min then 10 min at the last concentration. MS spectra were recorded with a ESI ion source.

1,3-Dipolar Cycloadditions in DMSO. A solution of dipolarophile **1** (0.10 mmol) and nitrones **2**−**5** (0.12 mmol) in DMSO (0.6 mL) was heated at 84 °C until the alkene was completely consumed as monitored by HPLC and TLC (see Table 1). The reaction mixture was cooled at room temperature and diluted with H_2O. Solvents were removed by lyophilization, and cycloadducts were isolated by flash column chromatography (FCC) on silica gel as white solids.

1,3-Dipolar Cycloadditions in Toluene. A suspension of dipolarophile **1** (0.10 mmol) and nitrones **2**−**5** (0.12 mmol) in toluene (0.6 mL) was heated at 87 °C until the alkene was completely consumed as monitored by HPLC and TLC (see Table 1). The reaction mixture was cooled at room temperature and diluted with MeOH. Solvents were removed on a rotavapor, and cycloadducts were isolated by FCC on silica gel as white solids.

6a: R_f = 0.60 (Et_2O−AcOEt−MeOH 100:50:1); t_R = 23.8 min; mp 205−207 °C ($CHCl_3$); $[\alpha]^{26}_D$ = −118.2 (c = 0.5, $CHCl_3$); ^{1}H NMR δ 10.82 (d, J = 1.4 Hz, 1H), 8.95 (d, J = 3.8 Hz, 1H), 8.23 (dd, J = 7.6, 5.5 Hz, 1H), 7.39 (d, J = 7.5 Hz, 1H), 7.33−7.12 (m, 12H), 7.09−7.05 (m, 2H), 6.99 (dd, J = 7.5, 7.2 Hz, 1H), 6.76 (d, J = 9.5 Hz, 1H), 4.87 (d, J = 9.7 Hz, 1H), 4.56 (ddd, J = 13.8, 11.5, 3.7 Hz, 1H), 4.05−4.00 (m, 1H), 3.95 (dt, J = 10.7, 3.8, Hz, 1H), 3.90 (br m, 1H), 3.78−3.71 (m, 2H), 3.50−3.47 (m, 2H), 3.17 (ddd, J = 11.5, 6.7, 6.0 Hz, 1H), 3.00 (dt, J = 11.5, 7.4 Hz, 1H), 2.87−2.77 (m, 4H), 2.62 (dd, J = 14.4, 11.5 Hz, 1H), 2.57 (dd, J = 14.8, 10.7 Hz, 1H), 2.22 (ddd, J = 13.3, 7.4, 6.0 Hz, 1H), 1.56−1.50 (m,1H), 1.36 (s, 9H); ^{13}C NMR δ 172.2 (s, 1C), 171.6 (s, 2C), 169.9 (s, 1C), 138.7 (s, 1C), 138.1 (s, 1C), 136.3 (s, 1C), 129.4 (d, 2C), 129.0 (d, 2C), 128.8 (d, 2C), 128.2 (d, 2C), 127.2 (s, 1C), 126.6 (d, 1C), 126.2 (d, 1C), 123.2 (d, 1C), 122.6 (d, 1C), 120.1 (d, 1C), 117.7 (d, 1C), 111.6 (d, 1C), 109.3 (s, 1C), 78.9 (d, 1C), 77.5 (d, 1C), 77.0 (d, 1C), 73.8 (s, 1C), 57.4 (d, 1C), 56.8 (d, 1C), 54.8 (t, 1C), 52.2 (d, 1C), 51.7 (d1C), 41.0 (t, 1C), 37.4 (t, 1C), 35.5 (t, 1C), 33.2 (t, 1C), 28.1 (q, 3C), 26.2 (t, 1C); ESI-MS m/z 721 ($MH^{\bullet+}$); ESI-MS/MS m/z 665 (47), 564 (100). Anal. Calcd for $C_{41}H_{48}N_6O_6$: C, 68.31; H, 6.71; N, 11.66. Found: C, 68.27; H, 6.31; N, 11.83.

6b: R_f = 0.80 (Et_2O−AcOEt−MeOH 100:50:1); t_R = 25.1 min; mp 129−130 °C ($CHCl_3$); $[\alpha]^{26}_D$ = −161.0 (c = 0.4, $CHCl_3$); ^{1}H NMR δ 10.90 (d, J = 2.0 Hz, 1H), 8.67 (dd, J = 7.9, 5.2 Hz, 1H), 8.19 (d, J = 3.9 Hz, 1H), 7.46 (d, J = 7.9 Hz, 1H), 7.35 (d, J = 8.1 Hz, 1H), 7.29−7.17 (m,10H), 7.12 (d, J = 2.0 Hz, 1H), 7.08 (dd, J = 8.1, 7.8 Hz, 1H), 7.05 (d, J = 9.9 Hz, 1H), 7.00 (dd, J = 7.9, 7.1 Hz, 1H), 6.81 (d, J = 9,5 Hz, 1H), 4.70 (d, J = 9.0 Hz, 1H), 4.55 (td, J = 10.7, 3.8 Hz, 1H), 4.05−3.99 (m, 2H), 3.97 (dt, J = 10.7, 3.8 Hz, 1H), 3.78 (ddd, J = 13.2, 7.9, 2.0 Hz, 1H), 3.70−3.67 (m, 2H), 3.43 (dd, J = 14.4, 3.8 Hz, 1H), 3.11 (dt, J = 12.6, 7.6 Hz, 1H), 2.96 (ddd, J = 12.6, 7.1, 5.5 Hz, 1H), 2.90−2.69 (m, 5H), 2.60 (dd, J = 14.4, 10.7 Hz, 1H), 2.21−2.14 (m,1H), 0.59−1.53 (m,1H), 1.10 (s, 9H); ^{13}C NMR δ 172.4 (s, 1C), 171.7 (s, 1C), 170.5 (s, 2C), 138.5 (s, 1C), 138.1 (s, 1C), 136.2 (s, 1C), 129.4 (d, 2C), 128.9 (d, 2C), 128.7 (d, 2C), 128.2 (d, 2C), 127.3 (s, 1C), 126.6 (d, 1C), 126.2 (d, 1C), 123.0 (d, 1C), 122.3 (d, 1C), 119.9 (d, 1C), 118.4 (d, 1C), 111.3 (d, 1C), 109.8 (s, 1C), 78.7 (d, 1C), 77.6 (d, 1C), 77.0 (d, 1C), 73.9 (s, 1C), 58.1 (d, 1C), 56.6 (d, 1C), 54.6 (t, 1C), 52.2 (d, 1C), 51.3 (d, 1C), 42.2 (t, 1C), 37.2 (t, 1C), 35.6 (t, 1C), 33.2 (t, 1C), 28.4 (q, 3C), 26.5 (t, 1C); ESI-MS m/z 721($MH^{\bullet+}$); ESI-MS/MS m/z 665 (100), 564 (5). Anal. Calcd for $C_{41}H_{48}N_6O_6$: C, 68.31; H, 6.71; N, 11.66. Found: C, 68.52; H, 7.05; N, 11.46.

7a: R_f = 0.60 (Et_2O−acetone 5:1); t_R = 27.0 min; mp 198−201 °C ($CHCl_3$); $[\alpha]^{27}_D$ = −84.2 (c = 0.7, $CHCl_3$); ^{1}H NMR δ 10.83 (d, J = 2.0 Hz, 1H), 8.95 (d, J = 4.0 Hz, 1H), 8.25 (dd, J = 7.7, 5.5 Hz, 1H), 7.39 (d, J = 7.8 Hz, 1H), 7.33−7.16 (m, 11H), 7.12 (d, J = 2.0 Hz, 1H), 7.10−7.05 (m, 2H), 6.98 (dd, J = 7.8, 7.2 Hz, 1H), 6.73 (d, J = 9.6 Hz, 1H), 4.98 (d, J = 10.0 Hz, 1H), 4.55 (ddd, J = 11.7, 10.5, 3.6 Hz, 1H), 4.04−3.99 (m, 1H), 3.88 (dt, J = 10.4, 3.6 Hz, 1H), 3.85 (dd, J = 10.0, 7.5 Hz, 1H), 3.81−3.71 (m, 2H), 3.61 (d, J = 3.4 Hz, 1H), 3.48 (dd, J = 14.6, 3.6 Hz, 1H), 3.38 (d, J = 7.5 Hz, 1H), 3.33−3.29 (m, 1H), 2.86−2.75 (m, 4H), 2.67 (t, J = 9.1 Hz, 1H), 2.62 (dd, J = 14.6, 11.7 Hz, 1H), 2.55 (dd, J = 14.8, 10.4 Hz, 1H), 1.11 (s, 9H), 0.59 (s, 9H); ^{13}C NMR δ 172.2 (s, 1C), 171.4 (s, 1C), 171.0 (s, 1C), 169.8 (s, 1C), 138.5 (s, 1C), 137.8 (s, 1C), 136.4 (s, 1C), 129.3 (d, 2C), 128.9 (d, 2C), 128.8 (d, 2C), 128.2 (d, 2C), 127.0 (s, 1C), 126.6 (d, 1C), 126.3 (d, 1C), 123.2 (d, 1C), 122.7 (d, 1C), 120.2 (d, 1C), 117.7 (d, 1C), 111.7 (d, 1C), 109.3 (s, 1C), 81.0 (d, 1C), 79.2 (d, 1C), 77.6 (d, 1C), 75.8 (d, 1C), 74.3 (s, 1C), 74.2 (s, 1C), 60.6 (t, 1C), 56.7 (d, 1C), 56.6 (d, 1C), 52.3 (d, 1C), 51.4 (d, 1C), 41.1 (t, 1C), 37.4 (t, 1C), 35.6 (t, 1C),28.3 (q, 3C), 27.9 (q, 3C), 26.4 (t, 1C); ESI-MS m/z 793 ($MH^{\bullet+}$); ESI-MS/MS m/z 737 (42), 564 (100). Anal. Calcd for $C_{45}H_{56}N_6O_7$: C, 68.16; H, 7.12; N, 10.60. Found: C, 68.16; H, 7.24; N, 10.55.

7b: R_f = 0.85 (Et_2O−acetone 5:1); t_R = 27.7 min; mp 132−134 °C ($CHCl_3$); $[\alpha]^{29}_D$ = −104.5 (c = 0.3, $CHCl_3$); ^{1}H NMR δ 10.87 (d, J = 2.0 Hz, 1H), 8.71 (dd, J = 7.3, 5.6 Hz, 1H), 8.02 (d, J = 4.5 Hz, 1H), 7.45 (d, J = 8.0 Hz, 1H), 7.35 (d, J = 8.0 Hz, 1H), 7.29−7.16 (m, 10H), 7.12 (d, J = 2.0 Hz, 1H), 7.09 (d, J = 9.9 Hz, 1H), 7.08 (dd, J = 8.0, 7.0 Hz, 1H), 6.99 (dd, J = 8.0, 7.1 Hz, 1H), 6.71 (d, J = 9.6 Hz, 1H), 4.93 (d, J = 9.9 Hz, 1H), 4.52 (ddd, J = 11.1, 9.9, 3.7 Hz, 1H), 4.04−3.99 (m, 2H), 3.86−3.83 (m, 2H), 3.77 (dd, J = 9.9, 7.0 Hz, 1H), 3.69 (ddd, J = 13.6, 7.3, 2.0 Hz, 1H), 3.58 (dd, J = 7.0, 1.3 Hz, 1H), 3.43 (dd, J = 14.6, 3.7 Hz, 1H), 3.31−3.38 (m, 1H), 2.93 (dd, J = 14.7, 10.6 Hz, 1H), 2.87−2.78 (m, 2H), 2.72 (dd, J = 13.8, 5.5 Hz, 1H), 2.67−2.64 (m, 2H), 2.60 (dd, J = 14.6, 11.1 Hz, 1H), 1.10 (s, 9H), 1.06 (s, 9H); ^{13}C NMR δ 171.5 (s, 1C), 171.3 (s, 2C), 170.1 (s, 1C), 138.3 (s, 1C), 137.9 (s, 1C), 136.4 (s, 1C), 129.4 (d, 2C), 128.8 (d, 4C), 128.1 (d, 2C), 127.0 (s, 1C), 126.6 (d, 1C), 126.2 (d, 1C), 122.9 (d, 1C), 122.4 (d, 1C), 119.8 (d, 1C), 118.6 (d, 1C), 111.2 (d, 1C), 109.8 (s, 1C), 80.9 (d, 1C), 78.9 (d, 1C), 78.0 (d, 1C), 75.2 (d, 1C), 74.5 (s, 1C), 74.3 (s, 1C), 60.6 (t, 1C), 56.6 (d, 1C), 55.7 (d, 1C), 52.2 (d, 1C), 51.1 (d, 1C), 42.1 (t, 1C), 37.1 (t, 1C), 35.6 (t, 1C), 28.4 (q, 3C), 28.3 (q, 3C), 26.9 (t, 1C); ESI-MS: m/z: 793 ($MH^{\bullet+}$); ESI-MS/MS m/z 737 (100), 564 (57). Anal. Calcd for $C_{45}H_{56}N_6O_7$: C, 68.16; H, 7.12; N, 10.60. Found: C, 68.05; H, 7.59; N, 10.40.

8a + 8d: R_f = 0.26 (Et_2O−$CHCl_3$−MeOH 50:50:1); t_R = 28.4 min; ^{1}H NMR δ 10.94 (br s, isom. **8d** 1H), 10.80 (br s, isom. **8a** 1H), 8.93 (d, J = 3.1 Hz, isom. **8a** 1H), 8.81−8.74 (br m, isom. **8d** 1H), 8.15 (dd, J = 7.4, 5.3 Hz, isom. **8a** 1H), 8.19 (br d, J = 2.8 Hz, isom. **8d** 1H), 7.34 (d, J = 8.0 Hz, isom. **8a** 1H), 7.44 (d, J = 8.0 Hz, isom. **8d** 1H), 7.30−7.15 (m, isom. **8a** 12H, isom. **8d** 12H), 7.10−6.99 (m, isom. **8a** 3H, isom. **8d** 5H), 6.96 (t, J = 7.2 Hz, isom. **8a** 1H), 6.85 (d, J = 9.5 isom. **8d** 1H), 6.77 (d, J = 9.2 Hz, isom. **8a** 1H), 4.78 (d, J = 9.3 Hz, isom. **8a** 1H), 4.68 (d, J = 9.9 isom. **8d** 1H), 4.60−4.53 (m, isom. **8a** 1H, isom. **8d** 1H), 4.04−3.90 (m, isom. **8a** 6H, isom. **8d** 6H), 3.80−3.76 (m, isom. **8a** 1H, isom. **8d** 13.29 (dd, J = 13.0, 6.8 Hz, isom. **8a** 1H), 3.08−3.03 (m, isom. **8d** 1H), 2.90−2.67 isom. **8a** 5H, isom. **8d** 5H), 2.63−2.56 (m, isom. **8a** 2H, isom. **8d** 2H), 1.10 (s, isom. **8d** 9H), 1.08 (s, isom. **8a** 9H, isom. **8d** 9H), 0.75 (s, isom. **8a** 9H); ESI-MS m/z 793 ($MH^{\bullet+}$); ESI-MS/MS m/z 737 (100), 564 (66). Anal. Calcd for $C_{45}H_{56}N_6O_7$: C, 68.16; H, 7.12; N, 10.60. Found: C, 68.08, H, 7.44, N, 10.95.

8b: R_f = 0.30 (Et_2O−$CHCl_3$−MeOH 10:10:1); t_R = 25.5 min; mp 178−179 °C ($CHCl_3$); $[\alpha]^{29}_D$ = −13.7 (c = 0.7, $CHCl_3$); ^{1}H

NMR δ 10.85 (d, J = 2.0 Hz, 1H), 8.72 (d, J = 4.7 Hz, 1H), 7.79 (br d, J = 4.5 Hz, 1H), 7.56 (br t, J = 7.5 Hz, 1H), 7.42 (d, J = 8.0 Hz, 1H), 7.35 (d, J = 8.0 Hz, 1H), 7.30−7.10 (m, 10H), 7.09 (dd, J = 8.0, 7.3 Hz, 1H), 7.00 (dd, J = 8.0, 7.3 Hz, 1H), 6.97 (d, J = 2.0 Hz, 1H), 6.81 (d, J = 8.5 Hz, 1H), 4.94 (d, J = 6.9 Hz, 1H), 4.40 (td, J = 9.9, 4.5 Hz, 1H), 4.15 (dt, J = 9.5, 4.7 Hz, 1H), 3.93 (ddd, J = 17.5, 8.5, 3.8 Hz, 1H), 3.83 (m, 2H), 3.71 (dd, J = 4.2, 3.4 Hz, 1H), 3.61 (dd, J = 6.9, 4.2 Hz, 1H), 3.40−3.36 (m, 2H), 3.31−3.26 (m, 1H), 3,16 (ddd, J = 13.5, 7.5, 3.8 Hz, 1H), 2.91−2.81 (m, 3H), 2.77−2.71 (m, 2H), 2.67 (dd, J = 14.2, 10.6 Hz, 1H), 1.15 (s, 9H), 1.13 (s, 9H); ^{13}C NMR 171.5 (s, 1C), 171.4 (s, 1C), 170.1 (s, 1C), 169.5 (s, 1C), 138.5 (s, 1C), 138.0 (s, 1C), 136.2 (s, 1C), 129.4 (d, 2C), 129.1 (d, 2C), 128.7 (d, 2C), 128.2 (d, 2C), 127.3 (s, 1C), 126.5 (d, 1C), 126.3 (d, 1C), 122.9 (d, 1C), 122.6 (d, 1C), 120.0 (d, 1C), 117.7 (d, 1C), 111.7 (d, 1C), 109.3 (s, 1C), 83.5 (d, 1C), 78.6 (d, 1C), 76.6 (d, 1C), 75.7 (d, 1C), 74.7 (s, 1C), 74.4 (s, 1C), 60.5 (t, 1C), 59.1 (d, 1C), 56.6 (d, 1C), 52.3 (d, 1C), 51.5 (d, 1C), 41.1 (t, 1C), 37.2 (t, 1C), 35.7 (t, 1C), 28.7 (q, 3C), 28.3 (q, 3C), 26.1 (t, 1C); ESI-MS m/z 793 ($MH^{\bullet+}$); ESI-MS/MS m/z 737 (41), 564 (100). Anal. Calcd for $C_{45}H_{56}N_6O_7$: C, 68.16; H, 7.12; N, 10.60. Found: C, 67.81; H, 7.28; N, 10.36.

9a: R_f = 0.50 (Et_2O−$CHCl_3$−MeOH 4:4:1); t_R = 24.5 min; mp 173−175 °C ($CHCl_3$); $[\alpha]^{29}_D$ = −44−6 (c = 0.8, $CHCl_3$); ^{1}H NMR δ 10.83 (br s, 1H), 9.04 (d, J = 3.9 Hz, 1H), 8.54 (dd, J = 7.0, 5.9 Hz, 1H), 7.40 (d, J = 7.9 Hz, 1H), 7.37 (d, J = 7.9 Hz, 1H), 7.32−7.08 (m, 15H), 7.03 (t, J = 7.9 Hz, 1H), 6.88 (d, J = 9.6 Hz, 1H), 6.82 (dt, J = 8.0, 4.0 Hz, 1H), 6.47 (d, J = 7.7 Hz, 1H), 5.03 (d, J = 10.3 Hz, 1H), 4.69 (d, J = 9.2 Hz, 1H), 4.57 (ddd, J = 11.5, 9.6, 3.5 Hz, 1H), 4.37 (dt, J = 11.5, 3.9 Hz, 1H), 4.05 (br t, J = 9.5 Hz, 1H), 3.83 (ddd, J = 13.2, 7.0, 2.0 Hz, 1H), 3.77 (dd, J = 10.3, 9.2 Hz, 1H), 3.50 (dd, J = 14.6, 3.5 Hz, 1H), 3.27−3.24 (m, 1H), 3.01−2.81 (m, 7H), 2.63 (dd, J = 14.6, 11.5 Hz, 1H), 2.56 (dd, J = 15.3, 11.5 Hz, 1H); ^{13}C NMR δ 171.9 (s, 1C), 171.4 (s, 1C), 170.5 (s, 1C), 169.5 (s, 1C), 138.5 (s, 1C), 137.7 (s, 1C), 136.3 (s, 2C), 132.7 (s, 1C), 131.9 (s, 1C), 129.2 (d, 1C), 128.8 (d), 128.4 (d, 1C), 128.2 (d, 1C), 127.4 (d, 1C), 126.7 (d, 1C), 126.2 (d, 1C), 122.9 (d, 1C), 122.7 (d, 1C), 120.1 (d, 1C), 117.9 (d, 1C), 111.5 (d, 1C), 109.0 (s, 1C), 79.3 (d, 1C), 67.9 (d, 1C), 59.4 (d, 1C), 56.3 (d, 1C), 52.4 (d, 1C), 51.5 (d, 1C), 48.8 (t, 1C), 41.5 (t, 1C), 37.4 (t, 1C), 35.8 (t, 1C), 28.4 (t, 1C), 26.4 (t, 1C); ESI-MS m/z 711 ($MH^{\bullet+}$); ESI-MS/MS m/z 564 (100), 536 (40), 435 (25), 231 (37). Anal. Calcd for $C_{42}H_{42}N_6O_5$: C, 70.97; H, 5.96; N, 11.82. Found: C, 70.99; H, 5.88; N, 12.21.

9b: R_f = 0.70 (Et_2O−$CHCl_3$−MeOH 4:4:1); t_R = 25.1 min; mp 150−152 °C ($CHCl_3$); $[\alpha]^{30}_D$ = −102.7 (c = 0.15, $CHCl_3$); ^{1}H NMR δ 10.89 (d, J = 1.7 Hz, 1H), 8.72 (dd, J = 7.5, 5.3 Hz, 1H), 8.26 (d, J = 4.2 Hz, 1H), 7.46 (d, J = 7.9 Hz, 1H), 7.36 (d, J = 8.1 Hz, 1H), 7.23−7.18 (m, 15H), 7.09 (dd, J = 8.1, 7.1 Hz, 1H), 7.00 (dd, J = 7.9, 7.1 Hz, 1H), 6.91−6.87 (m, 1H), 6.83 (d, J = 9.6 Hz, 1H), 5.01 (d, J = 10.6 Hz, 1H), 4.91 (d, J = 9.4 Hz, 1H), 4.53 (ddd, J = 10.7, 10.3, 3.5 Hz, 1H), 4.07−4.01 (m, 1H), 4.05 (dt, J = 10.8, 4.2, Hz, 1H), 3.92 (ddd, J = 13.4, 7.5, 2.0 Hz, 1H), 3.72 (dd, J = 10.6, 9.4 Hz, 1H), 3.48 (dd, J = 144.4, 3.5 Hz, 1H), 3.24−3.22 (m, 1H), 3.03−2.82 (m, 6H), 2.73−2.67 (m, 3H); ^{13}C NMR δ 173.5 (s, 1C), 172.9 (s, 1C), 172.8 (s, 1C), 171.8 (s, 1C), 140.2 (s, 2C), 139.8 (s, 1C), 138.5 (s, 1C), 135.1 (s, 1C), 134.1 (s, 1C), 131.6 (d, 2C), 130.8 (d, 4C), 130.4 (d, 1C), 130.1 (d, 2C), 129.6 (d, 1C), 128.9 (d, 1C), 128.7 (d, 2C), 128.2 (d, 1C), 124.8 (d, 1C), 124.6 (d, 1C), 122.0 (d, 1C), 120.8 (d, 1C), 113.3 (d, 1C), 112.1 (s, 1C), 80.9 (d, 1C), 68.8 (d, 1C), 61.3 (d, 1C), 57.6 (d, 1C), 54.1 (d, 1C), 53.2 (d, 1C), 50.7 (t, 1C), 44.1 (t, 1C), 39.2 (t, 1C), 37.5 (t, 1C), 30.3 (t, 1C), 29.2 (t, 1C); ESI-MS m/z 711 ($MH^{\bullet+}$); ESI-MS/MS m/z 564 (100), 540 (81). Anal. Calcd for $C_{42}H_{42}N_6O_5$: C, 70.97; H, 5.96; N, 11.82. Found: C, 70.90; H, 6.31; N, 11.66.

9c: R_f = 0.25 (Et_2O−$CHCl_3$−MeOH 4:4:1); t_R = 22.9 min; mp 172−174 °C ($CHCl_3$); ^{1}H NMR δ 10.90 (s, 1H), 8.85 (br s, 1H), 8,35 (br s, 1H), 7.42 (d, J = 8.1 Hz, 1H), 7.31−7.28 (m, 1H), 7.24−7.10 (m, 10H), 7.04 (br s, 1H), 6.97 (dd, J = 7.6, 7.3 Hz, 1H), 6.93 (d, J = 7.6 Hz, 1H), 6.86 (br s, 1H), 6.70 (br m, 1H), 6.58 (br s, 1H), 6.14 (br s, 1H), 4.92 (d, J = 9.6 Hz, 1H), 4.87 (br s, 1H), 4.53−4.43 (m, 2H), 4.23 (br m, 1H), 4.10−4.06 (m, 1H), 3.76 (ddd, J = 13.7, 8.3, 2.0 Hz, 1H), 3.51−3.45 (m, 1H), 3.40 (br s, 1H), 3.28−3.25 (m, 2H), 2.91−2.67 (m, 5H), 2.63−2.57 (m, 2H), 2.45−2.40 (m, 1H); ^{13}C NMR δ 171.8 (s, 1C), 171.0 (s, 2C), 169.5 (s, 1C), 138.5 (s, 2C), 138.0 (s, 1C), 137.9 (s, 1C), 136.2 (s, 1C), 134.3 (s, 1C), 129.2 (d), 128.9 (d), 128.6 (d), 128.5 (d, 1C), 128.2 (d), 127.4 (d, 1C), 126.8 (d, 1C), 126.2 (d, 1C), 125.8 (d, 1C), 123.1 (d, 1C), 122.8 (d, 1C), 120.3 (d, 1C), 117.6 (d, 1C), 11.7 (s, 1C), 81.1 (d, 1C), 56.3 (d, 1C), 52.2 (d, 1C), 51.1 (d, 1C), 41.0 (t, 1C), 37.4 (t, 1C), 35.9 (t, 1C), 29.7 (t, 1C), 28.1 (t, 1C), 25.8 (t, 1C); ESI-MS m/z 711 ($MH^{\bullet+}$); ESI-MS/MS m/z 564 (100), 540 (73). Anal. Calcd for $C_{42}H_{42}N_6O_5$: C, 70.97; H, 5.96; N, 11.82. Found: C, 70.91; H, 6.10; N, 12.05.

Drugs and Solutions. [^{125}I]NKA (specific activity, 2000 Ci mmol^{-1}) was purchased from Amersham (Amersham, U.K.). All the competing compounds were dissolved in DMSO (10 mM, stock solution) and then appropriately diluted in buffer B. Other reagents were of the highest purity available from commercial sources.

Binding Assays. CHO cells transfected with the human NK-2 receptor were provided by Dr. J. E. Krause (Washington University, School of Medicine, St. Louis, MO). Confluent cells from four petri dishes were harvested in phosphate-buffered saline, pelleted by centrifugation at 200g (4 °C), and homogenized using a Polytron PT3000 (Kinematica) at 13 000 rpm for 15 s, in 20 mL of 50 mM Tris-HCl buffer, pH 7.4, containing bacitracin (0.1 mg ml^{-1}), chymostatin (0.01 mg ml^{-1}), leupeptin (0.005 mg ml^{-1}), and 10 μM thiorphan (buffer A). The homogenate was centrifuged for 1 h at 25000g (4 °C) and the pellet resuspended in the binding buffer, composed of buffer A supplemented with 150 mM NaCl, 5 mM $MnCl_2$ and 0.1% bovine serum albumin (buffer B), at a protein concentration of ca. 0.35 mg ml^{-1}).[14] The membranes (70 μg protein/assay) were incubated for 30 min at 20 °C with 150 pM [^{125}I]NKA and various concentrations (0.01 nM−1 μM) of cold NKA (saturation experiments) or 10 different concentrations (0.01 nM−10 μM) of the competing compounds (competition experiments), in a final volume of 0.5 mL. All the experiments were performed in duplicate. Unlabeled NKA (1 μM) was used for defining nonspecific binding. The reaction was terminated by the addition of 4 mL of ice-cold 50 mM Tris-HCl buffer, pH 7.4, followed by rapid filtration through Whatman GF/B filter sheets (presoaked in 0.5% bovine serum albumin for at least 3 h) using a Brandel cell harvester. The filters were washed 3-fold with 4 mL of the same ice-cold buffer. The trapped radioactivity was determined using a γ-counter (Cobra, Canberra- Packard BioScience srl).

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(12) The only slight difference observed for the **7a**/**7b** formation in toluene (5:1 vs 4:1) might be due to competing steric hindrance induced by the added *tert*-butoxy group on nitrone **3**.

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