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Stereoselective Synthesis of Annular 9-*cis*-Retinoids and Binding Characterization to the Retinoid X Receptor

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Analogues of 9-*cis*-retinoic acid incorporating an alicyclic ring between the C19 and C10 positions have been synthesized and evaluated as ligands for the RXRα nuclear receptor. The stereocontrolled synthesis of these configurationally constrained retinoids combines a Stille cross-coupling and the Wittig reaction as key bond-forming steps. The palladium-catalyzed cross-coupling reaction of the *β*-bromo-α,*β*-unsaturated aldehydes **5** to dienylstannane **6** is very fast at room temperature, and takes place with preservation of the dienylstannane geometry. A highly stereoselective Wittig reaction afforded the C7−C8 bond connecting the hydrophobic ring to the retinoid side chain. The binding affinities of these compounds for the receptor were determined, and the structural and energetic rationale behind the affinity profile of the cyclic 9-*cis*-retinoic acid derivatives for the RXRα nuclear receptor was characterized by using Molecular Mechanics protocols.

Introduction

Native retinoids, the compounds related to vitamin A found in cells, are essential dietary substances that are needed by mammals for reproduction, normal embryogenesis, cell growth, maintenance of controlled cell proliferation and differentiation, vision, and integrity of the immune system.[1] The recent discovery and characterization of the retinoid receptors helped to understand the developmental and physiologic networks governed by vitamin A and its analogues.[2] It is now well-established that, within cells, retinoids regulate gene transcription acting through binding to the evolutionary distinct group of nuclear receptors called the retinoic acid receptors (RARs) and retinoid X receptors (RXRs),[1] which are ligand-dependent transcription regulatory factors. The RARs (isotypes RARα, RAR*β*, and RAR*γ*) are activated by both *trans*-retinoic acid **1** and 9-*cis*-retinoic acid **2** (Scheme 1) whereas only the latter ligand activates the RXRs (isotypes RXRα, RXR*β*, and RXR*γ*). Moreover, the RARs share a common mechanism of gene transcription with other members of the nuclear receptor superfamily[2] (vitamin D receptor (VDR), thyroid hormone receptor (TR), peroxisome proliferator-activated receptors (PPARs), and liver X receptor (LXR)) activated by small lipophilic ligands (vitamin D_3, thyroid hormone, fatty acids, and sterols, respectively), namely the formation of heterodimers with the RXRs. For retinoid signaling, the dimeric form of RXR/RAR binds to DNA sequences called retinoic acid response elements (RAREs) located in the promotor region of target genes. On the other hand, the RXRs can also act autonomously as homodimers and bind to 9-*cis*-retinoic acid response elements (RXREs), thus defining two different mechanisms by which retinoids influence gene transcription.[2]

The finding that RXR is a critical component of heterodimer formation has stimulated interest in the pathways of intracellular formation of its ligand 9-*cis*-retinoic acid **2**.[3] Although 9-*cis*-retinoids might be generated from dietary 9-*cis*-*β*-carotene, it is conceivable that 9-*cis*-retinoic acid **2** could be produced by intracellular isomerization (perhaps enzymatically catalyzed) of the thermodynamically more stable *trans*-retinoic acid **1**. It has recently been demonstrated that in bovine liver membranes (presumably mediated by thiol-containing catalysts) *trans*-retinoic acid **1** affords an equilibrium mixture containing stereoisomers with differing geometries around one and two double bonds, including 9-*cis*-

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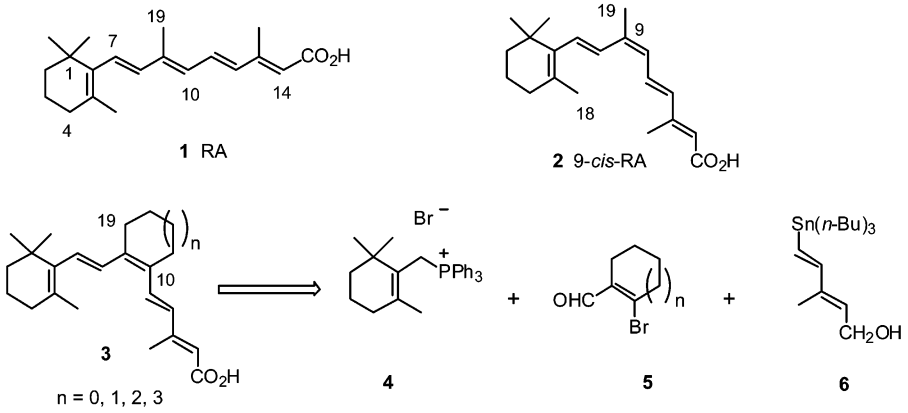
SCHEME 1. Native Ligands of the Retinoid Receptors and Disconnection of the 9-*cis*-Locked Retinoids



retinoic acid **2**.[4] In addition, it is plausible that, after its formation, the less stable 9-*cis*-retinoic acid **2** could be partially converted in vivo into the *trans*-isomer **1**, thus inducing biological responses not easily ascribed to either ligand. In this regard, 9-*cis*-retinoic acid analogues that are unable to isomerize might, at the outset, shed light into the relevance of in vivo isomerization of biologically active retinoids, allowing at the same time the biological effects attributable to 9-*cis*-retinoic acid **2** to be assessed.

With those considerations in mind, we initiated a program aimed to design and synthesize configurationally locked retinoic acid analogues,[5,6] focusing on the structure of the native RXR ligand 9-*cis*-retinoic acid **2**. The recently reported crystal structure of the ligand-binding domain of RXR bound to its cognate ligand 9-*cis*-retinoic acid **2**[7] has revealed a sharp conformational distortion of **2**, with the polyene side chain past C9 positioned almost perpendicular to the hydrophobic ring. The volume occupied by this ligand is only about 59% of the binding pocket total volume, indicating that there is additional space in the cavity for improving binding. Unoccupied volume is available in two regions: near the C19-methyl group close to Trp305 and Asn306, and in the vicinity of the C18 methyl, close to Val349. We set out to determine the effect on RXR binding affinities of configurationally locked 9-*cis*-retinoic acid analogues which incorporate an alicyclic ring near the C19 region. The additional ring of annular 9-*cis*-retinoic acid analogues (compounds **3**) encompass the C19 and C10 positions, thus constraining the retinoid side-chain into the 9-cis configuration, albeit an increase in ring size might allow unsaturated rings with trans geometries to be accommodated. Conformationally dependent RXR binding affinities[8] might then be assessed and molecular modeling studies of the binding of the ring analogues to the RXRα receptor could provide a rationale for the observed results.

Most of these configurationally locked 9-*cis*-retinoids (**3a**−**c**) were first synthesized in view of their suspected antithrombotic potential,[5a] and subsequently **3b** has also been shown to induce differentiation of HL-60 promielocytic leukemia cell lines.[5b] However, no RXR binding selectivities and no comparative studies have been carried out for these analogues, with the exception of **3b**.[5b]

Results and Discussion

Chemistry. Considering that the ring spanning the C9 and C10 double bond is positioned at the central region of the pentaene present in the desired targets **3**, we selected the disconnections shown in Scheme 1. The combination of a single bond (C10−C11) and a double bond (C7−C8) construction allows the convergent synthesis of the retinoids by coupling known fragments **4**,[9] **5**,[10] and **6**.[11]

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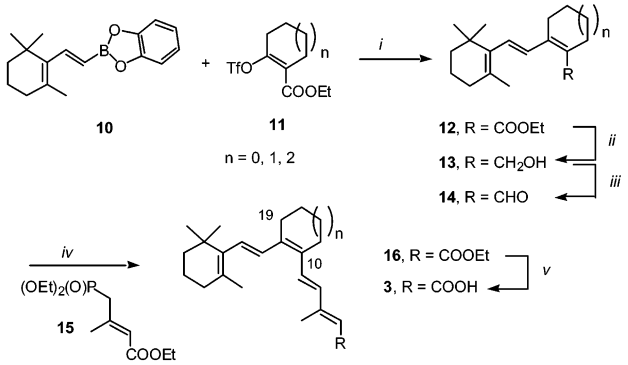

Reagents and conditions: (*i*) $Pd(PPh_3)_4$, KOH. (*ii*) LAH, THF. (*iii*) MnO_2. (*iv*) LDA, THF. (*v*) NaOH, EtOH.

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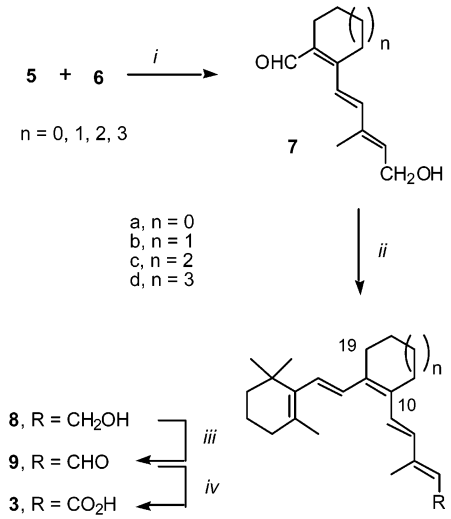
The generation of the C7−C8 double bond in the synthesis of retinoids poses no stereochemical concern due to the high *E*-stereoselectivity of the common double bond forming processes (Wittig, Julia), a selectivity enforced by the bulky trimethylcyclohexenyl ring.[12] On the other hand, the stereocontrolled C10−C11 single-bond construction demands the use of organometallic reactions. We have previously developed convergent approaches to highly conjugated olefins with either side-chain or ring modifications that afford geometrically homogeneous retinoids (trans- or 9-cis), as required to elicit biological responses.[13] These approaches are based on the palladium-catalyzed cross-coupling of alkenyl partners, organometallics (boronic acids or stannanes), and electrophiles (iodides, triflates). Both the Suzuki[14] and the Stille[15] coupling reactions display high levels of regio- and stereoselectivity and the reaction conditions are mild, which is especially well-suited for the preparation of unstable polyenes. For the case at hand, the Stille reaction was selected as a complementary approach to **3** by using Suzuki reactions described earlier.[5] Coupling of organostannanes to β-halo-unsaturated carbonyl derivatives is precedented in the literature.[16]

Following the described procedure, dienylstannane **6**[11] was prepared in good yield and excellent stereoselectivity by stannylcupration of the precursor alkyne with a "higher order"[17] tin cuprate according to the general procedure of Lipshutz,[18] followed by a MeOH quench. On the other hand, β-bromo-unsaturated aldehydes **5**[10] were easily obtained from the corresponding cycloalkanones with PBr_3 and DMF. Those aldehydes proved to be highly unstable, and were used immediately after their purification by distillation.

SCHEME 2. Stereocontrolled Synthesis of 9-*cis*-Locked Retinoids 3[a]



[a] Reagents and conditions: (*i*) $Pd_2(dba)_3$, $AsPh_3$, NMP, 25 °C, 5 min. (*ii*) *n*BuLi, THF, −78 °C, 1 h, 25 °C, 2 h. (*iii*) MnO_2, CH_2Cl_2, 25 °C. (*iv*) $NaClO_2$, NaH_2PO_4, 2-methylbut-2-ene, *t*BuOH−H_2O, 25 °C.

TABLE 1. Yields (%) for the Transformations Depicted in Scheme 2 and Overall Yields [%] of the Entire Sequence from Commercial Starting Materials

trienal **7**	retinol **8**	retinal **9**	retinoic acid **3**
7a (95%)	**8a** (55%)	**15a** (99%)	**3a** (80%); [25%]
7b (97%)	**8b** (59%)	**15b** (99%)	**3b** (83%); [24%]
7c (74%)	**8c** (69%)	**15c** (99%)	**3c** (81%); [20%]
7d (80%)	**8d** (73%)	**15d** (85%)	**3d** (77%); [11%]

For the coupling process, we selected the recent modifications of the Stille protocol introduced by Farina, a combination of "ligandless" palladium catalyst $Pd_2(dba)_3$ and the "soft" ligand $AsPh_3$ in NMP, which accelerates the coupling of organostannanes and electrophiles.[19] For the coupling of dienylstannane **6** and 2-bromocycloalkene carbaldehydes **5**, 5% $Pd_2(dba)_3$ and 5% $AsPh_3$ in NMP was employed, affording compounds **7** in high yields at room temperature in very short reaction times (1−5 min) (Scheme 2).[20]

Wittig reaction (Scheme 2) of conjugated aldehydes **7a**−**d** with excess (3 equiv)[21] phosphorane, generated upon treatment of β-cyclogeranylphosphonium bromide **4**[9] with *n*-BuLi at −30 °C, provided polyenes **8a**−**d** with high stereoselectivity as anticipated in the yields shown in Table 1.

The 9-*cis*-retinol analogues **8a**−**d** were then uneventfully converted to the final carboxylic acids **3a**−**d** through a two-step oxidation protocol. 9-*cis*-Retinal analogues

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TABLE 2. Dissociation Constants and Lowest Interaction Energy for the Annular 9-*cis*-Retinoic Acid Analogues

analogue	K_d (nM)	interaction energy (kcal/mol)
3a	199	−222.3
3b	89	−225.5
3c	1188	−223.9
3d	146	−224.3

9a−**d** were acquired after allylic oxidation of alcohols **8a**−**d** with MnO_2,[22] whereas the desired configurationally constrained 9-*cis*-retinoic acid analogues **3a**−**d** were obtained by oxidation of **9a**−**d** with Lindgrem conditions ($NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*-BuOH−H_2O, 25 °C)[23] in the yields listed in Table 1.[24]

RXR Binding Assay. The assay was performed as previously described.[25] Briefly, COS-7 cells were transfected with a pSG-derived expression vector encoding for human RXRα with the Polybrene technique. Cells were lysed, and the nuclei were recovered by centrifugation. For competition binding assays, nuclear extracts were incubated with [^{3}H]-9-*cis*-retinoic acid (50 nM) as the radioligand and various concentrations of the analogues. Separation of free and bound ligand was performed by using an hydroxylapatite gel. The dissociation constant (K_d value) for each retinoid was determined by nonlinear regression analysis with use of the Origin software (Microcalc Software Inc.). K_d values (nM; for comparison K_d = 50 nM for **2**) are listed in Table 2.

As seen from the values of Table 2, there is no monotonic variation of K_d with ring size. This points out the limitation in space near C19 imposed by the receptor. The best binder is the cyclohexenyl derivative **3b**, which in related studies has been described as a specific RXR ligand and activator of RXR-mediated pathways.[5b]

Molecular Modeling of the Binding Modes and Receptor Affinities. To rationalize the trends shown in Table 2, Molecular Modeling-based docking studies were carried out. As detailed in the Experimental Section, a large number of conformers were generated and docked into the receptor to determine their interaction energies.[26,27] This procedure allows the determination of a "preferred" binding conformation, the one having the lowest interaction energy. Table 2 lists the interaction energy between the receptor and the ligand in its preferred conformation for every analogue, which should be compared with the experimentally determined dissociation constants (see Table 2).

As seen from this comparison, the experimentally observed affinity ranking (for the aliphatic rings) abides by the following ring size order: **3b** > **3d** > **3a** ≫ **3c**.

Some of the differences in the experimental binding free energies are rather small. For instance, the differences in free energy of binding for **3c** and **3d** do not exceed 0.5 kcal/mol. Nevertheless, as seen from the values of Table 2, the interaction energy reproduces some of the features in the ranking of the dissociation constants, such as the affinity ranking order for analogues with an even number of bonds, and most notably the drop in the affinity shown by **3c** with respect to **3b** by 1.5 kcal/mol. This outcome indicates that **3c** is not able to generate an optimal set of interactions with the receptor as opposed to the other aliphatic analogues. Figure 1 displays the ligand receptor contacts for both the **3b** and **3c** analogues. The set of residues interacting with the ligands and the number of van der Waals contacts differ from one ligand to the other. While analogue **3b** interacts with residues Ala272, Trp305, Asn306, Leu309, and Leu436, analogue **3c** contacts residues Ala272, Trp305, Asn306, Leu309, Ile310, and Cys 432. Nevertheless, **2c** produces a larger number of van der Waals contacts (19) with a smaller number of residues (5) than the seven-member analogue **3c**, which contacts 6 residues producing only a total of 17 van der Waals interactions. The difference in the number of van der Waals contacts explains the difference in affinity. The folding of the ring fragment should play a role in allowing some of the analogues to optimize their contacts with the receptor.

The only discrepancy between the observed and calculated affinity rankings for the receptor resides in analogues **3a** and **3c**. As seen from Table 2, the K_d values show that the five-membered-ring analogue is a better binder than the seven-membered-ring analogue, while the interaction energies indicate the opposite. There may be several reasons for this disagreement. The results of the modeling could be affected by the force field used in our calculations. In the present study we have used CHARMM, which has been optimized for biomolecules in general rather than specifically for polyenic compounds. Nevertheless, it has been found that CHARMM-based molecular mechanics calculations of β-ionone, a retinoic acid metabolic precursor, reproduce well its preferred binding conformations to β-lactoglobulin, as observed in NMR experiments.[28]

Analysis of the unrestrained Molecular Dynamics (MD) simulations of the analogues at 300 K gives some clues about the source of the remaining disparity between the observed and calculated ranking of componds **3a** and **3c**. A possible rationale for this discrepancy can be found in the flexibility of both ligands in the unbound state as characterized by the root-mean-square (RMSD) fluctuations with respect to the average structure of a given analogue. Figure 2 depicts the time evolution of the RMSD fluctuations for both the five- (**3a**) and seven- (**3c**) membered-ring 9-*cis*-locked retinoids. The analysis was performed on 100 ps of MD trajectory and every frame was taken at every ps of this trajectory. As seen from

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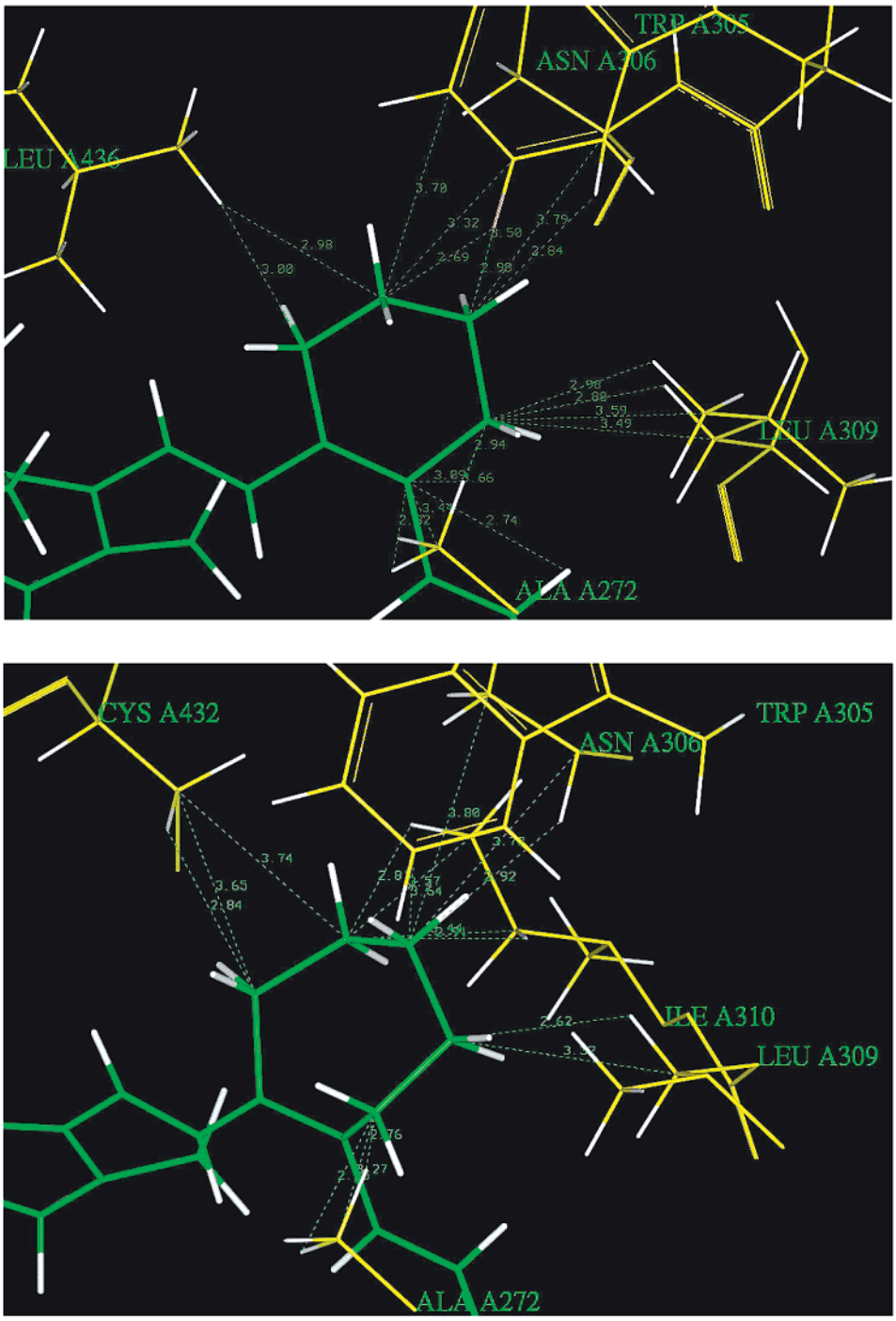

FIGURE 1. Close-up views of the van der Waals interactions (up to 3.8 Å) of the ring-heavy atoms (green) with the receptor (yellow) for analogues **3b** (upper panel) and **3c** (lower panel).

this figure, the RMSD fluctuations are larger for **3c** than for **3a**, in line with the larger number of degrees of freedom available to the former compound. These results indicate that the entropy penalty for binding **3c** to RXRα is larger than that for binding **3a**. Including a conformational entropy term in the prediction of the binding free energy could improve the agreement between the observed and calculated binding ranking of these compounds.

In summary, configurationally constrained 9-*cis*-retinoic acid analogues have been efficiently prepared by using a combination of Stille coupling and Wittig condensation reactions, followed by adjustment of the oxidation state. The binding affinity of these compounds has been determined and they have been rationalized by analysis of Molecular Modeling based docking of these compounds to the receptor. Moreover, the alicyclic binding rank was shown to be determined to a large extent by their interaction with the receptor. The outcome indicates that the available space is limited and it is not the only factor determining the binding capabilities. Including entropic terms that take into account the larger flexibility of those analogues with larger aliphatic rings could help to bridge the only remaining difference

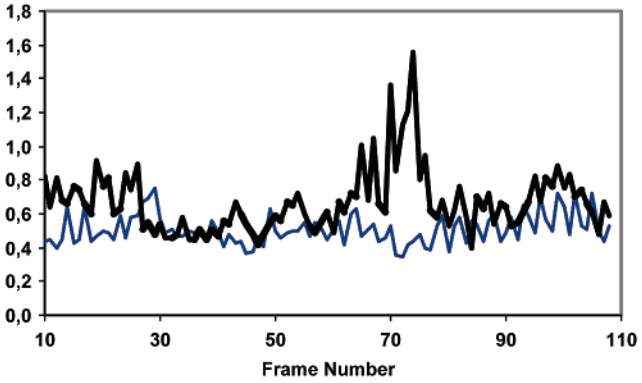

FIGURE 2. RMSD fluctuations of the structures of compounds **3a** (thin line) and **3c** (thick line) with respect to their averaged structure in an MD simulation, as a function of time.

between the observed dissociation constants and the predicted affinities.

Experimental Part

General Experimental Procedures: 2-[(1*E*,3*E*)-5-Hydroxy-3-methylpenta-1,3-dien-1-yl]cyclohex-1-en-carbaldehyde (7b). To a solution of *β*-bromoaldehyde **5b** (1.0 g, 5.29 mmol) in NMP (15 mL) were added $AsPh_3$ (65 mg, 0.21 mmol) and $Pd_2(dba)_3$ (194 mg, 0.21 mmol). After the mixture was stirred at room temperature for 10 min, a solution of vinylstannane **6** (2.37 g, 6.13 mmol) in NMP (24 mL) was added, and the reaction mixture was again stirred at room temperature for 5 min. The reaction was quenched by addition of a saturated aqueous KF solution (20 mL) and stirring was maintained for 30 min. The aqueous layer was extracted with ether (3×) and the combined organic layers were washed with saturated KF (3×) and H_2O (3×), dried ($MgSO_4$), filtered, and concentrated. The residue was purified by chromatography (silica, 70:30 hexane/ethyl acetate) to yield 1.06 g (97%) of aldehyde **7b** as an orange oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.5−1.6 (4H, m), 1.77 (3H, s), 2.22 (2H, t, J = 6.0 Hz), 2.37 (2H, t, J = 6.0 Hz), 4.26 (2H, d, J = 6.7 Hz), 5.74 (1H, t, J = 6.7 Hz), 6.45 (1H, d, J = 15.7 Hz), 7.10 (1H, d, J = 15.7 Hz), 10.26 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.6, 21.4, 21.9, 23.1, 27.5, 59.4, 122.6, 134.1, 135.2, 135.7, 137.6, 152.1, 190.6; IR (CH_2Cl_2) ν 3600−3100 (broad, OH), 1656 (s, C=O) cm^{-1}; UV (MeOH) λ_{max} 238, 312 nm; MS *m*/*z* (%) 206 (M^+, 4), 197 (3); HRMS [M^+] calcd for $C_{13}H_{18}O_2$ 206.1307, found 206.1307.

(2*E*,4*E*)-3-Methyl-5-[2-[(*E*)-2-(2,6,6-trimethylcyclohex-1-en-1-yl)ethenyl]-1-cyclohexen-1-yl}-2,4-pentadien-1-ol (8b). To a cooled (−30 °C) suspension of phosphonium salt **4** (1.52 g, 3.16 mmol) in THF (7 mL) was added *n*-BuLi (2.57 mL, 1.23 M in THF, 3.16 mmol) and the resulting solution was stirred at 0 °C for 1 h. Aldehyde **7b** (0.18 g, 0.87 mmol) in THF (3 mL) was then added via cannula at −78 °C, and the reaction mixture was stirred at −78 °C for 1 h and at 25 °C for 2 h. Water was added, and the aqueous layer was extracted with ether (3×). The combined organic layers were washed with H_2O (3×) and brine, dried ($MgSO_4$), and concentrated. Purification by chromatography (silica, 80:20 hexane/ethyl acetate) afforded 0.17 g (59%) of retinol **8b** as a yellow oil. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.03 (6H, s), 1.4−1.8 (8H, m), 1.74 (3H, s), 1.86 (3H, s), 1.9−2.0 (2H, m), 2.3−2.4 (4H, m), 4.29 (2H, d, J = 7.0 Hz), 5.70 (1H, d, J = 7.0 Hz), 6.14 (1H, d, J = 16.1 Hz), 6.28 (1H, d, J = 15.7 Hz), 6.70 (1H, d, J = 16.1 Hz), 6.95 (1H, d, J = 15.7 Hz); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 12.6, 19.2, 21.8, 22.6 (2×), 26.5, 26.8, 28.9 (2×), 33.0, 34.2, 39.5, 59.5, 126.4, 126.5, 128.9, 129.6, 130.8 (2×), 131.3, 134.1, 137.4, 138.5; IR (CH_2Cl_2) ν 3600−3100 (broad, OH) cm^{-1}; UV (MeOH) λ_{max} 240, 282 nm; MS *m*/*z* (%) 326 (M^+, 100), 311 (8), 295 (32); HRMS [M^+] calcd for $C_{23}H_{34}O$ 326.2610, found 326.2603.

(2*E*,4*E*)-3-Methyl-5-{2-[(*E*)-2-(2,6,6-trimethylcyclohex-1-en-1-yl)ethenyl]-1-cyclohexen-1-yl}-2,4-pentadienal (9b). To a mixture of alcohol **8b** (34 mg, 0.10 mmol) in CH_2Cl_2 (3 mL) was added MnO_2 (181 mg, 2.08 mmol) and the resulting suspension was stirred at room temperature for 16 h. The reaction mixture was filtered through Celite and the solvent was removed in vacuo to afford, after chromatography, retinal **9b** (33 mg, 99%) as a pale orange oil. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.04 (6H, s), 1.4−1.8 (8H, m), 1.76 (3H, s), 2.04 (2H, t, J = 5.9 Hz), 2.33 (3H, s), 2.3−2.4 (4H, m), 6.00 (1H, d, J = 8.2 Hz), 6.27 (1H, d, J = 16.1 Hz), 6.35 (1H, d, J = 15.7 Hz), 6.70 (1H, d, J = 16.1 Hz), 7.47 (1H, d, J = 15.7 Hz), 10.10 (1H, d, J = 8.2 Hz); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 13.1, 19.2, 21.8, 22.3 (2×), 26.3, 27.2, 29.0 (2×), 33.0, 34.2, 39.5, 128.9, 129.1 (2×), 129.8, 130.2, 131.0, 133.6, 138.3, 139.4, 155.6, 191.2; IR (CH_2Cl_2) ν 1726 (s, C=O) cm^{-1}; UV (MeOH) λ_{max} 370 nm; MS *m*/*z* (%) 324 (M^+, 100), 309 (18), 291 (14); HRMS [M^+] calcd for $C_{23}H_{32}O$ 324.2453, found 324.2451.

(2*E*,4*E*)-3-Methyl-5-{2-[(*E*)-2-(2,6,6-trimethylcyclohex-1-en-1-yl)ethenyl]-1-cyclohexen-1-yl}-2,4-pentadienoic Acid (3b). To a solution of aldehyde **9b** (38 mg, 0.12 mmol) and 2-methyl-2-butene (0.64 mL, 5.90 mmol) in *t*-BuOH (3 mL) was added, using a syringe pump, for a period of 73 min, 0.6 mL of a solution of $NaClO_2$ (67 mg, 0.74 mmol) and NaH_2PO_4 (53 mg, 0.44 mmol) in H_2O. The reaction mixture was stirred at room temperature for 10 h and then the pH was raised to pH ∼10 by addition of 3 M NaOH. The *t*-BuOH was evaporated under vacuum and the remaining residue was diluted with water, saturated with NaCl, and extracted with hexane. The aqueous layer was acidified to pH ∼3 with 0.5 N HCl and then extracted with ether (3×). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by chromatography (silica, 70:30 hexane/ethyl acetate) to afford retinoic acid **3b** (33 mg, 83%) as a yellow solid (mp 163−164 °C; lit.[5a] mp 165−167 °C). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.04 (6H, s), 1.5−1.7 (8H, m), 1.76 (3H, s), 2.0−2.1 (2H, m), 2.37 (3H, s), 2.3−2.4 (4H, m), 5.84 (1H, s), 6.25 (1H, d, J = 15.6 Hz), 6.32 (1H, d, J = 16.1 Hz), 6.72 (1H, d, J = 15.6 Hz), 7.39 (1H, d, J = 16.1 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.0, 19.2, 21.8 (2×), 22.4, 26.3, 27.1 (2×), 29.0 (2×), 33.0, 34.2, 39.4, 117.2, 128.2, 129.4, 129.8, 130.2, 130.8, 132.8, 138.1, 138.2, 156.0, 171.3; IR (CH_2Cl_2) ν 1682 (m, C=O) cm^{-1}; UV (MeOH) λ_{max} 266, 336 nm; MS *m*/*z* (%) 340 (M^+, 100), 325 (14); HRMS [M^+] calcd for $C_{23}H_{32}O_2$ 340.2402, found 340.2395.

Full details of the synthesis and spectroscopic characterization of analogues **3a**, **3c**, and **3d** are described in the Supporting Information.

Computational Methods

Preparation of Molecular Systems for the Calculations. The starting point for the calculations was the recently obtained structure of the RXRα receptor bound to its cognate ligand 9-*cis*-retinoic acid (PDB code 1FYB). The hydrogens were added to this complex by using the Biopolymer and Builder modules in the InsightII molecular display and handling package, at pH 7 and with the N and C end caps charged.[26]

Modeling the 9-*cis*-Retinoic Analogues. Using the Builder module in the MSI software package,[26] the 5-, 6-, 7-, and 8-membered alicyclic rings were created between atoms C19 and C10 of the molecular backbone of this molecule (see Scheme 1 for atom numbering). To generate multiple conformations of the aliphatic rings in these molecules, a molecular dynamic (MD) trajectory (100 ps in length) at 1000 K in an NVT ensemble was run, keeping most of the backbone atoms rigid, except for atoms C10 and C19. This MD simulation produced 100 conformations for each molecule, spaced at 1 ps. Additionally, 100 ps of a MD simulation, at 300 K (in a NVE ensemble) with all conformational internal degrees of freedom unconstrained in a vacuum, was run to determine the conformational phase space span by every ligand.

Estimating the Binding Affinities. Every single conformation of the 9-*cis*-retinoic derivatives obtained with the restrained MD was docked to the nuclear receptor keeping the same conformation as the original ligand by using the tools of the InsightII molecular display and handling software. The resulting complexes were subjected to a 1000-step energy minimization, using the steepest descent protocol and the CHARMM force field.[27] To rank the binding affinity of each of the 100 conformations of every ligand, we calculated the interaction energy between ligand and receptor using the same force field. This protocol allowed the prediction of the "best binding" conformation for every molecule, which was compared with the experimentally determined affinities.

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Supporting Information Available: Experimental procedures for the synthesis of **3a**, **3c**, and **3d** and ^{1}H and ^{13}C NMR spectra for compounds described in the Experimental Section. This material is available free of charge via the Internet at http://pubs.acs.org.

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