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A Designed Antagonist of the Thyroid Hormone Receptor

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Abstract—We synthesized an analogue of the thyromimetic GC-1 bearing the same hydrophobic appendage as the estrogen receptor antagonist ICI-164,384. While having reduced affinity for the thyroid hormone receptors compared to GC-1, it behaves in a manner consistent with a competitive antagonist in a transactivation assay.

Thyroid hormone, 3,3′,5-triiodo-L-thyronine (T_3, Fig. 1), plays important roles in development and homeostasis.[1] This hormone is synthesized in peripheral tissues by conversion of its precursor, thyroxine (3,5,3′,5′-tetraiodo-L-thyronine), derived from the thyroid gland, and to a lesser extent by the thyroid gland. T_3 is recognized in target tissues by thyroid hormone receptors (TRs)[2] which are members of the nuclear receptor superfamily of transcription regulators.[3] These receptors mediate the effects of thyroid hormone by positively and negatively regulating the transcription of target genes.[2]

Excess activity of the thyroid gland leads to hyperthyroidism that can result in cardiac arrhythmias, heart failure, weakness, and nervousness.[1] Current approaches to treating this disorder include surgical or radiological ablation of the thyroid gland, or the administration of drugs such as propylthiouracil and methimazole that inhibit the biosynthesis of T_3.[1] The cardiac antiarrhythmic drug amiodarone has antithyroid effects,[4,5] and its major metabolite desethylamiodarone (Fig. 1) antagonizes TR under certain in vitro conditions.[6–8] Desethylamiodarone, a non-competitive antagonist of TRβ, has been shown to interfere with the interaction of TRβ with p160 co-activator GRIP1.[9] However, desethylamiodarone has not been shown to be an antagonist in cells in culture or in animals. Amiodarone has a low affinity for the TR,[6] a slow onset of action, and results in serious side effects.[10] Thus, these compounds may not be true TR antagonists, and to our knowledge, no compound with clear TR antagonist activity has been reported.

Previously we reported the potent halogen-free thyromimetic GC-1 (Fig. 1) with a modest selectivity for TRβ over TRα.[11] In this study we report the design and synthesis of GC-1 analogues bearing substituents on the carbon atom that bridges the two aromatic rings.[12] The design of these compounds was based on using a

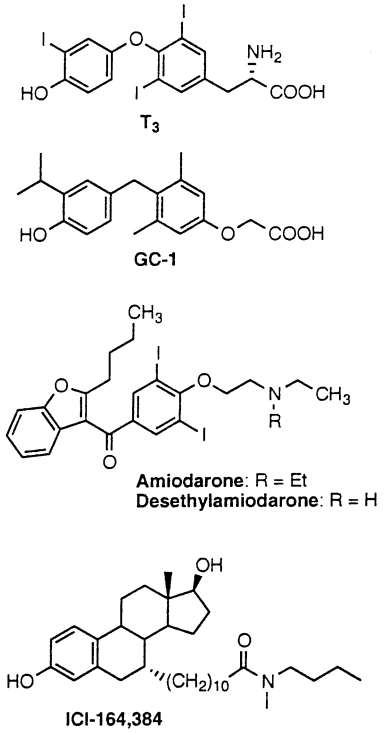

Figure 1. Chemical structures of thyroid hormone (T_3), thyromimetic GC-1, the antithyroid and cardiac antiarrhythmic drug amiodarone, and estrogen antagonist ICI-164,384.

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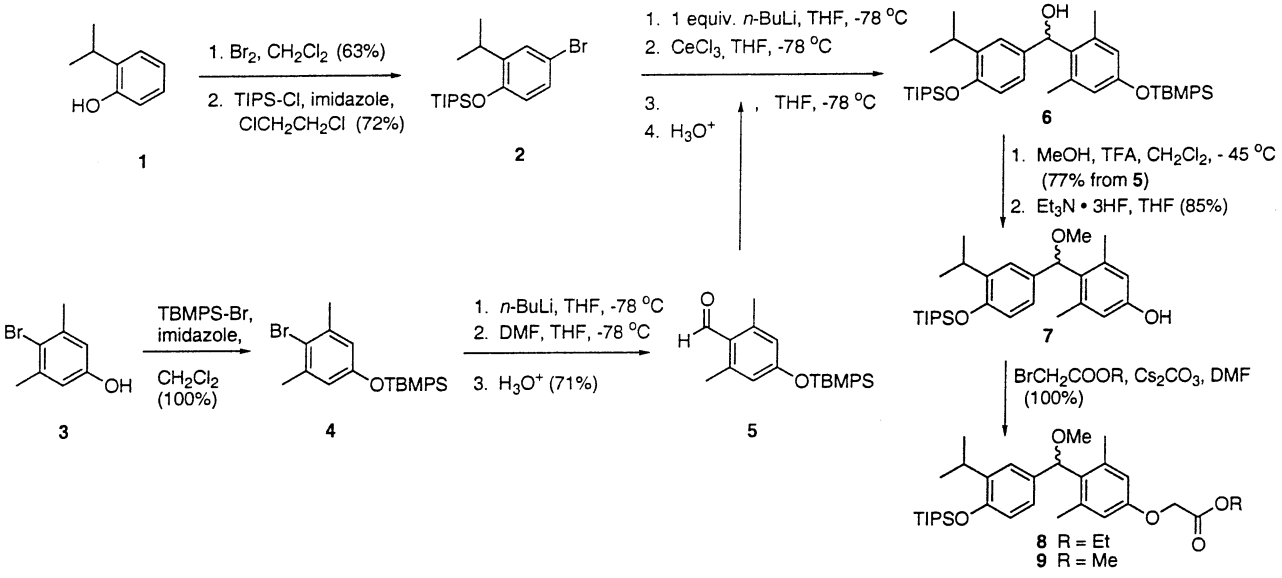
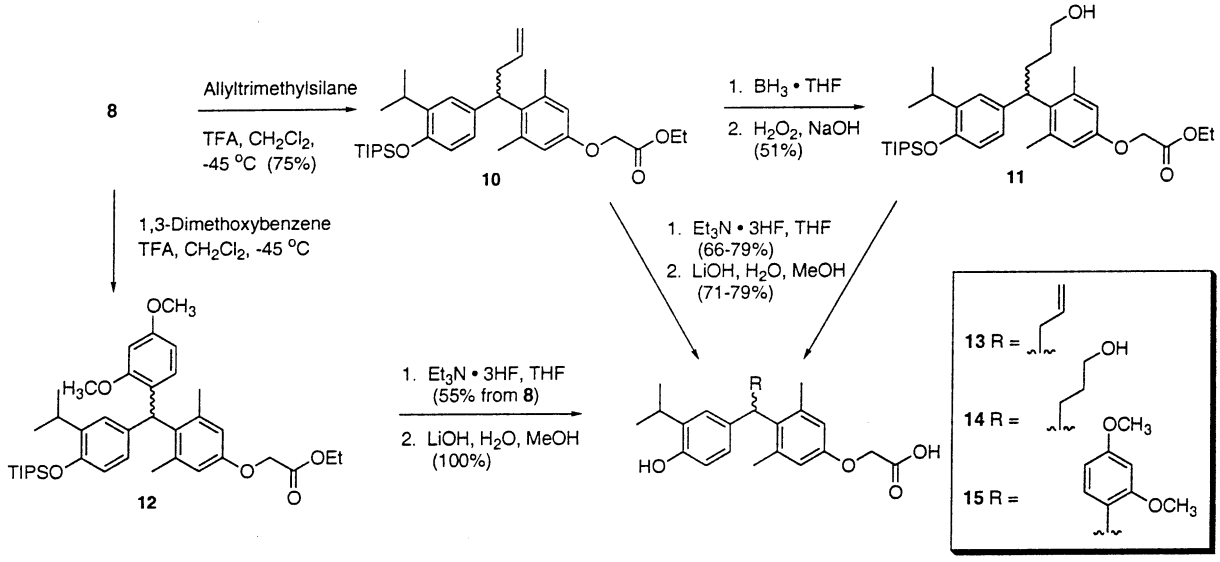
chemical structure that should bind to TR, but contain a side group to perturb the normal agonist-induced folding of the receptor as based on the TR X-ray crystal structure.[19] One of these analogues was found to antagonize T_3 in transactivation assays using cultured cells.

In this and previous[13] reports, we differentially protected the phenolic hydroxyls to improve a low-yielding step in the original synthesis of GC-1. Starting with 2-isopropylphenol (**1**, Scheme 1), bromination and treatment with triisopropylsilyl chloride yielded bromoarylsilyl ether **2**. 4-Bromo-3,5-dimethylphenol (**3**) was protected as the *tert*-butylmethoxyphenylsilyl (TBMPS) acetal, which can be selectively cleaved in the presence of silyl ethers.[14,15] Formylation of **4** to benzaldehyde **5** was followed by the coupling of the two aryl rings via the arylcerium(III) reagent prepared in situ from the aryllithium derived from arylbromide **2**. Using the arylcerium reagent[16,17] instead of its aryllithium precursor gave much higher yields with this sterically hindered electrophile. Crude carbinol **6** was reacted with methanol under acidic conditions, substituting the hydroxyl with a methoxyl group. The resulting product was more chromatographically mobile and easier to purify than **6**. Selective removal of the TBMPS protecting group with triethylamine trihydrofluoride produced phenol **7**, which was alkylated with either ethyl- or methylbromoacetate, yielding oxyacetic esters **8** and **9**, respectively.

Attempts at preparing GC-1 analogues with alkoxy substituents at the bridging carbon were unsuccessful, presumably due to the lability of the ether. The products of allylation (**10**, Scheme 2) or arylation (**12**) reactions of ethyl ester **8** via acid solvolysis were sufficiently stable to be deprotected, yielding GC-1 analogues **13** and **15**. Hydroboration and oxidation of butene



Scheme 1.



Scheme 2.

10 followed by the removal of TIPS and methyl ester protecting groups produced GC-1 analogue **14**.

TR binding data of analogues **13**–**15** are summarized in Table 1. Analogue **13**, having the highest affinity, was tested for its ability to activate TR-mediated transactivation; it exhibited low-potency agonistic activity with EC_{50} values of approximately 1 μM for TRβ and 10 μM for TRα (data not shown).

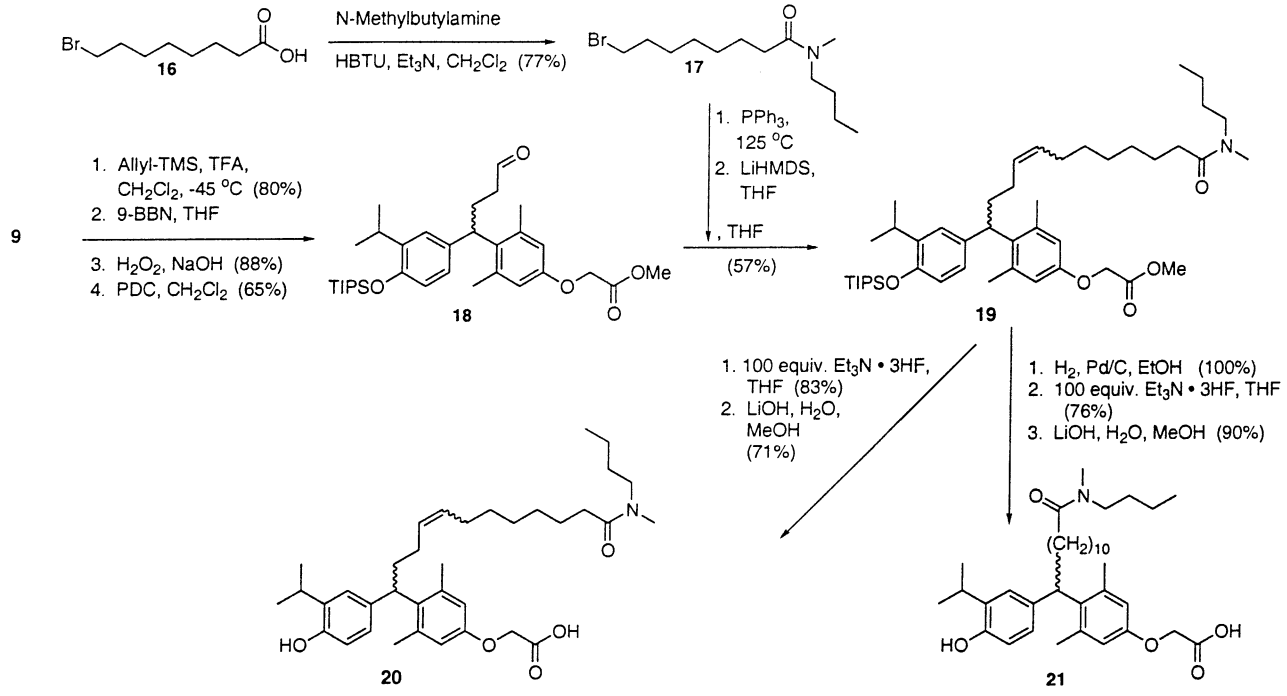
Inspection of the X-ray crystal structures of the ligand binding domains of estrogen receptor α[18] and rat TRα$_1$[19] suggested that the estrogen antagonist ICI-164,384[20,21] (Fig. 1) projects its long alkylamide appendage in a direction similar to the direction a substituent on the bridging carbon of GC-1 would project. This led us to synthesize a GC-1 analogue with the same alkylamide extension. As shown in Scheme 3, methyl ether **9** was allylated, hydroborated and oxidized to butanal **18**. 8-Bromooctanoic acid (**16**) was coupled with *N*-methylbutylamine using HBTU, then reacted neat with molten triphenylphosphine at 125 °C. The crude triphenylphosphonium bromide was deprotonated with lithium bis(trimethylsilyl)amide to generate the ylid and coupled with butanal **18**. The resulting olefin (**19**) was deprotected with and without first subjecting it to catalytic hydrogenation, yielding GC-1 analogues **20** and **21**.

Table 1. Binding affinities of GC-1 bridge-substituted analogues for TR

Compd	K_d (hTRα$_1$)±SE (nM)	K_d (hTRβ$_1$)±SE (nM)
T_3	0.17±0.02	0.14±0.01
13	430±60	90±10
14	1400±800	3600±140
15	1400±700	4200±200
20	240±50	720±100
21	112±18	148±13

The tighter binding analogue **21** (Table 1) was tested for its ability to activate TR-mediated transcription. The assay consisted of JEG-3 cells transiently transfected via electroporation with a TR-expression plasmid and a DR-4-driven luciferase reporter construct.[22] Having only the slightest ability to activate transcription, its ability to block agonist-induced transactivation was examined. The competition curve against 300 pM T_3 (Fig. 2a) indicates antagonism, with increasing concentrations of **21** resulting in reduced transactivation. To assess whether the decrease in transactivation was due to competition with T_3 for TR rather than the inhibition of another factor required for transactivation or reporter activity, dose–response curves of T_3 in the presence and absence of 10 μM **21** were obtained (Fig. 2b). The similarity of the curves, differing only in potency (EC_{50}: 0.64±0.14→4.7±1.3 nM), is indicative of competition between T_3 and **21**. Similar results were observed with TRα$_1$ (data not shown), with the EC_{50} of T_3 shifting from 0.17±0.05 nM to 2.2±1.1 nM in the presence of 10 μM **21**. Antagonism was observed when the method of transfection was either electroporation or calcium phosphate but not when using lipofection, presumably due to the sequestration of **21** by residual lipofection reagent.

With its structural similarity to thyroid hormone and competitive binding to the receptor, **21** likely binds in the same ligand binding pocket as agonist ligands. The recently published X-ray crystal structure of ERβ·ICI-164,384[23] shows the steroid core in the ligand binding pocket with the alkylamide side chain protruding from the pocket in a manner similar to that observed for the hydrophobic extensions of other estrogen receptor



Scheme 3.

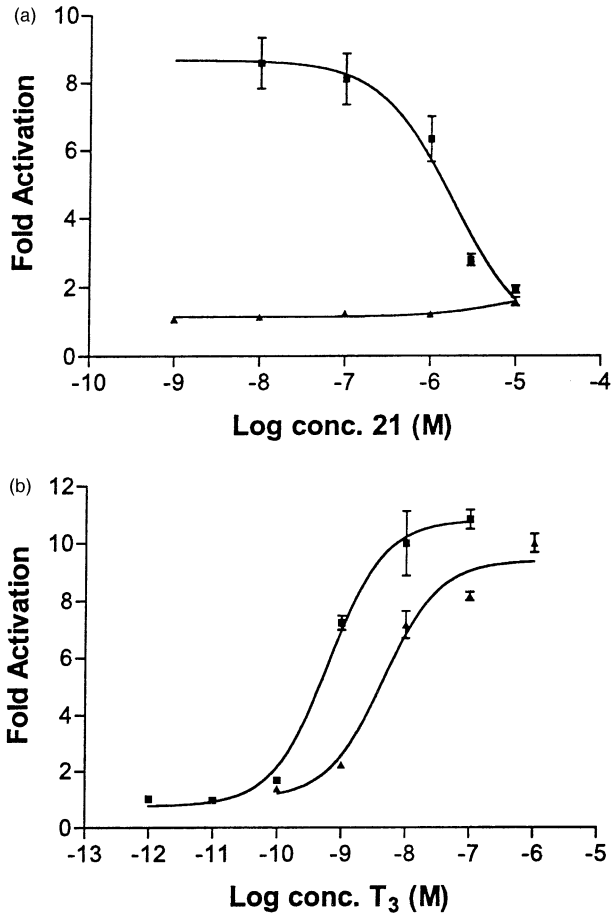


Figure 2. Antagonism by **21** of TR-mediated transactivation of luciferase under the control of a DR4-type thyroid hormone response element in transiently transfected JEG-3 cells: (a) dose–response of **21** alone (triangles) or in competition with 300 pM T_3 (squares); (b) dose–response of T_3 in presence (triangles) and absence (squares) of 10 μM **21**.

modulators complexed with ER,[18,24] resulting in structural perturbations to a surface of the ligand binding domain that interacts with co-regulatory factors. This binding mode requires the steroid core of ICI-164,384 to rotate by approximately 180° from the orientation found in the ERα·estradiol structure. For **21** to bind TR in such a fashion would require a similar change in the orientation of its thyronine core, which, with its bent profile incurred by the sp^3-hybridized carbon linking the two aromatic rings, may have a higher energetic cost than the comparatively planar steroid estradiol. A significant penalty with regard to binding affinity is incurred with substitution at the bridging carbon (Table 1). However, comparing analogue **13** to **21**, increasing the side chain length from 3 to 16 carbons has no additional cost in binding affinity and changes the analogue from an agonist to an antagonist.

All analogues were synthesized as racemic mixtures with the substitution reactions of **8** and **9** proceeding under solvolytic conditions through a presumed carbocation intermediate. Attempts to prepare enantiopure versions of **21**, via resolution of diastereomeric derivatives of **21** or intermediates to **21**, were unsuccessful. The enantiomers may have differing activities, as in the case of the estrogen antagonist ICI-164,384 and its analogues. For the ICI series, only the α-epimers are active estrogen antagonists,[21] indicating that the direction in which the alkylamide extension protrudes is critical for activity. Since the one stereocenter of **21** is the carbon which bears the alkylamide appendage, each enantiomer will project the appendage in a different direction and interact differently with TR.

Unlike GC-1, analogues **14**, **15**, **20**, and **21** do not show a preference in binding to the β subtype of TR, although they share many structural features, including an oxyacetic acid side chain, which may be responsible for conferring GC-1's β-selectivity.[25] Indeed, they have varying degrees of preference for TRα (Table 1). Thus in the context of non-isosteric thyroid hormone analogues, additional factors may influence the preferential binding to one TR subtype.

These results support the notion that the design principles of other nuclear receptor antagonists[26] can also be successfully applied to the thyroid hormone receptor. Thyroid hormone analogue **21** represents a prototype for a new class of antithyroid compounds that will serve as useful pharmacological probes of thyroid hormone action.

Acknowledgements

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References and Notes

1. Braverman, L. E.; Utiger, R. D.; Werner, S. C.; Ingbar, S. H. *Werner and Ingbar's the Thyroid: a Fundamental and Clinical Text*, 6th ed.; Lippincott: Philadelphia, 1991.
2. Zhang, J.; Lazar, M. A. *Annu. Rev. Physiol.* **2000**, *62*, 439.
3. Mangelsdorf, D. J.; Thummel, C.; Beato, M.; Herrlich, P.; Schütz, G.; Umesono, K.; Blumberg, B.; Kastner, P.; Mark, M.; Chambon, P. *Cell* **1995**, *83*, 835.
4. Singh, B. N.; Vaughan Williams, E. M. *Br. J. Pharmacol.* **1970**, *39*, 657.
5. Burger, A.; Dinichert, D.; Nicod, P.; Jenny, M.; Lemarchand-Béraud, T.; Vallotton, M. B. *J. Clin. Invest.* **1976**, *58*, 255.
6. Latham, K. R.; Sellitti, D. F.; Goldstein, R. E. *J. Am. Coll. Cardiol.* **1987**, *9*, 872.
7. Bakker, O.; Vanbeeren, H. C.; Wiersinga, W. M. *Endocrinology* **1994**, *134*, 1665.
8. Vanbeeren, H. C.; Bakker, O.; Wiersinga, W. M. *Mol. Cell. Endocrinol.* **1995**, *112*, 15.

9. van Beeren, H. C.; Bakker, O.; Wiersinga, W. M. *FEBS Lett*. **2000**, *481*, 213.
10. Morse, R. M.; Valenzuela, G. A.; Greenwald, T. P.; Eulie, P. J.; Wesley, R. C.; McCallum, R. W. *Ann. Intern. Med*. **1988**, *109*, 838.
11. Chiellini, G.; Apriletti, J. W.; Yoshihara, H. A. I.; Baxter, J. D.; Ribeiro, R. C. J.; Scanlan, T. S. *Chem. Biol*. **1998**, *5*, 299.
12. Yoshihara, H. A. I.; Chiellini, G.; Mitchison, T. J.; Scanlan, T. S. *Bioorg. Med. Chem*. **1998**, *6*, 1179.
13. Chiellini, G.; Nguyen, N. H.; Yoshihara, H. A. I.; Scanlan, T. S. *Bioorg. Med. Chem. Lett*. **2000**, *10*, 2607.
14. Guindon, Y.; Fortin, R.; Yoakim, C.; Gillard, J. W. *Tetrahedron Lett*. **1984**, *25*, 4717.
15. Gillard, J. W.; Fortin, R.; Morton, H. E.; Yoakim, C.; Quesnelle, C. A.; Daignault, S.; Guindon, Y. *J. Org. Chem*. **1988**, *53*, 2602.
16. Imamoto, T.; Sugiura, Y.; Takiyama, N. *Tetrahedron Lett*. **1984**, *25*, 4233.
17. Imamoto, T.; Kusumoto, T.; Tawarayama, Y.; Sugiura, Y.; Mita, T.; Hatanaka, Y.; Yokoyama, M. *J. Org. Chem*. **1984**, *49*, 3904.
18. Brzozowski, A. M.; Pike, A. C. W.; Dauter, Z.; Hubbard, R. E.; Bonn, T.; Engstrom, O.; Ohman, L.; Greene, G. L.; Gustafsson, J.Å.; Carlquist, M. *Nature (London)* **1997**, *389*, 753.
19. Wagner, R. L.; Apriletti, J. W.; McGrath, M. E.; West, B. L.; Baxter, J. D.; Fletterick, R. J. *Nature (London)* **1995**, *378*, 690.
20. Bowler, J.; Lilley, T. J.; Pittam, J. D.; Wakeling, A. E. *Steroids* **1989**, *54*, 71.
21. Wakeling, A. E.; Bowler, J. *J. Endocrinol*. **1987**, *112*, R7.
22. Transfection assay: JEG-3 cells were grown to ~80% confluency in MEM Eagle's with Earle's BSS with 5% FBS, penicillin and streptomycin. Approximately 1.5×10^6 cells were transfected by electroporation (BioRad Gene Pulser, 0.22 kV, 960 μF, 0.4 cm cuvettes) in 500 μL electroporation buffer with 5 μg of a DR4-pGL3 reporter construct, 1 μg of pRL-TK (Promega) and 1 μg of hTR in a pSG5 vector, per transfection. Cells were plated in 12-well plates in growth medium. After 6 h, medium was changed to a version containing charcoal-stripped FBS and drugs or vehicle (EtOH) were added in triplicate. After 24 h, cells were harvested and assayed for luciferase activity using the Promega Dual Luciferase kit and Analytical Luminescence Laboratory Monolight® 3010 luminometer. Binding assay: the TR-binding assay was performed as previously described.[11]
23. Pike, A. C. W.; Brzozowski, A. M.; Walton, J.; Hubbard, R. E.; Thorsell, A. G.; Li, Y. L.; Gustafsson, J. A.; Carlquist, M. *Structure* **2001**, *9*, 145.
24. Shiau, A. K.; Barstad, D.; Loria, P. M.; Cheng, L.; Kushner, P. J.; Agard, D. A.; Greene, G. L. *Cell* **1998**, *95*, 927.
25. Wagner, R. L.; Huber, B. R.; Shiau, A. K.; Kelly, A.; Cunha Lima, S. T.; Scanlan, T. S.; Apriletti, J. W.; Baxter, J. D.; West, B. L.; Fletterick, R. J. *Mol. Endocrinol*. **2001**, *15*, 398.
26. Weatherman, R. V.; Fletterick, R. J.; Scanlan, T. S. *Annu. Rev. Biochem*. **1999**, *68*, 559.