

Synthesis and binding affinity studies of muscarinic receptor antagonists related to dehydrohimbacine

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Dedicated to Professor Georges Hoornaert on his 65th birthday

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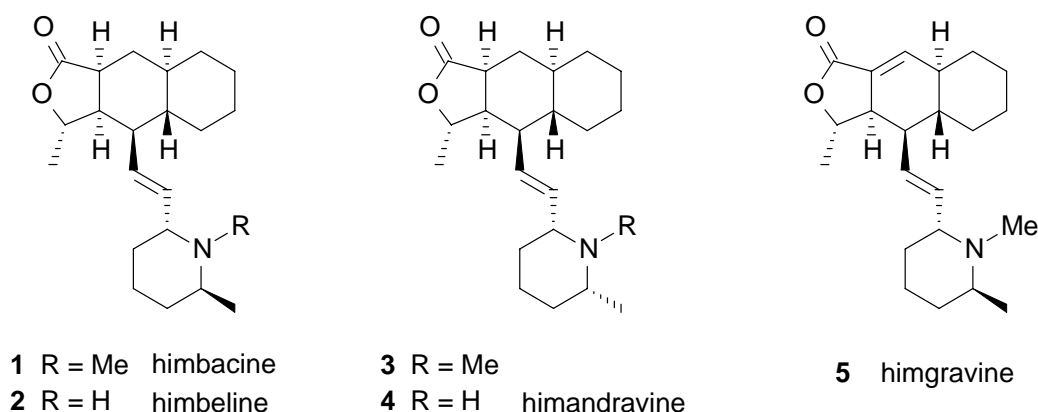
Abstract

The intramolecular Diels–Alder reaction of bromo-substituted nonatrienoate **24** leads to a mixture of the *anti*-adducts **25a** and **25b**, in which the *trans*-fused lactone **25b**, the result of an expected *exo*-addition, predominates (6.2:1 stereoselectivity). Further introduction of a 2,6-*trans*-disubstituted piperidine ring (**26** and **27**) via palladium catalyzed cross-coupling reactions (Stille and Sonogashira) affords intermediates that are easily transformed into dehydrohimbacine derivatives **14a**, **14b**, **15a**, and **15b**. Binding affinity studies for the muscarinic receptors M₁, M₂, M₃, and M₄ show that **15a** possesses a 18-fold selectivity for the M₂ relative to the M₁ receptor, but with concomitant loss in affinity compared to the naturally occurring (+)-himbacine (**1**), a recognized muscarinic receptor antagonist.

Keywords: Himbacine, muscarinic receptor, synthesis

Introduction

Since memorable times natural products with characteristic biological activities have constituted attractive targets for total synthesis, especially when their structures offer some challenging features with regard to connectivity, functionality and/or stereochemistry. In this context recently there has been interest in the synthesis of a series of piperidine alkaloids **1-5** (Figure 1), several of which have been isolated from the bark of *Galbulimima baccata*, a species belonging to the magnolia family found in New Guinea and North Queensland.¹ The structure of (+)-himbacine (**1**),² the most representative member of the family, was determined in 1962 via an X-ray diffraction study of the corresponding hydrobromic salt.³

**Figure 1**

Medicinal interest in himbacine originates from the finding that it is a potent muscarinic receptor antagonist that displays selectivity for the M_2 receptor.⁴ Already in 1914 Dale proposed that acetylcholine (ACh) acts through two major receptor subtypes defined as nicotinic and muscarinic.⁵ These receptors differ in location, function, structure and receptor-effector coupling mechanisms. Whereas nicotinic receptors are ligand-gated cation channels, muscarinic receptors are members of a superfamily, also including e.g. the opsins, which transmit their signal through GTP-binding proteins. This receptor family is structurally characterized by seven hydrophobic regions which span the membrane.⁶

In the late 1900s, five different subtypes m_1 to m_5 of the muscarinic receptor were identified using molecular biology techniques.⁷ Their expression in cultured cells devoid of any endogenous muscarinic receptors led to the pharmacological and biological characterization of the five subtypes M_1 - M_5 .⁸ The pharmacological properties of the cloned receptors are, as a rule, in excellent agreement with the binding as well as with the functional properties of the naturally expressed receptors M_1 - M_4 . These are distributed throughout the body where they regulate vital functions in both the central and autonomic nervous system. The M_2 and M_3 receptors are widely expressed in peripheral tissues, where the effector organs include heart (M_2), smooth muscle and exocrine glands (M_3). All five muscarinic receptor mRNAs have been identified in the central nervous system by in situ hybridization.⁹

The muscarinic receptor subtypes M_1 - M_5 can further be divided into two categories, both on the basis of their sequence homology and of their signaling mechanism. The M_1 , M_3 , M_5 form a first homologous group that is known to activate phospholipase C, which results in increased intracellular Ca^{2+} concentrations, and cellular excitation. The M_2 , M_4 receptors form a second homologous group that inhibits adenylate cyclase, closes Ca^{2+} and open K^+ channels, resulting in the hyperpolarization of the membrane and inhibitory signaling.

The age-related neurodegenerative diseases, such as Alzheimer's disease, have been associated with loss of cholinergic innervation and reduced levels of synaptic acetylcholine in several brain areas.¹⁰ The cholinergic approach to the treatment of the disease is directed towards

the enhancement of ACh levels. Whereas this goal can in principle be achieved by inhibitors of cholinesterase,¹¹ the use of postsynaptic M₁ agonists has also been proposed,¹² especially since the M₁ subtype is almost absent in peripheral effector organs and the number of postsynaptic receptors seems not to be altered. An alternative strategy focuses on the blocking of the presynaptic M₂ receptor subtypes, activation of which inhibits the release of ACh.¹³ Obviously, in the latter case M₂ antagonists with M₁/M₂ selectivity are required.

Several antagonists have been useful in the classification of muscarinic receptor subtypes.¹⁴ Next to pirenzepine whose selective M₁ antagonistic activity was discovered in the early 1980s,¹⁵ others have included 4-diphenylacetoxy-*N*-methylpiperidine methiodide (4-DAMP), hexahydro-siladifenidol (HHSiD), gallamine, the benzodiazepinone derivative AF-DX 116 and himbacine.¹⁶ Interestingly, it has also been demonstrated that the blocking action of gallamine and himbacine on the heart differed from competitive inhibition, but could be rationalized by an allosteric mechanism.¹⁷

In general the known muscarinic agonists and antagonists are not very selective.¹⁸ Himbacine however has been reported to bind to M₂ receptors with K_d values of 4.6-10 nM and with a 10-20 fold selectivity against the M₁ receptor. The full selectivity pattern M₁-M₄ of himbacine in binding studies with rat brain receptor subtypes has been reported.¹⁹

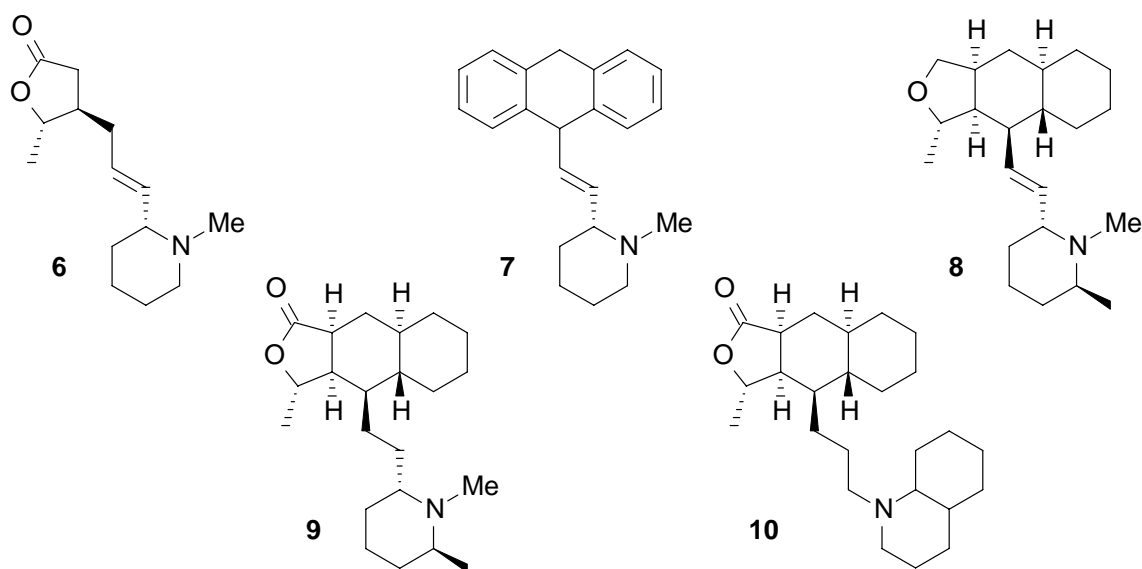


Figure 2

In an early structure-activity relationship study the natural alkaloids (**1**, **2**, **4**, **5**) and their corresponding dihydro-derivatives were evaluated as antagonists of muscarinic receptors in guinea-pig ileal longitudinal muscle and electrically stimulated left atrium.²⁰ Himbacine was the most potent compound and displayed a 15-fold selectivity for the M₂ receptor. Reduction of the double bond in himbacine or removal of the *N*-methyl group (cf. himbeline, **2**) led to reduced selectivity. The same trend was observed in subsequent structure-activity relationship studies

aiming at the development of himbacine analogues with high potency (better than 1 nM) and selectivity (better than 100-fold).²¹ Structures **6-8** (Figure 2) are representative molecules and their binding affinities at M₂ sites and M₁/M₂ selectivities are given in Table 1. Compared to himbacine the severely truncated analogue **6** lost both affinity for the M₂ receptor (about 260-fold) and selectivity.^{21a}

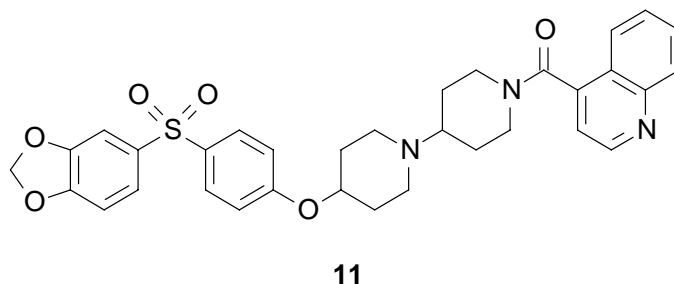
Table 1. K_i values (nM) reported for himbacine (**1**) and compounds **6-13**

Compound	M ₂	M ₁ /M ₂
1 ^a	11	6.5
6 ^b	1210	8
7 ^c	3.2	0.5
8 ^d	353	0.5
9 ^e	4.3	7.5
10 ^e	13	2.9
11 ^f	1.0	140
12 ^g	310	10.4
13 ^g	170	10.2

^a Competition experiments with [³H]-*N*-methyl scopolamine at 25 °C in a 50 mM sodium phosphate/2 mM MgCl₂ buffer (pH 7.4) at rat muscarinic receptors: M₁ in brain, M₂ in heart.

^b Reference 21a. ^c Reference 21b. ^d Reference 21c. ^e Reference 22. ^f Reference 23. ^g Reference 24.

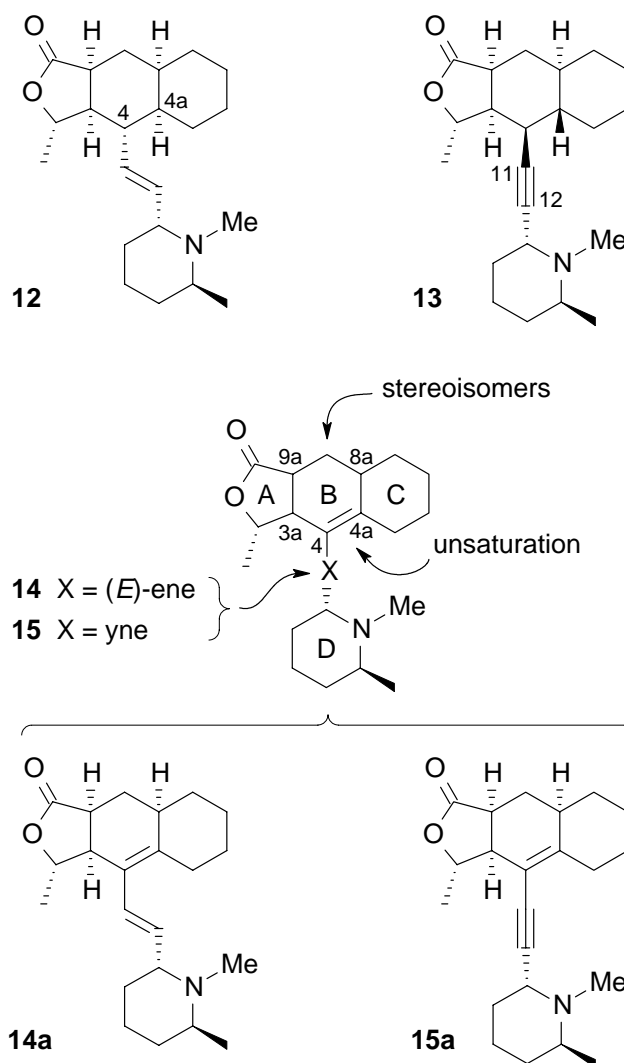
Whereas the replacement of the original tricyclic moiety of himbacine with a dihydroanthracene core as in **7** leads to an increase in affinity at the M₂ receptor subtype, a preferential binding at the M₁ receptor subtype is now observed.^{21b} The importance of the structural integrity of the ABC-ring part, including the heterocyclic ring, for optimal M₂ selectivity was also confirmed by the observation that the removal of the carbonyl oxygen of himbacine affords a molecule (**8**) with reduced affinity and inversed selectivity.^{21c} Finally, an interesting series of analogues has been developed by Chackalamannil on the basis of their finding that dihydrohimbacine (**9**) has a potency and selectivity comparable to that of himbacine in cloned human M₁ and M₂ receptors.²² Various *N*-alkylated derivatives such as **10**, in which the nitrogen atom is located at the same relative position to the intact ABC-ring as in himbacine, were prepared. Several of these analogues display a potency comparable to that of himbacine, but again at the expense of selectivity. In essence, so far, analogue development on the basis of the himbacine skeleton has not met the expectations. In contrast, in another structural series, highly active compounds such as **11** (Figure 3) with >100-fold selectivity against M₁, M₃, and M₅ receptors have been developed.²³

**Figure 3**

Results and Discussion

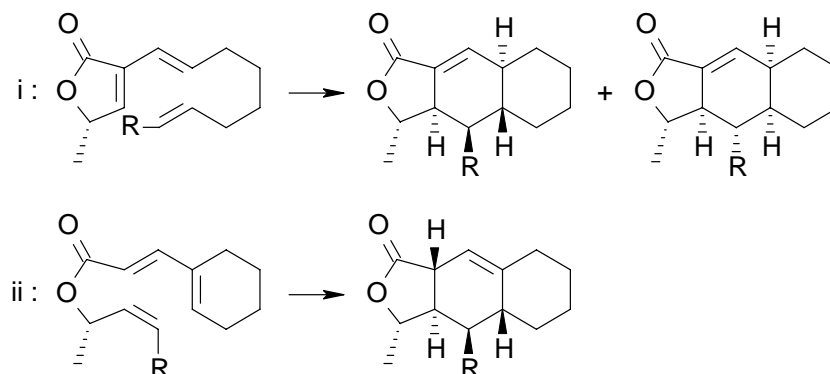
Inspired by the above SAR studies that indicate that the deletion of substantial parts of the skeleton and/or functionality of the natural derivative results in loss of selectivity, we recently became interested in the synthesis of intact stereoisomers of himbacine with focus on the stereogenic centers in the tricyclic system of the molecule.²⁴ Also variations in the geometry of the two carbon interconnecting unit between the two cyclic parts of the molecule were investigated, i.e. the incorporation of an acetylenic and of a (*Z*)-ethylenic next to the natural (*E*)-ethylenic bridge. Three different isomeric series were synthesized, which, in combination with the three different above mentioned interconnecting units, afforded nine derivatives, including synthetic (+)-himbacine. Interestingly, the binding affinity studies showed that, next to himbacine, the two analogues **12** and **13** (Figure 4) possess a moderate 10-fold selectivity for the M₂ receptor, albeit with reduced potency (Table 1). Analogue **12** is epimeric at C-4 and at C-4a relative to himbacine whereas analogue **13** corresponds to 11,12-dehydrohimbacine. In a further search at optimizing both the potency and M₂/M₁ selectivity of himbacine-like derivatives we wish to report now on the synthesis of a few stereoisomeric derivatives **14** and **15** characterized by the presence of (i) a double bond at C-4,C-4a and of (ii) an interconnecting two carbon unit of the (*E*)-ene or yne type, and in which the other structural features of himbacine are kept intact. At the conceptual level, derivatives within the **a**-configurational series, such as **14a** and **15a**, were preferred. They respect the structural integrity of the two cyclic parts of himbacine and include a bridging unit, which by its nature imposes an extended geometry to the molecule, presumably a crucial factor in determining the biological activity.

For a synthetic chemist, himbacine (**1**) possesses an attractive structure. Its skeleton consists of a tricyclic ABC-ring system, to which is connected, via a (*E*)-double bond, the *N*-methyl piperidine D-ring. The ABC-ring part itself consists of a *trans*-fused perhydonaphthalene with a *cis*-fused γ -lactone. The two cyclic parts also display interesting stereochemical patterns: in the ABC-ring part six contiguous stereocenters are present, while the D-ring features a less common 2,6-*trans*-disubstituted piperidine ring. The first enantioselective total synthesis of himbacine was reported by Hart and Kozikowski in 1995.²⁵ Other enantioselective syntheses of himbacine have been subsequently reported by Chackalamannil,²⁶ by Terashima,²⁷ and by De Clercq.²⁸

**Figure 4**

So far the various total synthetic approaches towards himbacine have all involved the use of a Diels-Alder reaction in establishing the central B-ring nucleus of the ABC-ring skeleton.^{25,26,27,28} Among the six different bond construction sets that one may distinguish following this particular cycloaddition strategy, two sets correspond to the intermolecular reaction mode, one of which has been realized in practice,²⁷ and the four remaining construction sets correspond to intramolecular versions of this popular construction reaction (IMDA).²⁹ Symptomatic of how pervasive this reaction type has become in terms of cyclic skeleton construction, especially when involving stereochemical constraints, is the observation that the three synthetic approaches that were first reported almost simultaneously in 1995, involved pathway i (Scheme 1).^{25,30,31} One of the benefits of this particular cycloaddition route resides in the obtainment of the functionalized ABC-ring system as present in himgravine (**5**), whose selective reduction to himbacine (**1**) has been reported before.² Also, depending on the reaction

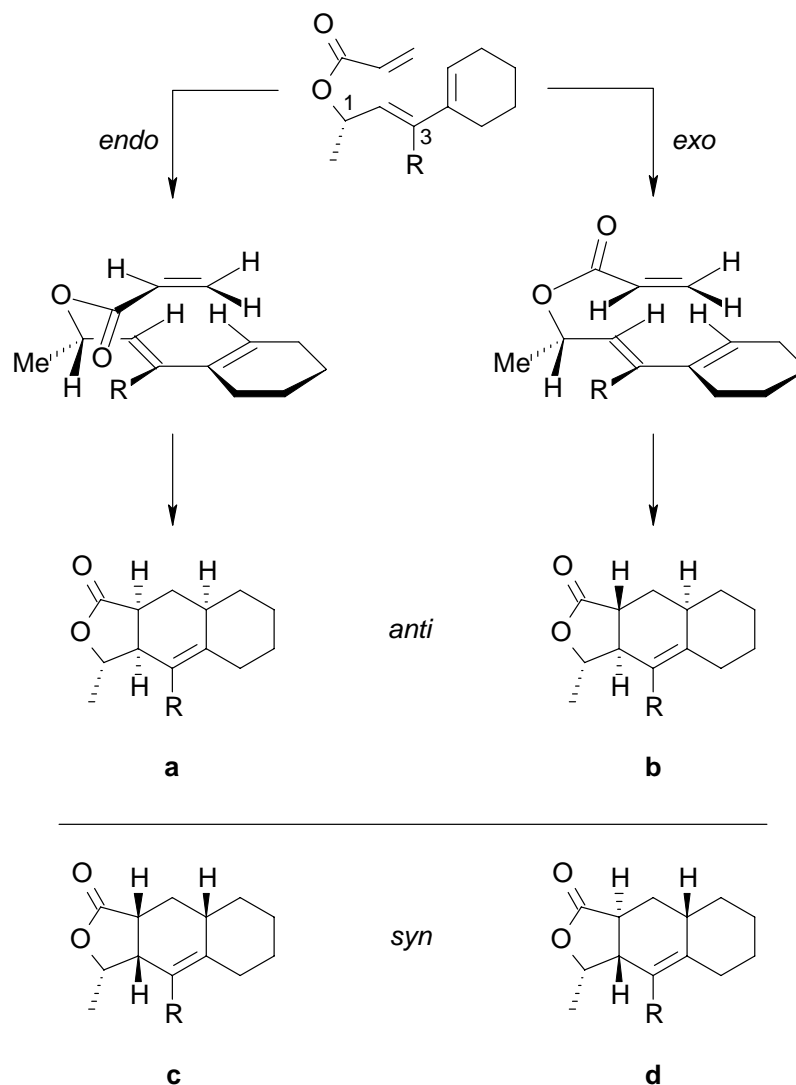
conditions and the nature of the R group, the stereochemical outcome of the process could be directed towards the himbacine-like configuration.^{25b} A second construction set (ii) was exploited successfully by Chackalamannil in the total syntheses of himbacine and more recently of himandravine.^{26,32} This cycloaddition whereby the R group already contains the intact D-ring leads exclusively to one stereoisomer in which the γ -lactone is *trans*-fused, the result of an *anti,exo*-approach (vide infra).



Scheme 1

In the present study we wish to elaborate on the construction set shown in Scheme 2.³³ This third pathway bears some analogy with the previous approach since in both cases the intramolecular cycloaddition leads to the simultaneous formation of the A- and B-rings. This novel strategy involves as a key step the cycloaddition of an acrylate derived from a 3,4,5-trisubstituted pentadienyl alcohol bearing a stereocenter (i.e., at C-1) in the diene/dienophile tether that is expected to play a stereodirecting role in the process. Following our preliminary report on this work,^{33a} an almost identical synthetic approach has been disclosed by Sherburn and co-workers.^{33b} The stereochemical outcome of the Diels–Alder reaction is determined by (1) the *anti*- or *syn*-approach of the dienophile relative to the orientation of the methyl group at C-1 on the planar pentadienyl moiety, and by (2) the *exo*- or *endo*-mode of addition. On one hand the *anti*-approach should be preferred on the basis of steric considerations, especially when R is voluminous, thus favoring the **a**- and **b**-configurations; on the other hand the *exo*-mode of addition is expected here on the basis of a literature precedent dealing with the synthesis of drimane sesquiterpenes.³⁴ Hence at the outset the preferred *anti,exo*-formation of the **b**-type stereoisomeric relationship was anticipated. As will be discussed later in more detail a very recent joint synthetic computational investigation into the optimization of the stereoselectivity in the IMDA reaction of pentadienyl acrylates has corroborated this expectation.³⁵ Subsequent isomerization of the *trans*-fused lactone (**b**) to the more stable *cis*-configuration should afford the desired stereochemistry (**a**) as present in derivatives **14a** and **15a**. A further strategic choice allowing for the convergent synthesis of both **14** and **15**, which only differ in the connecting unit, consists of the incorporation of a vinylic halogen atom (i.e., R = Br), whose replacement using

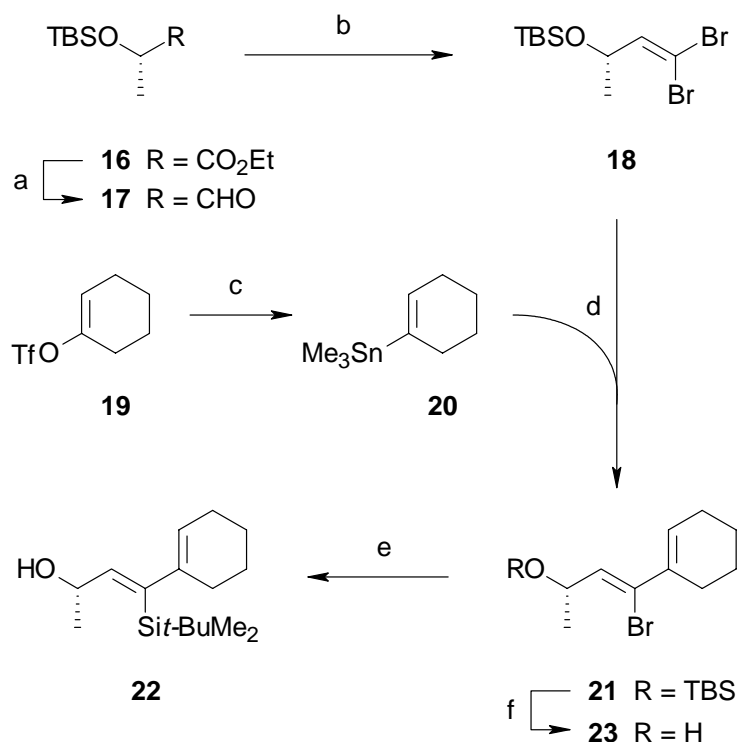
one of the several existing cross-coupling reactions, should allow for the completion of the carbon framework of the molecule possessing the genuine D-ring as in **14** and **15**.



Scheme 2

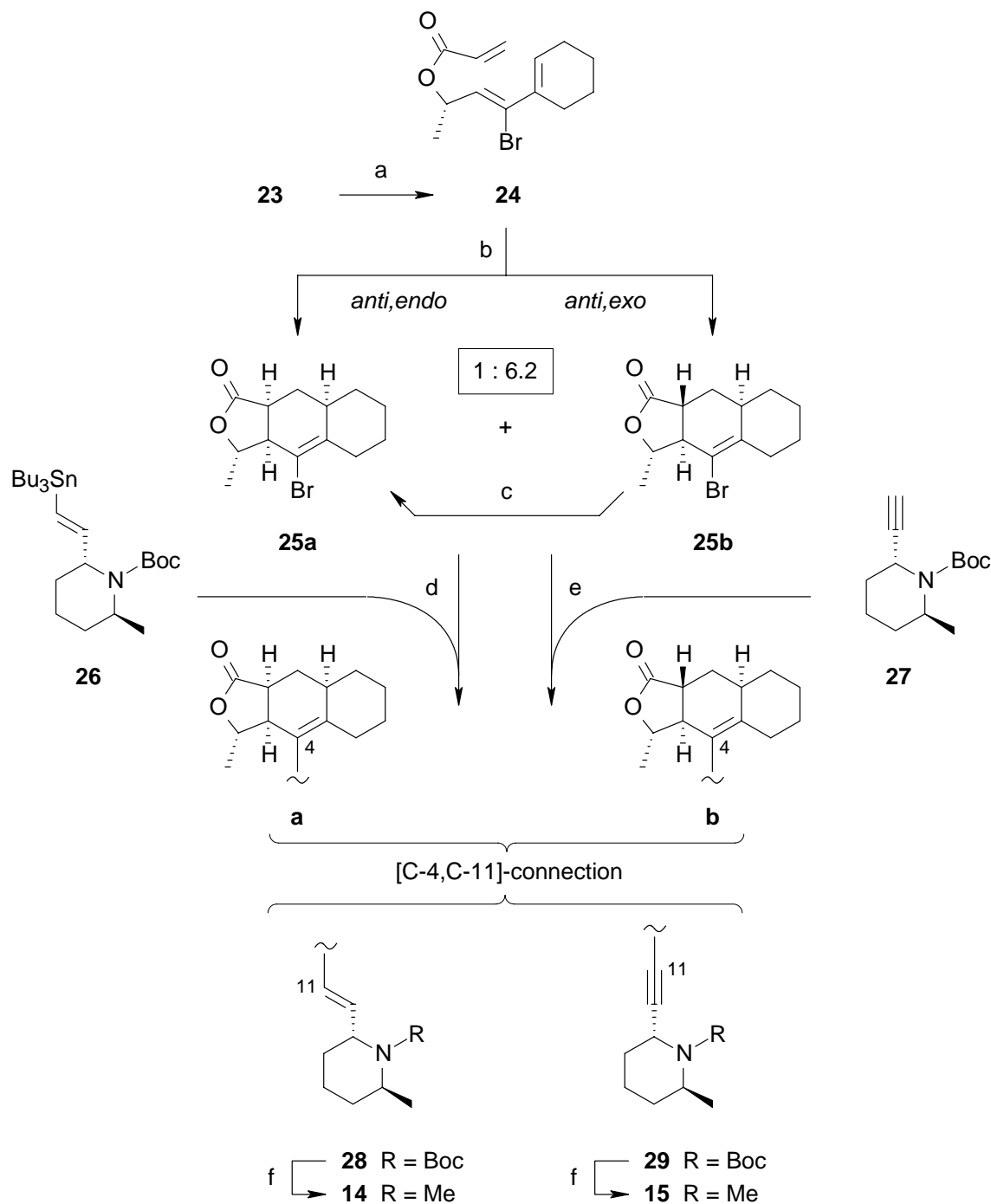
Central in the approach following the above strategy stands alcohol **23**, whose synthesis is outlined in Scheme 3. After protection of the hydroxyl group in (*S*)-ethyl lactate as *tert*-butyldimethylsilyl ether, the resulting ester **16** was reduced with diisobutylaluminum hydride affording aldehyde **17** (80% yield).³⁶ Conversion of the latter using the protocol of Corey–Fuchs³⁷ gave the known geminal dibromo substituted alkene **18** (84% yield).³⁸ The stereoselective introduction of the cyclohexenyl moiety was then realized via a Stille coupling with stannane **20**.³⁹ The latter was obtained in 79% yield by reaction of enol triflate **19** with hexamethylditin (tetrakis(triphenylphosphine)palladium(0), lithium chloride, THF, 60 °C).⁴⁰ Several studies have recently appeared involving the replacement of one or both halogen atoms in 1,1-dibromo-1-alkenes.⁴¹ In particular the stereoselective replacement of the (*E*)-halogen atom

using the Stille reaction has been studied in detail by Shen and Wang.^{41a} Using the same optimized reaction conditions, tris(dibenzylideneacetone)dipalladium(0) and tri-2-furylphosphine in refluxing toluene, vinyl bromide **21** was obtained in 71% yield. Although the (*Z*)-stereochemistry at the trisubstituted double bond was expected, its unambiguous configurational assignment followed from the stereospecific transformation of **21** into C-silylated alcohol **22** after treatment with *tert*-butyllithium at -78 °C. This rearrangement that follows a metal-halogen exchange implies, of course, a *cis*-relationship between the halogen atom and the silyloxyethyl group. Desilylation of **21** with tetrabutylammonium fluoride afforded alcohol **23** in 88% yield.



Scheme 3. (a) DIBAL-H, toluene, -78 °C, 2 h (80%); (b) CBr_4 , PPh_3 , CH_2Cl_2 , -78 °C, 2 h (84%); (c) $\text{Me}_3\text{SnSnMe}_3$, $\text{Pd}(\text{PPh}_3)_4$, LiCl , THF, 60 °C, 3.5 h (79%); (d) Pd_2dba_3 , $(\text{fur})_3\text{P}$, toluene, reflux, 4 h (71%); (e) *t*-BuLi, THF, -78 °C, 10 min (42%); (f) TBAF, THF, rt, 1 h (88%).

The further conversion of **23** into himbacine analogues **14a**, **14b**, **15a** and **15b** is shown in Scheme 4. First the alcohol **23** was converted into the corresponding acrylate **24** using acryloyl chloride and diisopropylethylamine (DIPEA) in dichloromethane (77% yield). The Diels–Alder reaction of **24** required high temperatures (185 °C, toluene) and the presence of both an antioxidant (TEMPO) and a base (DIPEA).⁴² After 24 h a mixture of the two stereoisomeric vinylic bromides **25a** and **25b** was obtained in 79% yield.

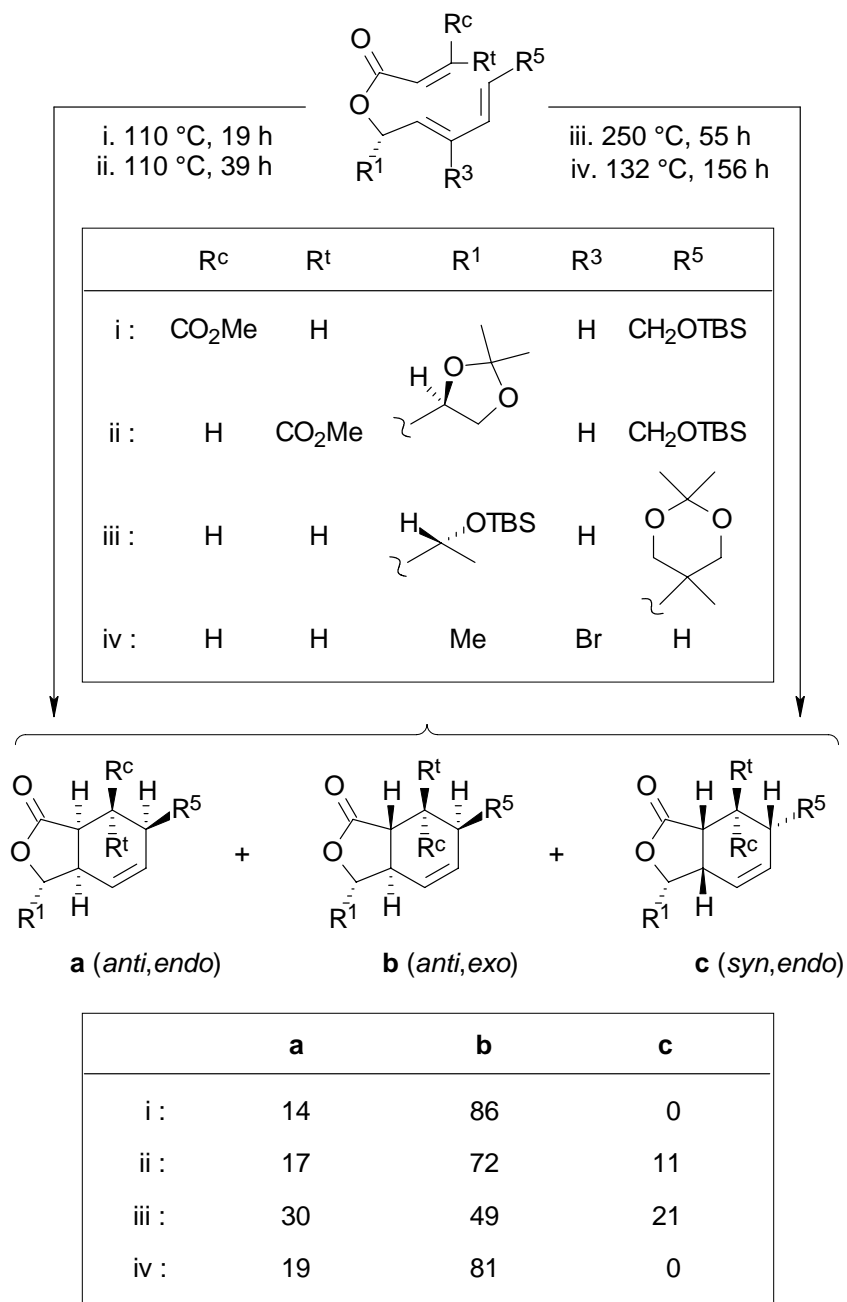


Scheme 4. (a) $\text{CH}_2=\text{CHCOCl}$, DIPEA, CH_2Cl_2 , $-50\text{ }^\circ\text{C}$, 10 min (77%); (b) TEMPO, DIPEA, toluene, $185\text{ }^\circ\text{C}$, 24 h (79%); (c) NaH, THF, rt, 2 h (82%); (d) Pd_2dba_3 , $(\text{fur})_3\text{P}$, DIPEA, toluene, reflux, 2 h (**25a** \rightarrow **28a**: 80%; **25b** \rightarrow **28b**: 77%); (e) $\text{Pd}(\text{PPh}_3)_2(\text{OAc})_2$, Et_3N , CH_3CN , reflux, 1 h (**25a** \rightarrow **29a**: 76%; **25b** \rightarrow **29b**: 73%); (f) TFA, CH_2Cl_2 , rt, 1 h; 37% aqueous H_2CO , NaBH_3CN , CH_3CN , rt, 1 h (**14a**: 81%; **14b**: 80%; **15a**: 75%; **15b**: 78%).

The expected *anti,exo*-adduct (**25b**) predominated (ratio **25a:25b** = 1:6.2). The structural assignment of both adducts follows from the X-ray crystal structure determination of **25b** and from the observation that upon basic treatment the *trans*-fused lactone in **25b** is isomerized into the more stable *cis*-fused adduct **25a**.^{33a} In line with this experimental result, force field calculations indicate a relative steric energy difference of 9.9 kJ mol⁻¹ in favour of **25a**.⁴³

The transformation of **25a** and of **25b** into the desired analogues **14a**, **15a** and **14b**, **15b**, respectively, involves palladium catalyzed cross-coupling reactions. The Stille reaction between bromides **25a** and **25b**, and the vinyltributylstannane **26** using tris(dibenzylideneacetone)-dipalladium(0) and tri-2-furylphosphine in the presence of DIPEA (toluene, reflux) afforded dienes **28a** (80% yield) and **28b** (77% yield), respectively. The synthesis of vinylstannane **26** proceeded by reaction of a higher order tributyltin cuprate derivative, obtained from copper(I) cyanide, *n*-butyllithium and tributyltinhydride,⁴⁴ with the known terminal alkyne **27** (80% yield).²⁸ The Sonogashira coupling involved reaction of the same bromides **25a** and **25b** with alkyne **27** (bis(triphenylphosphine)palladium(II) acetate, triethylamine in acetonitrile) and afforded enynes **29a** (76% yield) and **29b** (73% yield), respectively.⁴⁵ It is worth noting that the above conditions are the result of thorough experimentation.⁴⁶ The final conversion of the *N*-Boc derivatives **28a**, **28b**, **29a** and **29b** into the corresponding *N*-methylated analogues **14a**, **14b**, **15a**, and **15b** proceeded in two steps: after acidic removal of the Boc protective group (trifluoroacetic acid, dichloromethane), the resulting crude secondary amines were treated with 37% aqueous formaldehyde and sodium cyanoborohydride in acetonitrile to yield **14a** (81% yield), **14b** (80% yield), **15a** (75% yield) and **15b** (78% yield), respectively.

At this point it is interesting to focus in more detail on the stereochemical outcome of the Diels–Alder reaction of the pentadienyl acrylate system as present in precursor **24**.⁴⁷ Very recently a few related cases have been reported in considerable detail. The relevant transformations dealing with C-1 substituted derivatives are depicted in Scheme 5. The reaction of the double activated dienophile substrates was studied by Paddon–Row and Sherburn:⁴⁸ (i) the more reactive maleate gave isomers **a** and **b** in the same ratio (1:6.1) as observed in the reaction of **24**; (ii) the fumarate led to the formation of the three isomers **a**, **b** and **c** with a distinct preference for the *anti,exo*-adduct **b**. The cycloaddition of the acrylate studied by White and Snyder (iii) required harsher reaction conditions and led to a mixture of the **a**, **b** and **c** stereoisomers,⁴⁹ although the observed stereoselectivity was poor, the *anti,exo*-adduct **b** again predominated. Lastly, the cycloaddition of a C-3 bromine substituted pentadienyl acrylate, described by Paddon–Row and Sherburn (iv) leads to the corresponding stereoisomers **a** and **b**, with again a clear preference for the *anti,exo*-adduct **b**.³⁵ Interestingly in the previous studies elaborate DFT calculations have corroborated the experimental results. Since the cycloadditions were shown to proceed under kinetic control, the relative energies of the different transition states were calculated. Our result is almost identical to the one observed in (iv). This is not really surprising in view of the presumed determining influence of the steric interference, related to ^{1,3}A-strain,⁵⁰ between the C-1 alkyl and C-3 bromine substituents. The latter substituent has been termed a “steric directing group” by Paddon–Row and Sherburn in this particular context.³⁵



Scheme 5

Binding affinity studies

The binding studies of synthetic (+)-himbacine **1** and of the four derivatives **14a**, **14b**, **15a** and **15b** for the four muscarine receptor subtypes were carried out by analysis of competition experiments with [³H]-*N*-methylscopolamine (NMS) at 25 °C in a 50 mM sodium phosphate/2 mM magnesium chloride buffer (pH 7.4).⁵¹ The [³H]-NMS saturation curves in each cell line were compatible with recognition of a single receptor subtype. In the present set of experiments,

the [^3H]-NMS K_D values were 0.26, 0.50, 0.42 and 0.18 nM at M_1 , M_2 , M_3 and M_4 muscarinic receptors, respectively. Representative competition curves are included in the supporting information section. The corresponding pK_i values are given in Table 2.

Table 2. K_i values (nM) and pK_i values (between parentheses) at cloned CHO cells expressing hM₁, hM₂, hM₃, and hM₄ muscarine receptors^a

Compound	hM ₁	hM ₂	hM ₃	hM ₄
1 ^b	160 (6.80)	4.7 (8.33)	100 (6.98)	8.7 (8.06)
14a	700 (6.16)	1400 (5.87)	2500 (5.61)	1500 (5.81)
14b	2500 (5.61)	2600 (5.59)	6500 (5.19)	6000 (5.22)
15a	1800 (5.74)	100 (7.00)	1700 (5.77)	270 (6.57)
15b	8500 (5.07)	2600 (5.59)	17000 (4.77)	3600 (5.44)

^a Since the distribution of the K_i values is lognormal, pK_i values, $-\log(K_i \text{ concentration units})$, are also mentioned; the calculated s.e.m. values vary between ± 0.01 and ± 0.40 . ^b synthetic (+)-himbacine, see reference 24.

The results of the binding studies indicate that all analogues possess a lower affinity for each receptor subtype when compared with himbacine. Analogue **15a** shows an 18-fold selectivity for the M_2 relative to the M_1 receptor. It is interesting to note that the structure of **15a** involves an acetylenic connecting bridge instead of the natural (*E*)-ethylenic moiety.

Conclusions

We have developed a concise convergent approach towards the synthesis of 4,4a-dehydrohimbacine derivatives. The key step in the synthesis involves an intramolecular Diels–Alder reaction of a substituted pentadienyl acrylate (**24**), which afforded with good stereoselectivity the *trans*-fused lactone bromide **25b**, the adduct resulting from the expected preferred *anti,exo*-addition. Subsequent attachment of the piperidine D-ring involved Stille (vinylstannane **26**) and Sonogashira (alkyne **27**) coupling reactions.

Experimental Section

General Procedures. All air sensitive reactions were run under Ar or N₂ atmosphere and reagents were added through septa using oven dried syringes. Et₂O and THF were distilled from benzophenone ketyl prior to use. *N,N*-Diisopropylethylamine (DIPEA), Et₃N, CH₃CN and HMPA were distilled from CaH₂ and CH₂Cl₂ was distilled from P₂O₅. TLC were run on glass plates pre-coated with silica gel (Merck, 60F254). Column chromatography was performed on

silica gel (Merck, 230-400 mesh) and HPLC separations were performed on Bio-Sil D 90-10, 10- μm columns (Bio-Rad) of 1×25 cm and 2.2×25 cm. ^1H NMR chemical shifts are reported in ppm (δ) relative to CDCl_3 (7.26 ppm) or C_6D_6 (7.16 ppm) as an internal reference. ^{13}C NMR chemical shifts are reported in ppm (δ) relative to CDCl_3 (77.16 ppm) or C_6D_6 (128.06 ppm) as an internal reference. Mass spectra were recorded at 70 eV.

(Z,3S)-1-Bromo-3-(tert-butyldimethylsilyloxy-1-(cyclohex-1-enyl)but-1-ene (21). To a solution of tris(dibenzylideneacetone)dipalladium(0) (Pd_2dba_3 , 0.372 g, 0.4 mmol) and tri-2-furylphosphine (0.404 g, 1.7 mmol) in toluene (35 mL) was added dibromide **18** (2.0 g, 5.8 mmol). After stirring at rt for 15 min, tin derivative **20** (1.5 g, 6.1 mmol) was dropwise added and the reaction mixture was refluxed for 4 h (color change from yellow to dark red-brown). The reaction mixture was cooled to rt, toluene was evaporated under reduced pressure and the residue was purified by column chromatography on silica gel (isooctane/EtOAc 9:1) to give **21** (1.42 g, 71%) as a light-yellow oil: R_f (isooctane/EtOAc 9:1) 0.7; $[\alpha]_D^{20}$ -2.7 (c 1.000, CHCl_3); IR (KBr film) ν 2929, 2886, 2857, 1255, 1140, 1078, 998, 834, 776 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 6.28 (1H, br s), 5.92 (1H, d, $J = 7.2$ Hz), 4.79 (1H, dq, $J = 6.5, 6.5$ Hz), 2.24-2.18 (4H, m), 1.71-1.66 (2H, m), 1.61-1.55 (2H, m), 1.25 (3H, d, $J = 6.3$ Hz), 0.89 (9H, s), 0.08 (3H, s), 0.06 (3H, s); ^{13}C NMR/DEPT (50 MHz, CDCl_3) δ 134.0 (C), 132.4 (CH), 130.9 (CH), 125.8 (C), 70.1 (CH), 26.9 (CH_2), 25.9 (CH_3), 25.8 (CH_2), 23.3 (CH_2), 22.8 (CH_2), 22.0 (CH_2), 18.2 (C), -4.5 (CH_3), -4.8 (CH_3); MS m/z 288 (28), 287 (30), 273 (1), 245 (1), 225 (1), 183 (100), 181 (100), 139 (34), 137 (34), 91 (46), 75 (78), 73 (78). Anal. Calcd for $\text{C}_{16}\text{H}_{29}\text{BrOSi}$: C, 55.64; H, 8.46. Found: C, 55.81; H, 8.57.

(Z,2S)-4-tert-Butyldimethylsilyl-4-(cyclohex-1-enyl)but-3-en-2-ol (22). To a solution of bromide **21** (25 mg, 0.072 mmol) in dry THF (0.9 mL) at -78 $^\circ\text{C}$ was dropwise added $t\text{-BuLi}$ (1.7 M in pentane, 0.053 mL, 0.09 mmol). After stirring for 10 min, the yellow-colored reaction mixture was poured into water (5 mL). The aqueous phase was extracted with Et_2O and the combined organic layers were dried over anhydrous MgSO_4 . The solvent was removed under reduced pressure and the residue was purified by HPLC on silica gel (n -pentane/EtOAc 9:1) to afford pure **22** (8 mg, 42%): R_f (isooctane/EtOAc 9:1) 0.15; IR (KBr film) ν 2956, 2828, 2856, 1249, 1053, 834, 823, 765 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 5.92 (1H, d, $J = 9.8$ Hz), 5.23 (1H, br s), 4.43 (1H, dq, $J = 9.8, 6.1$ Hz), 2.04-2.01 (2H, m), 1.24 (3H, d, $J = 6.1$ Hz), 0.94 (9H, s), 0.13 (3H, s), 0.09 (3H, s); ^{13}C NMR/DEPT (50 MHz, CDCl_3) δ 146.7 (CH), 145.4 (C), 143.4 (C), 121.4 (CH), 67.7 (CH), 30.7 (CH_2), 27.6 (CH_3), 25.2 (CH_2), 23.1 (CH_2), 23.0 (CH_3), 22.2 (CH_2), 17.7 (C), -2.6 (CH_3), -2.7 (CH_3); MS m/z 209 (2), 191 (1), 133 (14), 105 (3), 91 (5), 75 (100).

(Z,2S)-4-Bromo-4-(cyclohex-1-enyl)but-3-en-2-ol (23). Tetrabutylammonium fluoride (TBAF, 1 M in THF, 5.43 mL, 5.43 mmol) was dropwise added to a solution of bromide **21** (1.5 g, 4.34 mmol) in dry THF (12 mL) at rt. After stirring for 1 h, the reaction mixture was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel (isooctane/EtOAc 95:5 to 8:2) to give alcohol **23** (0.882 g, 88%) as a colorless oil: R_f (isooctane/EtOAc 8:2) 0.1; $[\alpha]_D^{20}$ -20.1 (c 0.990, CHCl_3); IR (KBr film) ν 3322, 2970, 2928, 2858,

2830, 1634, 1613, 1448, 1436, 1366, 1183, 1169, 1138, 1063, 880, 801 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 6.34 (1H, t, $J = 4.1$ Hz), 5.95 (1H, d, $J = 7.3$ Hz), 4.84 (1H, dq, $J = 6.6, 6.6$ Hz), 2.25-2.18 (4H, m), 1.79 (1H, br s), 1.70-1.66 (2H, m), 1.60-1.56 (2H, m), 1.33 (3H, d, $J = 6.4$ Hz); ^{13}C NMR/DEPT (50 MHz, CDCl_3) δ 133.9 (C), 131.7 (CH), 130.7 (CH), 128.7 (C), 68.6 (CH), 26.9 (CH_2), 25.9 (CH_2), 22.7 (CH_2), 22.2 (CH_3), 21.9 (CH_2); MS m/z 230 (1) [$\text{M}^+ - \text{H}$], 215 (1), 151 (9), 133 (7), 123 (1), 107 (7), 93 (18), 91 (18), 79 (17), 67 (12), 43 (100). Anal. Calcd for $\text{C}_{10}\text{H}_{15}\text{BrO}$: C, 51.97; H, 6.54. Found: C, 52.48; H, 6.77.

(Z,2S)-2-[4-Bromo-4-(cyclohex-1-enyl)]but-3-enyl Acrylate (24). To a solution of alcohol **23** (0.85 g, 3.68 mmol) in dry dichloromethane (16 mL) at -50 $^\circ\text{C}$ were added acryloyl chloride (0.6 mL, 7.36 mmol) and DIPEA (1.28 mL, 7.36 mmol). Stirring was continued for 10 min at -50 $^\circ\text{C}$ and the reaction mixture was poured into a saturated aqueous NH_4Cl solution (10 mL). The aqueous phase was extracted with dichloromethane (2 \times) and the combined organic layers were dried over anhydrous MgSO_4 . The solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel (isooctane/EtOAc 9:1) to give ester **24** (0.81 g, 77%) as a light-yellow oil: R_f (isooctane/EtOAc 9:1) 0.43; $[\alpha]_D^{20}$ -9.75 (c 0.120, CHCl_3); IR (KBr film) ν 2931, 2860, 1726, 1636, 1618, 1404, 1294, 1269, 1193, 1150, 1050, 921, 876, 809 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 6.41 (1H, dd, $J = 1.4, 17.3$ Hz), 6.37 (1H, t, $J = 4.1$ Hz), 6.11 (1H, dd, $J = 10.4, 17.3$ Hz), 5.95 (1H, d, $J = 7.5$ Hz), 5.87 (1H, dq, $J = 6.7, 6.7$ Hz), 5.82 (1H, dd, $J = 1.4, 10.4$ Hz), 2.24 (2H, m), 2.19 (2H, m), 1.70-1.53 (4H, m), 1.39 (3H, d, $J = 6.4$ Hz); ^{13}C NMR/DEPT (50 MHz, CDCl_3) δ 165.3 (C), 133.7 (C), 132.2 (CH), 130.7 (CH), 129.7 (C), 128.6 (CH), 126.6 (CH), 72.0 (CH), 26.8 (CH_2), 25.9 (CH_2), 22.7 (CH_2), 21.9 (CH_2), 19.7 (CH_3); MS m/z 285 (1), 241 (1), 229 (1), 214 (3), 212 (3), 205 (4), 151 (9), 133 (25), 105 (17), 91 (22), 77 (16), 55 (100). Anal. Calcd for $\text{C}_{13}\text{H}_{17}\text{BrO}_2$: C, 54.75; H, 6.01. Found: C, 55.89; H, 6.25.

(3S,3aR,8aR,9aS)-4-Bromo-3-methyl-3a,5,6,7,8,8a,9,9a-octahydro-3H-naphtho[2,3-c]furan-1-one (25a) and **(3S,3aR,8aR,9aR)-4-Bromo-3-methyl-3a,5,6,7,8,8a,9,9a-octahydro-3H-naphtho[2,3-c]furan-1-one (25b)**. A mixture of ester **24** (0.25 g, 0.88 mmol), 2,2,6,6-tetramethyl-1-piperidinyloxy free radical (TEMPO, 10 mg) and a drop of DIPEA in toluene (20 mL) was flushed with Ar and heated in a sealed metal tube at 185 $^\circ\text{C}$ for 24 h. After cooling to rt, the reaction mixture was passed through a short silica gel column eluted with isooctane/EtOAc 3:1 (50 mL). The solvents were removed under reduced pressure and the mixture was separated by HPLC on silica gel (isooctane/EtOAc 10:1) to yield Diels-Alder adducts **25a** (0.028 g, 11%) as a colorless oil and **25b** (0.17 g, 68%) as white crystals, next to unreacted **24** (0.02 g, 8%). **25a**: R_f (isooctane/EtOAc 4:1) 0.38; $[\alpha]_D^{20}$ $+16.1$ (c 1.320, CHCl_3); IR (KBr film) ν 2929, 2855, 1778, 1448, 1384, 1313, 1196, 1064, 957, 934, 769 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 4.56 (1H, dq, $J = 7.7, 6.2$ Hz), 3.02 (1H, m), 2.96 (1H, dd, $J = 7.8, 7.8$ Hz), 2.79 (1H, ddd, $J = 5.1, 7.8, 11.8$ Hz), 2.09 (1H, ddd, $J = 5.3, 5.3, 13.6$ Hz), 1.89-1.76 (4H, m), 1.64 (3H, d, $J = 6.2$ Hz), 1.56 (1H, m), 1.42-1.20 (2H, m), 1.05 (1H, ddt, $J = 3.6, 12.7, 12.7$ Hz); ^{13}C NMR/DEPT (50 MHz, CDCl_3) δ 177.2 (C), 141.7 (C), 113.5 (C), 81.3 (CH), 51.4 (CH), 40.8 (CH), 39.1 (CH), 34.8 (CH_2), 33.4 (CH_2), 29.0 (CH_2), 25.3 (CH_2), 25.0 (CH_2), 23.2 (CH_3); MS m/z 285 (7) [M^+], 241 (4), 239 (4),

214 (44), 212 (43), 172 (23), 170 (23), 149 (4), 133 (17), 105 (15), 91 (100), 77 (25), 43 (26); ESMS m/z 302 and 304 $[M + NH_3]^+$. Anal. Calcd for $C_{13}H_{17}BrO_2$: C, 54.75; H, 6.01. Found: C, 55.81; H, 6.28. **25b**: R_f (isooctane/EtOAc 4:1) 0.44; mp 87 °C; $[\alpha]_D^{20}$ -0.92 (c 1.090, $CHCl_3$); IR (KBr film) ν 2924, 2854, 1796, 1444, 1386, 1291, 1140, 1085, 1027, 962, 839, 717 cm^{-1} ; 1H NMR (500 MHz, $CDCl_3$) δ 4.39 (1H, dq, $J = 9.7, 5.9$ Hz), 2.96 (1H, ddd, $J = 4.0, 4.0, 9.4$ Hz), 2.67 (2H, m), 2.39 (1H, m), 1.90-1.68 (6H, m), 1.66 (3H, d, $J = 6.0$ Hz), 1.51-1.40 (2H, m), 1.28-1.24 (1H, m); ^{13}C NMR/DEPT (50 MHz, $CDCl_3$) δ 175.0 (C), 143.0 (C), 110.5 (C), 80.5 (CH), 53.5 (CH), 45.0 (CH), 41.8 (CH), 35.5 (CH_2), 34.0 (CH_2), 28.5 (CH_2), 27.1 (CH_2), 26.5 (CH_2), 20.2 (CH_3); ESMS m/z 302 and 304 $[M + NH_3]^+$. Anal. Calcd for $C_{13}H_{17}BrO_2$: C, 54.75; H, 6.01. Found: C, 54.71; H, 6.16.

Isomerization of 25b to 25a. To a solution of **25b** (50 mg, 0.18 mmol) in dry THF (1.25 mL) was added NaH (60% dispersion in mineral oil, 35 mg, 0.88 mmol) in one portion. The reaction mixture was stirred at rt for 2 h, then poured into a mixture of a saturated aqueous NH_4Cl solution (10 mL) and Et_2O (10 mL). The aqueous phase was extracted with Et_2O (3×10 mL) and the combined extracts were dried over anhydrous $MgSO_4$. After filtration and concentration under reduced pressure, the residue was chromatographed on silica gel (isooctane/EtOAc 8:1) to give **25a** (41 mg, 82%) as a colorless oil, identical to the minor isomer obtained in the above Diels-Alder reaction of **24**.

***tert*-Butyl (2*S*,6*R*)-6-[(*E*)-2-(Tributylstannyl)ethenyl]-2-methylpiperidinecarboxylate (26).**

To a solution of $CuCN$ (0.20 g, 2.24 mmol) in dry THF (6 mL) at -78 °C under Ar was dropwise added $n-BuLi$ (2.5 M in hexanes, 1.91 mL, 4.77 mmol). After stirring at -78 °C for 15 min, Bu_3SnH (1.39 g, 4.77 mmol) was dropwise added to the reaction mixture (color change to light yellow). After stirring at -78 °C for 10 min, alkyne **27** (0.50 g, 2.24 mmol) in dry THF (2 mL) was dropwise added (color change to light orange). After stirring at -78 °C for an additional 10 min, the reaction mixture was poured into an aqueous NH_4Cl/NH_4OH buffer solution (pH 12). The precipitated salts were filtered off using a pad of Celite, the filtrate was dried over anhydrous $MgSO_4$ and the solvents were removed under reduced pressure. The residue was purified by column chromatography on silica gel (isooctane/EtOAc 95:5) followed by HPLC on silica gel (isooctane/EtOAc 98:2) to yield tin derivative **26** (0.99 g, 86%) as a colorless oil: R_f (isooctane/EtOAc 9:1) 0.45; $[\alpha]_D^{20}$ +48.9 (c 2.120, $CHCl_3$); IR (KBr film) ν 2956, 2927, 1692, 1464, 1389, 1374, 1178, 1151, 1112 cm^{-1} ; 1H NMR (500 MHz, $CDCl_3$) δ 5.95 (2H, s), 4.43 (1H, br s), 4.06 (1H, m), 1.97-1.85 (3H, m), 1.61-1.51 (3H, m), 1.50-1.42 (6H, m), 1.44 (9H, s), 1.33-1.25 (6H, m), 1.24 (3H, d, $J = 6.6$ Hz), 0.94-0.86 (15H, m); ^{13}C NMR/DEPT (50 MHz, $CDCl_3$) δ 155.4 (C), 150.1 (CH), 125.3 (CH), 78.8 (C), 55.4 (CH), 47.0 (CH), 29.1 (CH_2), 28.4 (CH_3), 27.2 (CH_2), 26.2 (CH_2), 25.0 (CH_2), 20.8 (CH_3), 13.7 (CH_3), 13.6 (CH_2), 9.4 (CH_2); MS m/z 514 (1) $[M^+]$, 458 (8), 402 (22), 356 (1), 327 (1), 251 (6), 242 (3), 177 (14), 121 (15), 57 (100). Anal. Calcd for $C_{25}H_{49}NO_2Sn$: C, 58.38; H, 9.60; N, 2.72. Found: C, 59.20; H, 9.68; N, 2.72.

(3*S*,3*aR*,8*aR*,9*aS*)-4-[(*E*)-2-((2*S*,6*R*)-1-*tert*-Butyloxycarbonyl-2-methylpiperidin-6-yl)ethenyl]-3-methyl-3*a*,5,6,7,8,8*a*,9,9*a*-octahydro-3*H*-naphtho[2,3-*c*]furan-1-one (28*a*). To a mixture of tris(dibenzylideneacetone)dipalladium(0) (Pd₂dba₃, 8 mg, 0.009 mmol), tri-2-furylphosphine (12 mg, 0.052 mmol) and DIPEA (0.05 mL, 0.28 mmol) in toluene (2.5 mL) was added bromide **25a** (25 mg, 0.088 mmol) followed by tin derivative **26** (68 mg, 0.13 mmol). The mixture was flushed 3 times with Ar and then refluxed under Ar for 2 h (color change to brown). The reaction mixture was cooled to rt, diluted with *n*-pentane (5 mL) and filtered through a pad of silica gel, which was rinsed with isooctane/EtOAc 3:1 (20 mL). After concentration under reduced pressure, the residue was purified by HPLC on silica gel (isooctane/EtOAc 5:1) to afford **28a** (36 mg, 80%) as a colorless oil: R_f (isooctane/EtOAc 4:1) 0.23; [α]_D²⁰ +15.2 (*c* 0.250, CHCl₃); IR (KBr film) ν 2972, 2931, 2856, 1771, 1682, 1454, 1391, 1364, 1316, 1267, 1251, 1176, 1114, 1089, 1064, 956, 736 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 6.34 (1H, d, *J* = 16.0 Hz), 5.61 (1H, dd, *J* = 5.8, 16.0 Hz), 4.49 (1H, m), 4.37 (1H, dq, *J* = 7.6, 6.3 Hz), 4.06 (1H, m), 2.94 (1H, m), 2.92 (1H, m), 2.68 (1H, ddd, *J* = 5.3, 7.4, 11.3 Hz), 2.15-1.15 (15H, m), 1.48 (3H, d, *J* = 6.2 Hz), 1.44 (9H, s), 1.25 (3H, d, *J* = 6.6 Hz), 1.10 (1H, ddd, *J* = 3.7, 12.8, 25.2 Hz); ¹H NMR (500 MHz, C₆D₆) δ 6.37 (1H, d, *J* = 16.0 Hz), 5.46 (1H, dd, *J* = 6.2, 16.0 Hz), 4.55 (1H, m), 4.15 (1H, m), 4.08 (1H, dq, *J* = 7.5, 6.3 Hz), 2.90 (1H, d, *J* = 14.4 Hz), 2.43 (1H, t, 7.7 Hz), 2.28 (1H, m), 1.46 (9H, s), 1.23 (6H, d, *J* = 6.3 Hz), 1.80–0.80 (16H, m); ¹³C NMR/DEPT (50 MHz, CDCl₃) δ 179.0 (C), 155.2 (C), 141.1 (C), 132.6 (CH), 126.6 (CH), 122.8 (C), 81.8 (CH), 79.2 (C), 52.9 (CH), 47.2 (CH), 43.9 (CH), 39.0 (CH), 37.7 (CH), 35.8 (CH₂), 30.0 (CH₂), 28.5 (CH₃), 28.1 (CH₂), 27.4 (CH₂), 26.6 (CH₂), 26.3 (CH₂), 26.1 (CH₂), 21.3 (CH₃), 20.6 (CH₃), 13.9 (CH₂).

(3*S*,3*aR*,8*aR*,9*aR*)-4-[(*E*)-2-((2*S*,6*R*)-1-*tert*-Butyloxycarbonyl-2-methylpiperidin-6-yl)ethenyl]-3-methyl-3*a*,5,6,7,8,8*a*,9,9*a*-octahydro-3*H*-naphtho[2,3-*c*]furan-1-one (28*b*). Bromide **25b** was reacted with tin derivative **26** as described above for bromide **25a** to give **28b** (77%) as a waxy solid: R_f (isooctane/EtOAc 4:1) 0.25; [α]_D²⁰ +56.1 (*c* 1.090, CHCl₃); IR (KBr film) ν 2961, 2933, 2856, 1777, 1686, 1389, 1361, 1279, 1251, 1174, 1112, 1088, 1030, 1002, 959, 916, 882, 767 cm⁻¹; ¹H NMR/COSY (500 MHz, C₆D₆) δ 5.66 (1H, d, *J* = 15.9 Hz), 5.40 (1H, dd, *J* = 5.7, 15.9 Hz), 4.50 (1H, dd, *J* = 4.0, 8.9 Hz), 4.06 (1H, m), 3.95 (1H, dq, *J* = 10.0, 6.0 Hz), 2.84 (1H, d, *J* = 13.2 Hz), 2.14 (1H, dt, *J* = 2.8, 13.0 Hz), 2.03 (1H, t, *J* = 11.2 Hz), 1.86 (1H, m), 1.46 (9H, s), 1.42 (3H, d, *J* = 6.1 Hz), 1.17 (3H, d, *J* = 6.6 Hz), 1.80-1.00 (15H, m); ¹³C NMR/DEPT (50 MHz, C₆D₆) δ 175.4 (C), 155.1 (C), 139.6 (C), 136.6 (CH), 127.1 (C), 125.8 (CH), 79.3 (CH), 78.9 (C), 52.7 (CH), 51.8 (CH), 47.6 (CH), 42.6 (CH), 40.1 (CH), 36.4 (CH₂), 31.4 (CH₂), 29.6 (CH₂), 28.7 (CH₂), 28.6 (CH₃), 27.4 (CH₂), 27.0 (CH₂), 26.6 (CH₂), 20.9 (2CH₃), 14.3 (CH₂); ESMS *m/z* 881 (100) [2M⁺ + Na], 859 (10) [2M⁺ + H], 673 (20), 483 (15), 452 (20) [M⁺ + Na], 430 (5) [M⁺ + H], 330 (10).

(3*S*,3*aR*,8*aR*,9*aS*)-4-[(*E*)-2-((2*S*,6*R*)-1,2-Dimethylpiperidin-6-yl)ethenyl]-3-methyl-3*a*,5,6,7,8,8*a*,9,9*a*-octahydro-3*H*-naphtho[2,3-*c*]furan-1-one (14*a*). To a solution of **28a** (0.020 g, 0.047 mmol) in dichloromethane (2 mL) was added CF₃COOH (0.5 mL). The resulting mixture was stirred at rt for 1 h and the solvent was removed under reduced pressure. The residue was dissolved in CH₃CN (2 mL) and aqueous formaldehyde (37%, 0.25 mL) followed by NaBH₃CN

(0.04 g) were added. The reaction mixture was stirred at rt for 1 h, brought to pH 7 using CH₃COOH and stirring was continued for another 2 h. The mixture was concentrated under reduced pressure, the residue was dissolved in dichloromethane (10 mL) and NaOH (2.5 M in water, 5 mL) was added. This mixture was extracted with dichloromethane (3 × 10 mL) and the combined extracts were dried over anhydrous K₂CO₃. After filtration and concentration, the residue was chromatographed on silica gel (isooctane/acetone 1:1) to give **14a** (0.013 g, 81%) as a colorless oil: R_f (isooctane/acetone 2:3) 0.28; [α]²⁰_D -61.5 (c 1.049, CHCl₃); UV (MeOH) λ_{max} 245 nm; IR (KBr film) ν 2930, 2854, 2772, 1771, 1670, 1448, 1383, 1323, 1269, 1239, 1183, 1142, 1092, 1065, 1031, 956, 734 cm⁻¹; ¹H NMR/COSY (500 MHz, C₆D₆) δ 6.27 (1H, d, *J* = 16.1 Hz), 5.40 (1H, dd; *J* = 8.2, 16.1 Hz), 4.13 (1H, dq, *J* = 6.3, 6.3 Hz), 2.81 (1H, br d, *J* = 14.0 Hz), 2.42 (2H, m), 2.26 (1H, m), 2.19 (3H, s), 1.86 (1H, m), 1.70-1.00 (16H, m), 1.19 (3H, d, *J* = 6.3 Hz), 1.07 (3H, d, *J* = 6.1 Hz); ¹³C NMR/DEPT (50 MHz, C₆D₆) δ 177.7 (C), 140.8 (C), 135.8 (CH), 127.8 (CH), 123.7 (C), 80.8 (CH), 69.2 (CH), 59.7 (CH), 43.7 (CH), 41.0 (CH₃), 38.6 (CH), 38.1 (CH), 36.2 (CH₂), 35.3 (CH₂), 35.0 (CH₂), 30.5 (CH₂), 28.0 (CH₂), 27.9 (CH₂), 26.7 (CH₂), 24.7 (CH₂), 21.9 (CH₃), 21.1 (CH₃); MS *m/z* 343 (22) [M⁺], 328 (20), 314 (5), 300 (12), 270 (6), 230 (10), 199 (10), 171 (8), 149 (32), 125 (48), 112 (100), 91 (55), 58 (90).

(3*S*,3*aR*,8*aR*,9*aR*)-4-[(*E*)-2-((2*S*,6*R*)-1,2-Dimethylpiperidin-6-yl)ethenyl]-3-methyl-3*a*,5,6,7,8,8*a*,9,9*a*-octahydro-3*H*-naphtho[2,3-*c*]furan-1-one (14b). Removal of the *N*-Boc protecting group of **28b** was carried out as described above for **28a** to give **14b** (80%) as a solid: R_f (isooctane/acetone 2:3) 0.20; UV (MeOH) λ_{max} 247 nm; [α]²⁰_D -52.2 (c 1.425, CHCl₃); IR (KBr film) ν 2926, 2843, 2773, 1784, 1445, 1384, 1278, 1179, 1139, 1088, 1068, 1032, 998, 958, 908, 873, 762 cm⁻¹; ¹H NMR/COSY (500 MHz, C₆D₆) δ 5.56 (1H, d, *J* = 16.0 Hz), 5.37 (1H, dd; *J* = 8.4, 16.0 Hz), 3.88 (1H, dq, *J* = 10.0, 6.0 Hz), 2.86 (1H, d, *J* = 13.0 Hz), 2.37 (1H, dd, *J* = 8.1, 14.0 Hz), 2.25 (3H, s), 2.15 (1H, dt, *J* = 2.9, 13.0 Hz), 1.98 (1H, t, *J* = 12.8 Hz), 1.89 (2H, m), 1.76 (1H, dd, *J* = 1.8, 13.0 Hz), 1.70-0.90 (14H, m), 1.29 (3H, d, *J* = 6.0 Hz), 1.12 (3H, d, *J* = 6.1 Hz); ¹³C NMR/DEPT (50 MHz, C₆D₆) δ 175.2 (C), 139.5 (C), 139.4 (CH), 126.7 (C), 126.4 (CH), 78.8 (CH), 68.7 (CH), 59.4 (CH), 51.6 (CH), 42.4 (CH), 40.8 (CH₃), 39.9 (CH), 36.2 (CH₂), 35.2 (CH₂), 34.5 (CH₂), 31.4 (CH₂), 29.4 (CH₂), 28.6 (CH₂), 27.2 (CH₂), 24.6 (CH₂), 21.8 (CH₃), 20.5 (CH₃); MS *m/z* 343 (8) [M⁺], 328 (7), 300 (5), 272 (3), 255 (2), 232 (4), 199 (2), 163 (4), 149 (25), 125 (18), 112 (30), 84 (70), 49 (100).

(3*S*,3*aR*,8*aR*,9*aS*)-4-((2*S*,6*R*)-1-*tert*-Butyloxycarbonyl-2-methylpiperidin-6-yl)ethynyl-3-methyl-3*a*,5,6,7,8,8*a*,9,9*a*-octahydro-3*H*-naphtho[2,3-*c*]furan-1-one (29a). To a mixture of bis(triphenylphosphine)palladium(II) acetate (Pd(PPh₃)₂(OAc)₂, 8.5 mg, 0.001 mmol), bromide **25a** (15 mg, 0.05 mmol) and Et₃N (0.5 mL) in CH₃CN (2 mL) was added a solution of alkyne **27** (23.5 mg, 0.1 mmol) in CH₃CN (0.5 mL) over a period of 30 min. The reaction mixture was refluxed under Ar for 1 h (color change to brown), then cooled to rt and the volatiles were removed under reduced pressure. The residue was dissolved in isooctane/EtOAc 3:1 (5 mL) and passed through a pad of silica gel, which was rinsed with isooctane/EtOAc 3:1 (20 mL). After concentration, the residue was purified by HPLC on silica gel (isooctane/EtOAc 5:1) to yield **29a** (17 mg, 76%) as a colorless oil: R_f (isooctane/EtOAc 4:1) 0.22; [α]²⁰_D +77.1 (c 0.854, CHCl₃);

IR (KBr film) ν 2933, 2859, 1778, 1694, 1454, 1387, 1361, 1306, 1273, 1250, 1176, 1111, 1065, 1028, 954, 862, 773, 732, 641 cm^{-1} ; ^1H NMR/COSY (500 MHz, CDCl_3) δ 4.96 (1H, t, $J = 3.4$ Hz), 4.45 (1H, dq, $J = 8.0, 6.1$ Hz), 3.93 (1H, m), 3.10 (1H, m), 2.64 (2H, m), 2.20-1.70 (12H, m), 1.61 (3H, d, $J = 6.1$ Hz), 1.47 (9H, s), 1.55-1.20 (3H, m), 1.27 (3H, d, $J = 6.7$ Hz), 1.01 (1H, ddd, $J = 3.5, 12.5, 25.0$ Hz); ^{13}C NMR/DEPT (50 MHz, CDCl_3) δ 178.1 (C), 154.9 (C), 149.9 (C), 109.4 (C), 94.7 (C), 81.4 (CH), 80.8 (C), 79.7 (C), 47.9 (CH), 46.7 (CH), 43.9 (CH), 39.0 (CH), 36.8 (CH), 34.7 (CH_2), 31.9 (CH_2), 28.8 (CH_2), 28.5 (CH_3), 27.4 (CH_2), 27.1 (CH_2), 26.5 (CH_2), 25.7 (CH_2), 21.1 (CH_3), 20.7 (CH_3), 15.0 (CH_2); MS m/z 371 (24), 326 (8), 299 (10), 271 (14), 240 (8), 213 (10), 149 (10), 142 (30), 98 (35), 57 (100).

(3S,3aR,8aR,9aR)-4-((2S,6R)-1-tert-Butyloxycarbonyl-2-methylpiperidin-6-yl)ethynyl-3-methyl-3a,5,6,7,8,8a,9,9a-octahydro-3H-naphtho[2,3-c]furan-1-one (29b). Bromide **25b** was reacted with alkyne **27** as described above for bromide **25a** to give **29b** (73%) as a solid: R_f (isooctane/EtOAc 4:1) 0.32; mp 129 $^\circ\text{C}$; $[\alpha]_D^{20} +57.2$ (c 0.552, CHCl_3); IR (KBr) ν 2938, 2852, 1771, 1687, 1478, 1450, 1397, 1363, 1326, 1307, 1285, 1264, 1236, 1180, 1150, 1113, 1081, 1040, 1002, 962, 909, 882, 864, 847, 770, 701, 608 cm^{-1} ; ^1H NMR/COSY (500 MHz, CDCl_3) δ 4.97 (1H, t, $J = 3.0$ Hz), 4.36 (1H, dq, $J = 10.2, 6.1$ Hz), 3.93 (1H, m), 3.00 (1H, m), 2.37 (2H, m), 2.13 (1H, m), 1.61 (3H, d, $J = 6.1$ Hz), 1.46 (9H, s), 2.00-1.20 (15H, m), 1.26 (3H, d, $J = 6.7$ Hz); ^{13}C NMR/DEPT (50 MHz, CDCl_3) δ 176.0 (C), 154.9 (C), 151.7 (C), 110.3 (C), 95.8 (C), 80.1 (CH), 79.7 (C), 78.3 (C), 49.9 (CH), 47.9 (CH), 43.8 (CH), 42.2 (CH), 39.6 (CH), 36.0 (CH_2), 33.0 (CH_2), 28.5 (CH_2), 28.4 (CH_3), 27.6 (CH_2), 27.3 (CH_2), 27.1 (CH_2), 26.6 (CH_2), 21.1 (CH_3), 19.6 (CH_3), 14.8 (CH_2); MS m/z 427 [M^+], 371 (22), 354 (2), 326 (12), 284 (16), 256 (5), 230 (5), 185 (8), 142 (50), 115 (20), 98 (62), 57 (100).

(3S,3aR,8aR,9aS)-4-((2S,6R)-1,2-Dimethylpiperidin-6-yl)ethynyl-3-methyl-3a,5,6,7,8,8a,9,9a-octahydro-3H-naphtho[2,3-c]furan-1-one (15a). Removal of the *N*-Boc protecting group of **29a** was carried out as described above for **28a** to give **15a** (75%) as a colorless oil: R_f (isooctane/acetone 2:3) 0.38; $[\alpha]_D^{20} +10.1$ (c 0.976, CHCl_3); IR (KBr film) ν 2932, 2858, 2787, 1778, 1453, 1382, 1333, 1287, 1252, 1194, 1129, 1065, 1032, 954, 834, 800, 728, 662, 598 cm^{-1} ; ^1H NMR/COSY (500 MHz, C_6D_6) δ 4.21 (1H, dq, $J = 7.8, 6.1$ Hz), 3.70 (1H, br s), 3.24 (1H, br d, $J = 14.1$ Hz), 2.50 (1H, dtd, $J = 2.5, 6.3, 17.0$ Hz), 2.28 (3H, s), 2.24 (2H, m), 1.80-1.00 (15H, m), 1.43 (3H, d, $J = 6.1$ Hz), 0.99 (3H, d, $J = 6.2$ Hz), 0.75 (1H, ddd, $J = 3.6, 12.8, 25.3$ Hz, 1H); ^{13}C NMR/DEPT (50 MHz, C_6D_6) δ 176.8 (C), 148.8 (C), 110.5 (C), 92.1 (C), 85.9 (C), 80.5 (CH), 55.6 (CH), 53.2 (CH), 47.2 (CH), 41.3 (CH_3), 39.1 (CH), 36.9 (CH), 35.2 (CH_2), 35.0 (CH_2), 32.6 (CH_2), 31.8 (CH_2), 28.8 (CH_2), 27.0 (CH_2), 26.0 (CH_2), 21.3 (CH_2), 21.1 (CH_3), 21.0 (CH_3); MS m/z 341(29) [M^+], 326 (100), 312 (15), 298 (20), 270 (25), 242 (12), 197 (10), 155 (15), 149 (35), 115 (48), 96 (70), 64 (40), 42 (90).

(3S,3aR,8aR,9aR)-4-((2S,6R)-1,2-Dimethylpiperidin-6-yl)ethynyl-3-methyl-3a,5,6,7,8,8a,9,9a-octahydro-3H-naphtho[2,3-c]furan-1-one (15b). Removal of the *N*-Boc protecting group of **29b** was carried out as described above for **28a** to give **15b** (78%) as a colorless oil: R_f (isooctane/acetone 2:3) 0.40; $[\alpha]_D^{20} -2.0$ (c 0.589, CHCl_3); IR (KBr film) ν 2932, 2854, 2782, 1787, 1446, 1388, 1333, 1286, 1171, 1142, 1093, 1050, 1031, 1003, 962, 912, 888, 691, 595

cm⁻¹; ¹H NMR/COSY (500 MHz, C₆D₆) δ 4.08 (1H, dq, *J* = 9.8, 6.0 Hz), 3.69 (1H, br s), 3.17 (1H, dd, *J* = 2.3, 13.5 Hz), 2.48 (1H, dtd, *J* = 2.4, 6.3, 17.2 Hz), 2.27 (3H, s), 2.05 (1H, dt, *J* = 2.9, 12.9 Hz), 1.98 (1H, m), 1.80-0.80 (16H, m), 1.52 (3H, d, *J* = 6.0 Hz), 0.98 (3H, d, *J* = 6.2 Hz); ¹³C NMR/DEPT (50 MHz, C₆D₆) δ 174.6 (C), 150.7 (C), 111.5 (C), 93.5 (C), 83.3 (C), 79.2 (CH), 55.7 (CH), 53.2 (CH), 50.2 (CH), 42.3 (CH₃), 41.3 (CH), 39.7 (CH), 36.0 (CH₂), 35.2 (CH₂), 33.6 (CH₂), 31.8 (CH₂), 29.1 (CH₂), 27.9 (CH₂), 26.9 (CH₂), 21.3 (CH₂), 21.0 (CH₃), 20.0 (CH₃); MS *m/z* 341 (29) [M⁺], 326 (100), 312 (14), 298 (15), 270 (18), 254 (10), 226 (10), 197 (15), 155 (12), 141 (20), 115 (30), 96 (60), 42 (75).

Binding affinity studies. Materials. [³H]-NMS (about 80 Ci/mmol) was obtained from Du Pont-New England Nuclear. Atropine sulfate was obtained from Sigma (St. Louis, FL). The cell culture medium, flasks and foetal calf serum were obtained from GIBCO Life Technologies (Gent, Belgium). All other chemicals were of the highest grade available. The drug stock solutions were prepared in 50% ethanol water at a 10 mM concentration. All subsequent dilutions were performed in water, in order to keep the ethanol concentration in the assays below 1%; at these concentrations ethanol does not interfere with muscarinic binding. Addition of an approximately equimolar HCl concentration was necessary to maintain the drugs in solution in water.

Cell culture. CHO cell lines, transfected respectively with plasmids encoding each of the four muscarinic receptor subtypes (hM₁, hM₂, hM₃, and hM₄), were obtained from Dr. N. Buckley (London, UK). They were maintained in Dulbecco's MEM enriched with 10% Foetal calf serum, 200 I.U./mL penicillin and 100 µg/mL streptomycin. The stock culture medium contained 0.5 µg/mL geneticin to maintain selective pressure; the subcultures prepared for binding studies were maintained in a medium without geneticin. Confluent cells were harvested by scraping with a rubber policeman and centrifuged. The pellet was homogenized in a 1 mM sodium bicarbonate solution, and stored in liquid nitrogen until use.

[³H]-NMS binding. CHO cell homogenates (at a concentration equivalent to about 0.05 to 0.1 nM muscarinic receptors in the binding assays) were incubated at 25 °C, in the presence of [³H]-NMS and of the indicated concentrations of the unlabelled drugs, in 1.0 mL of a 50 mM sodium phosphate buffer (pH 7.4) enriched with 2 mM MgCl₂. Non-specific binding was defined as binding in the presence of 10 µM atropine. The tracer concentrations used were 0.25 nM for competition curves with hM₁, hM₃ or hM₄ receptors, or 0.8 nM for competition curves with hM₂ receptors. To verify the tracer affinities at these receptors, saturation curves were obtained by varying the [³H]-NMS concentration between 0.05 and 3.0 nM. At 25 °C, an incubation period of 2 h for incubations with hM₁, hM₂ or hM₄ receptors, 4 h for incubations with hM₃ receptors was sufficient to achieve equilibrium tracer binding. The incubations were terminated by filtration over glass microfiber filters C (K-LAB), pre-soaked in 0.01% polyethyleneimine (Sigma, St Louis, FL) to reduce non-specific binding. The filters were rinsed 3 times with 2 mL of ice-cold 50 mM sodium phosphate buffer (pH 7.4) then soaked at least 4 h in "Lumagel Plus" (Lumac Lsc.) and the radioactivity counted in a Packard 1500 Tricarb liquid scintillation analyser. The total tracer binding (in the absence of competitor) was kept below 20% (typically between 10

and 15%) of the radioactivity offered, to avoid alterations of the free tracer concentration with increasing competitor concentrations. Non-specific binding was always below 1% of the radioactivity offered (that is, below 10% of the bound radioactivity). Duplicate determinations were always within 5% (typically 3%) of each other.

Data Analysis. All competition curves were performed in duplicate, and repeated twice. The experimental data were analyzed by non-linear curve fitting, using the Graph Pad program. The tracer affinity was determined in the same experiment by saturation curve analysis. The unlabeled drugs K_i values were calculated assuming that they inhibited competitively [^3H]-NMS binding, using the Cheng and Prusoff equation.

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