

## Article

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*ACS Chem. Neurosci.*, **Just Accepted Manuscript** • DOI: 10.1021/acscchemneuro.7b00216 • Publication Date (Web): 10 Jul 2017

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# Ligand dependent switch from RXR homo- to RXR-NURR1 heterodimerization

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**Abstract** Retinoid X Receptors (RXRs) play key roles in many physiological processes in both the periphery and central nervous system. In addition, RXRs form heterodimers with other nuclear receptors to exert their physiological effects. The Nuclear Receptor Related 1 protein (NURR1) is particularly interesting because of its role in promoting differentiation and survival of dopamine neurons. However only a small number of RXR-heterodimer selective modulators are available, with limited chemical diversity. This work describes the synthesis, biochemical evaluation and structural elucidation of a novel series of RXR ligands with strongly biased interactions with RXR $\alpha$ -NURR1 heterodimers. Targeted modifications to the small molecule biaryl scaffold caused local RXR $\alpha$  side-chain disturbances and displacement of secondary structural elements upon ligand binding. This resulted in the repositioning of protein helices in the heterodimer interface of RXR $\alpha$ , alterations in homo- versus heterodimer formation, and modulation of activation function 2 (AF2). The data provide a rationale for the design of RXR ligands consisting of a highly conserved hydrophilic region, strongly contributing to the ligand affinity, and a variable hydrophobic region, which efficiently probes the effects of structural changes at the level of the ligand on co-regulator recruitment or the RXR $\alpha$ -NURR1 dimerization interface.

**Key Words** NUCLEAR RECEPTORS; HETERODIMERIZATION; LIGAND BINDING DOMAIN; RETINOID X RECEPTOR; NUCLEAR RECEPTOR RELATED 1

## Introduction.

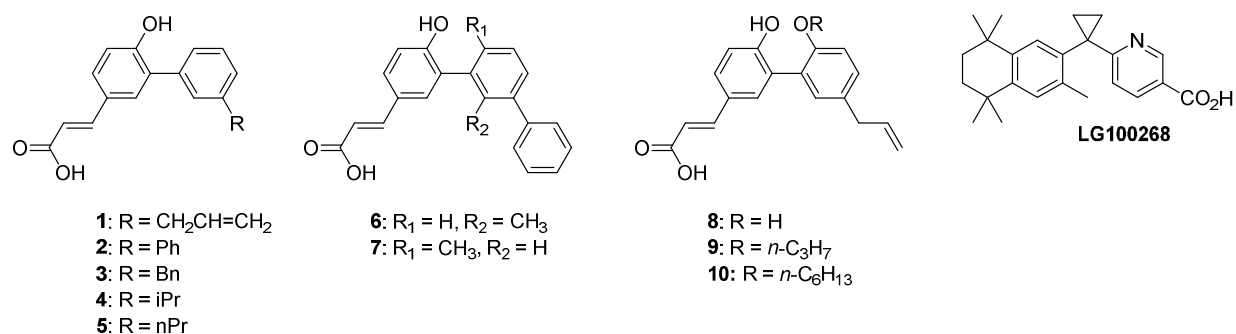
The retinoid X receptor (RXR) plays a key role as a transcriptional regulator through formation of heterodimers with other nuclear receptor partners. Activation of RXR heterodimers exerts neuroprotective effects in animal models of neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease and multiple sclerosis.<sup>1-4</sup> The activity of RXRs is influenced by a class of compounds related to the naturally occurring 9-cis-13,14-dihydroretinoic acid.<sup>5</sup> The L-shaped ligand binding pocket is unique to RXRs, and structural information derived from X-ray crystallographic data of the RXR ligand binding domains in the apo- or holo- state<sup>6,7</sup> has aided the design of specific ligands for this receptor.<sup>8</sup> Comprehensive overviews of the structure, biology and therapeutic implications of targeting RXRs with small molecule ligands are available.<sup>8-12</sup> However, the chemical diversity of these ligands is limited by the structural constraints placed by the RXR ligand binding pocket and the availability of synthetic methodologies to access designed ligands.<sup>13</sup> Controlled RXR heterodimerization and RXR partial agonism are contemporary biomedical challenges,<sup>11,14</sup> both of which could in principle be addressed via exploration of an appropriate structural class.

Three RXR subtypes are known – RXR $\alpha$  (subject of this study), RXR $\beta$ , and RXR $\gamma$  (NR2B1, NR2B2, and NR2B3) – and all three interact similarly with many co-regulator proteins, and with several nuclear receptors to form heterodimers. Ligand binding to RXR can induce the transcriptional activity of some of its heterodimeric partner receptors (NURR1, NGFIB, FXR, LXR, CAR, and PPAR).<sup>15</sup> Thus, RXR-selective ligands that only activate specific RXR heterodimers may have greater therapeutic potential, because they would be expected to cause fewer side effects compared to ligands that cause general activation of RXR–NR heterodimers.<sup>5,9,11</sup> In this respect, RXR–PPAR and RXR–LXR heterodimers have gained a lot of

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3 attention, as the clearance of A $\beta$  through apoE in Alzheimer's mouse models is believed to be  
4 facilitated by the activation of these heterodimers.<sup>16,17</sup> For example, the ligand **LG101506** was  
5 identified as the most potent of a series of selective RXR–PPAR heterodimer activators<sup>18,19</sup>,  
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7 whereas it did not activate the RXR-LXR heterodimer. The potential of this selective RXR  
8 modulator as a treatment for type 2 diabetes with reduced side effects was shown in mice.  
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10 Activation of RXR–nuclear receptor heterodimers with RXR receptor ligands also provides an  
11 important strategy for activating orphan nuclear receptors which themselves do not readily bind  
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13 ligands (e.g. NURR1).  
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22 The Nuclear Receptor Related 1 protein or NURR1 (also referred to as NR4A2) controls the  
23 development, function, and survival of dopaminergic neurons.<sup>20–22</sup> NURR1 knockout mice have  
24 reduced dopaminergic neurons and show perinatal lethality.<sup>23,24</sup> Loss of function NURR1 genetic  
25 polymorphisms in patients are linked to familial Parkinson's disease.<sup>25,26</sup> The crystal structure of  
26 the LBD of NURR1 reveals a 'closed' ligand binding pocket, with the C-terminal helix 12 in a  
27 canonical fold analogous to agonist bound ligand binding domains,<sup>27</sup> which may not easily allow  
28 access to small molecule ligands.<sup>28</sup> Therefore, modulation of NURR1 activity via RXRs would  
29 constitute a viable entry point for NURR1 activation.<sup>4,29–31</sup> A limited number of RXR–NURR1  
30 heterodimer activators have been reported with promising selectivity over other RXR-  
31 heterodimer pairs.<sup>4,32,33</sup> Notwithstanding these important advances in developing selective RXR-  
32 heterodimer modulators, there is a demand for a broader portfolio of RXR–NURR1 modulators,  
33 both for use as research tools, to address open question such as regarding the resulting effect on  
34 RXR signaling via other heterodimers and the potential presence of physiological ligands with  
35 similar profiles, and as potential drugs.<sup>22</sup>  
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Here we report the synthesis, biophysical evaluation and structural elucidation of a new series of RXR $\alpha$  ligands with a strong bias towards promoting RXR $\alpha$ –NURR1 heterodimer versus RXR $\alpha$ –RXR $\alpha$  homodimer formation. The work makes use of the knowledge derived from studies on the natural product<sup>34</sup> honokiol derived RXR $\alpha$  agonists described previously.<sup>35</sup> The biaryl scaffold of this series is straightforward to derivatize, which in principle enables a rapid pharmacophore mapping of the RXR $\alpha$  ligand binding pocket. In this present study, ligands **1** – **5** (Figure 1) were designed to probe the hydrophobic region of the RXR $\alpha$  ligand binding pocket, whilst keeping the polar interactions intact, to access the flexibility and local displacements of amino acid side-chains of the ligand binding domain. Ortho-substituted ligands **6** and **7** were synthesized to investigate how constraining the conformation of the biaryl system would affect the activity of these ligands. Finally, a canonical side-chain extension<sup>36</sup> of the biaryl scaffold in the direction of the RXR $\alpha$  helix 12 produced compounds **9** and **10**, for the purpose to investigate for possible RXR $\alpha$  partial and full antagonism. This compact set of chemical probes was subsequently evaluated as RXR $\alpha$  ligands, using a fluorescence-based polarization assay and cellular mammalian two-hybrid assay. In addition, their effectiveness in modulating RXR $\alpha$ –NURR1 heterodimers over RXR $\alpha$ –RXR $\alpha$  homodimers was tested using cell-based bioluminescence resonance energy transfer (BRET2) assays.<sup>2,33</sup> To corroborate these results and to elucidate the binding mode and conformational changes in the protein induced by these compounds, the X-ray structures of five of these novel ligands bound to RXR $\alpha$  were solved.

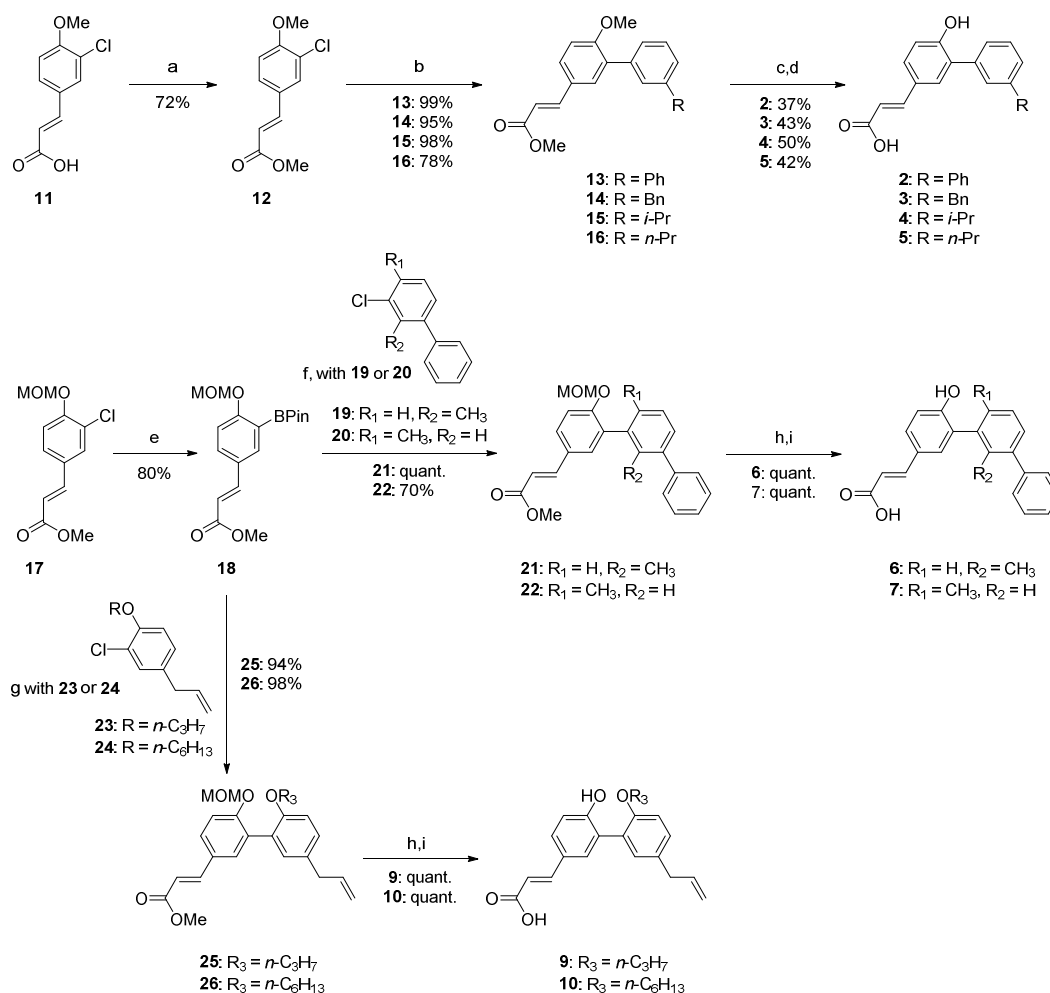


**Figure 1.** Designed biaryl-based RXR ligands **1-10** & established potent RXR agonist LG100268.

## Results and Discussion

**Synthesis.** The synthesis and co-crystal structure of ligand **1** is described previously.<sup>35</sup> The allyl side chain of **1** partly occupies the lipophilic pocket in the ligand binding domain of RXR $\alpha$ , analogously to the tetramethyl-cyclohexene unit found in typical RXR ligands, leading to closure of the ligand binding pocket via repositioning of helix 12 in an agonist conformation and subsequent increased binding towards co-activators. The high binding affinity and low molecular weight of **1** makes it an ideal scaffold to explore modifications targeting the lipophilic pocket (Table 1). Ligands **2–5** were therefore synthesized bearing structural variations in the hydrophobic side chain. The synthesis of compounds **2–5** relied on efficient palladium-catalyzed cross coupling reactions (Scheme 1). The cinnamic acid derivative **11** was treated with thionyl chloride in methanol to obtain the methyl cinnamate derivative **12**. Four boronic acids or esters were then reacted with **12** using a Suzuki coupling (Buchwald-modified) to provide intermediates **13–16** in excellent yields (78–99%).<sup>35,37</sup> The biaryls **13–16** were thereafter demethylated using boron tribromide and hydrolyzed using sodium hydroxide, yielding ligands **2–5** in reasonable yields with high purities after a preparative HPLC purification. Molecules **6** and **7** were designed and synthesized to access the contorted conformation necessary for the biaryl ligands to fit within the ligand binding pocket of RXR $\alpha$ .<sup>8</sup> To enable a more efficient synthesis, the methyl protection of the phenol (**11**) was replaced by a methoxymethyl acetal (MOM) group (**17**). Subsequent Miyaura borylation enabled the key palladium cross coupling with **19** or **20**, accessible in a single step in moderate yields (51–57%). The biaryl products were then treated with dilute hydrochloric acid (3 M) to deprotect the phenol in quantitative yields. Finally, the methyl ester was efficiently hydrolyzed using sodium hydroxide, yielding ligands **6** and **7**.





**Scheme 1.** Synthesis of novel biaryl RXR $\alpha$  ligands. The syntheses of **1** and **8** have been previously reported.<sup>35</sup> Conditions: (a) thionyl chloride, MeOH, 0 °C; (b) arylboronic acid or arylboronic ester, Pd<sub>2</sub>(dba)<sub>3</sub>, SPhos, KF, dioxane/H<sub>2</sub>O (10:1 v/v), 110 °C; (c) BBr<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub> -78 °C. (d) NaOH, dioxane/MeOH (14:5 v/v), 40 °C; (e) Bis(pinacolato)diboron, Pd(OAc)<sub>2</sub>, XPhos, KOAc, dioxane 110 °C; (f) **19** or **20**, Pd<sub>2</sub>(dba)<sub>3</sub>, SPhos, KF, dioxane/H<sub>2</sub>O (6:1 v/v), 110 °C; (g) **23** or **24**, Pd<sub>2</sub>(dba)<sub>3</sub>, SPhos, KF, dioxane/H<sub>2</sub>O (6:1 v/v), 110 °C; (h) HCl, THF, room temperature; (i) NaOH, dioxane/MeOH (14:5 v/v).

In our efforts to selectively drive RXR $\alpha$  homodimers, and not RXR $\alpha$ –NURR1 heterodimers, towards an antagonistic conformation, we applied the previously validated strategy by Nahoum and coworkers for inducing RXR antagonism.<sup>36</sup> Compound **8** (Figure 1) was previously described by us and found to lack significant RXR $\alpha$  activity, because of the additional polar phenolic functionality, which points towards a lipophilic environment.<sup>35</sup> X-ray crystallographic data have demonstrated that alkylation of the appropriate phenol displaces the position of helix 12 towards a (partial) antagonistic fold, influencing the position of L436, which plays a determining role in the communication with helix 12.<sup>36,38</sup> Therefore, using the biaryl scaffold, agonist **8** was modified with two different length alkoxy chains. The length of the alkoxy chain was hypothesized to be directly correlated with the displacement of helix 12 and therefore its antagonist properties.<sup>36</sup> The antagonists **9** and **10** were synthesized using intermediate **18** for the Suzuki couplings, using the ligands introduced by Buchwald,<sup>35,37</sup> with either **23** or **24**. Intermediates **23** and **24** were each made in two steps in excellent yields via sp<sup>2</sup>-sp<sup>3</sup> Pd-catalyzed cross couplings on the bromide group to introduce the allyl-substituted group. Finally, intermediates **25** and **26** were treated with hydrochloric acid in THF for the deprotection of the MOM-group and subsequently with sodium hydroxide for the hydrolysis of the methyl ester to provide the antagonists **9** and **10**.

**Pharmacological evaluation.** The activity of the ligands on RXR $\alpha$  was initially profiled using a fluorescence-based co-activator recruitment polarization (FP) assay and then in a more biologically relevant mammalian two-hybrid (M2H) assay.. The FP and M2H assays revealed an  $EC_{50(FP)} = 260$  nM and  $EC_{50(M2H)} = 6.3$  nM for **1**, which compares favorably to the established, but more bulky, full agonist **LG100268**:  $EC_{50(FP)} = 150$  nM and  $EC_{50(M2H)} = 5.1$  nM (Table 1). The differences in measured  $EC_{50}$  affinities between the FP assay and the M2H assay are a common phenomenon because of intrinsic differences between the two assay formats, the protein concentrations and the co-regulator peptide.<sup>35,39</sup> Besides **1**, ligands **2** – **5** also displayed full agonism in both assays with the measured affinities ( $EC_{50}$ ) in the nanomolar range (Table 1). Replacing the allyl group with the closely related *i*-propyl (**4**), or *n*-propyl (**5**) did not strongly affect the ligand affinity for RXR $\alpha$ . The aromatic phenyl (**2**) and benzyl (**3**) substituents displayed a 10-fold decrease in potency in the M2H cell-based assay compared to the smaller propyl substituents. Nevertheless, also these ligands still activate RXR $\alpha$  with nanomolar potencies. The RXR $\alpha$  binding of **1**–**5** is thus dominated by the hydrophilic portion of the biaryl ligands, *i.e.* the hydroxyl-cinnamic acid moiety. The hydrophobic substituents tune the ligand affinity but are not crucial for high ligand affinity. As such, ligand modifications at this part of the molecule could provide an entry to affect the homo- vs. heterodimer preference of RXR $\alpha$ .

The terphenyl ligand **2**, provides an interesting platform to study the importance and effects of the rotation around the two phenyl-phenyl bonds. Addition of a single methyl group at the central phenyl (**6** and **7**) was thought to direct the rotation to preferred orientations in complex with RXR $\alpha$ . Ligands **6** and **7** both displayed full agonism in the FP assay and the M2H assay, albeit with potencies in the 1-10  $\mu$ M range (Table 1). Comparison with the nanomolar affinities observed for ligand **2**, demonstrates that the addition of the single methyl groups strongly

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3 impacts affinity for RXR $\alpha$ . This very strict SAR is typical for this class of biphenyls, as in our  
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5 previous studies the placement of a hydroxyl functionality, such as in **8**, similarly impacted  
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7 affinity by changes over 100-fold.<sup>35</sup> In the case of **6** and **7** the decrease in affinity might be  
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9 caused by a suboptimal conformational match of the ligand for binding to the protein in the  
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11 conformation befitting the binding pocket (*vide infra*).  
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15 We previously studied ligand **8**, which demonstrated full agonism, but with only moderate  
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17 affinity for RXR $\alpha$  in FP as well as M2H assays.<sup>35</sup> The design of **9** and **10** prompted us to study  
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19 these compounds in a competition format to profile their antagonist characteristics. RXR $\alpha$  was  
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21 therefore stimulated with the full agonist **LG100268** and the subsequent impact of ligands **9** and  
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23 **10** on co-regulator recruitment was studied via fluorescence polarization studies and on  
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25 transcription via M2H assays. The addition of ligands **9** or **10** to the agonist-stimulated RXR $\alpha$   
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27 resulted in decreased fluorescence polarization, indicating lowered co-activator recruitment via  
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29 displacement of the agonist and stabilization of an inactive RXR $\alpha$  conformation. Ligand **10**  
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31 demonstrated full antagonism, while ligand **9** showed partial antagonism (Figure 3a). Consistent  
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33 with its partial antagonist activity, ligand **9** also displayed partial agonism in an agonist assay  
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35 format. The measured affinities of **9**: IC<sub>50(FP)</sub> = 48.5  $\pm$  4.6  $\mu$ M and **10**: IC<sub>50(FP)</sub> = 46.9  $\pm$  5.9  $\mu$ M at  
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37 the RXR $\alpha$  receptor were approximately 25-fold lower than the known antagonist **UVI3003**:  
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39 IC<sub>50(FP)</sub> = 1.8  $\pm$  0.6  $\mu$ M. Compounds **9** and **10** were also RXR $\alpha$  antagonists in the cell-based M2H  
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41 assay. M2H competition experiments showed a clear decrease in luciferase expression upon  
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43 addition of either ligand in the 10 – 40  $\mu$ M range after stimulation with agonist **LG100268**  
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45 (Figure 3b).  
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**Table 1.** Summary of fluorescence polarization (FP) and mammalian two-hybrid (M2H) data for the RXR $\alpha$  agonists

| Compound   | FP / EC <sub>50</sub> (nM) | M2H (Luciferase) / EC <sub>50</sub> (nM) |
|--|----------------------------|--|
| <b>LG100268</b>                                    | 150 $\pm$ 40               | 5.1 $\pm$ 2.0                            |
| <b>1</b> (R = CH <sub>2</sub> CH=CH <sub>2</sub> ) | 260 $\pm$ 110              | 6.3 $\pm$ 4.0                            |
| <b>2</b> (R = Ph)                                  | 140 $\pm$ 23               | 85 $\pm$ 9                               |
| <b>3</b> (R = Bn)                                  | 142 $\pm$ 9                | 92 $\pm$ 36                              |
| <b>4</b> (R = <i>i</i> Pr)                         | 89 $\pm$ 7                 | 5.8 $\pm$ 1.8                            |
| <b>5</b> (R = <i>n</i> Pr)                         | 170 $\pm$ 80               | 18 $\pm$ 10                              |
| <b>6</b>   | 9900 $\pm$ 2500            | > 2500                                   |
| <b>7</b>   | 1020 $\pm$ 60              | 14600 $\pm$ 1800                         |

EC<sub>50</sub> values for **LG100268** and ligands **1–7**. See experimental section for details of the assays. The 20- to 30-fold difference between the FP and M2H data for the more potent compounds is a common phenomenon,<sup>35,40</sup> which can be explained by intrinsic differences between the two different assay formats, in particular, the different protein and peptide concentrations used.

The potency and efficacy of the biaryl ligands to induce RXR $\alpha$ –RXR $\alpha$  homodimer and RXR $\alpha$ –NURR1 heterodimer conformational changes was determined using cellular BRET2 assays<sup>2,33</sup> (Table 2). Agonist ligands **1–5** all displayed remarkably strong potencies (single digit and sub nanomolar EC<sub>50</sub>'s) and high efficacies, comparable to the chemically optimized agonist **LG100268** (Figure 1). Ligands **1–5** all feature preferential affinity for RXR $\alpha$ –NURR1 heterodimers. Ligand **4** displayed an encouraging 25-fold higher potency at RXR $\alpha$ –NURR1 over RXR $\alpha$ –RXR $\alpha$ , with a pEC<sub>50</sub> of 9.1. It should be noted that in these same assays both the well-studied RXR ligand bexarotene and the recently developed dihydrobenzofuran-based ligands only showed 2- to 7-fold selectivity.<sup>2,33</sup> In contrast, the methylated terphenyl ligands **6** and **7** showed higher potency at RXR $\alpha$ –RXR $\alpha$  homodimers. The addition of the single methyl group to **2** *ortho* to the biphenyl bonds, resulting in **6** and **7**, thus leads to a reversal in homo- vs. heterodimer affinity.

The potency and efficacy of the antagonistic biaryl ligands **9** and **10** to selectively induce RXR $\alpha$ –RXR $\alpha$  homodimers over RXR $\alpha$ –NURR1 heterodimers towards antagonistic conformational changes, was also determined. Partial antagonist **9** shows a profile similar to **6** and **7**, but with further biased interactions towards RXR $\alpha$  homodimers over RXR $\alpha$ –NURR1 heterodimers by a factor of 10. Additionally, ligand **9** featured a lower efficacy consistent with its partial antagonist character. The full antagonist **10** did not show appreciable activity in the BRET2 assays.

**Table 2.** Pharmacological evaluation of hetero- and homodimerization using BRET2 assays.

| Compound        | RXR $\alpha$ –NURR1    |           | RXR $\alpha$ –RXR $\alpha$ |           | NURR1–RXR $\alpha$<br>vs. RXR $\alpha$ –RXR $\alpha$<br>selectivity |
|-----------------|------------------------|-----------|----------------------------|-----------|---|
|                 | pEC <sub>50</sub> (SD) | %Eff (SD) | pEC <sub>50</sub> (SD)     | %Eff (SD) | fold  |
| <b>LG100268</b> | 9.3 (0.3)              | 100 (7)   | 8.2 (0.2)                  | 100 (11)  | 13  |
| <b>1</b>        | 8.6 (0.2)              | 129 (14)  | 7.5 (0.1)                  | 269 (32)  | 13  |
| <b>2</b>        | 8.5 (0.2)              | 148 (15)  | 7.5 (0.1)                  | 283 (15)  | 10  |
| <b>3</b>        | 8.6 (0.2)              | 178 (18)  | 7.7 (0.1)                  | 286 (44)  | 8   |
| <b>4</b>        | 9.1 (0.4)              | 129 (4)   | 7.7 (0.1)                  | 291 (28)  | 25  |
| <b>5</b>        | 8.3 (0.2)              | 141 (9)   | 7.0 (0.0)                  | 286 (37)  | 20  |
| <b>6</b>        | 6.1 (0.2)              | 330 (38)  | 6.8 (0.2)                  | 236 (11)  | 0.2   |
| <b>7</b>        | 7.1 (0.4)              | 123 (12)  | 7.5 (0.1)                  | 175 (24)  | 0.4   |
| <b>9</b>        | 6.8 (0.2)              | 41 (25)   | 7.7 (0.2)                  | 66 (6)    | 0.1   |
| <b>10</b>       | <5.0                   | 32 (19)   | <5.0                       | 42 (18)   | -   |

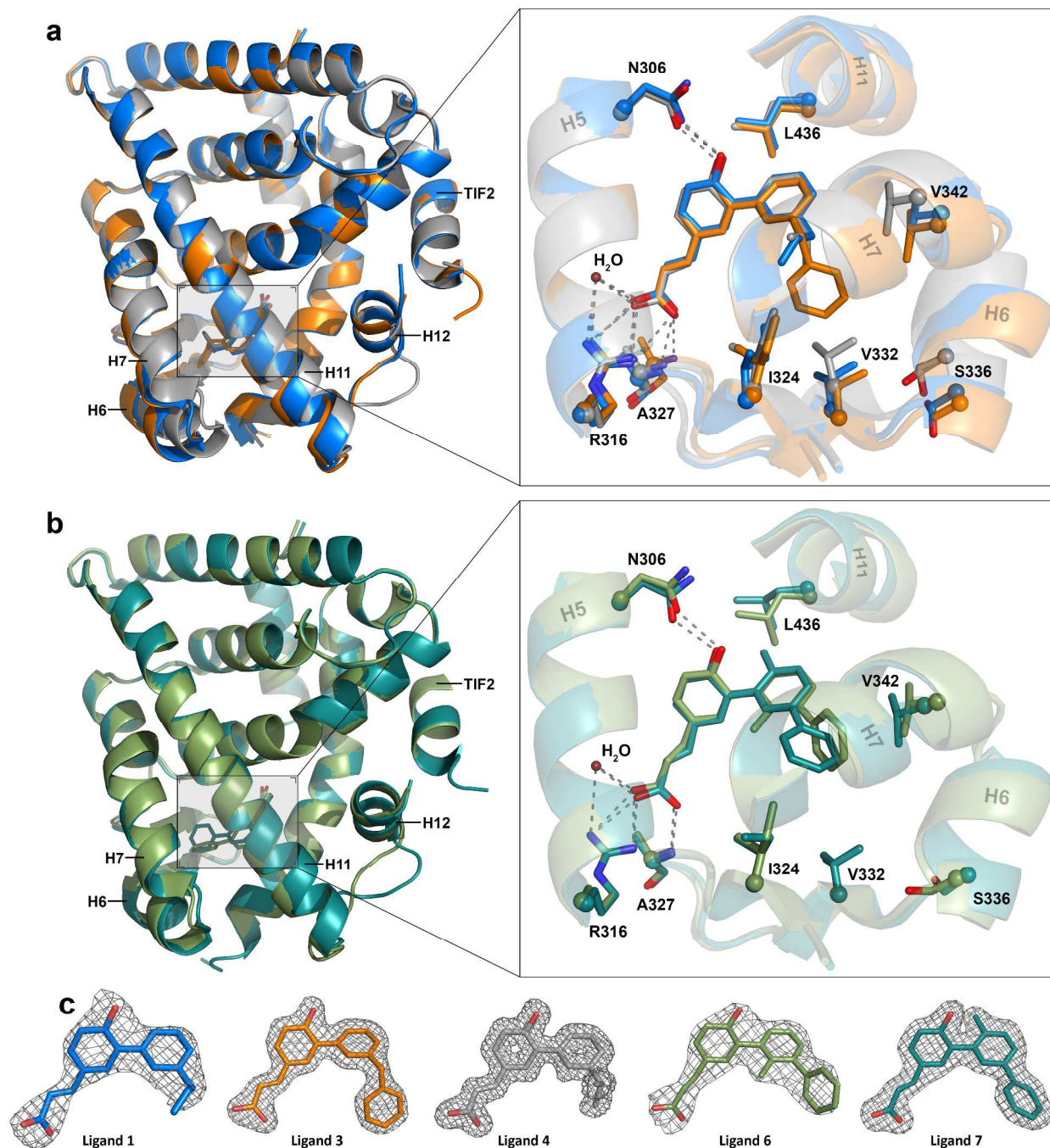
The BRET2 assay was performed as described previously.<sup>33,41</sup>

**Structural evaluation.** The co-crystallization of ligands **1**, **3** and **4** with RXR $\alpha$  showed the canonical interactions of the carboxylate group of the ligands with Arg316, the backbone nitrogen of Ala327 and a conserved water molecule (Figure 2a). The free hydroxyl group on the ligands makes a hydrogen bond with Asn306. This hydrogen bonding network is conserved for all the ligands and directs the positioning of the hydrophobic part of the molecules. The hydrophobic component of **1**, **3**, and **4** occupies the lipophilic region of the ligand binding pocket. In this region, ligand-dependent RXR $\alpha$  amino acid reorientations can be observed. Especially ligand **4** (*i*-propyl substitution) repositions Ile324, Val332, Ser336 and Val342 compared to ligands **1** and **3**, creating a smaller ligand binding pocket (Figure 2a, zoom and Supporting Figure 60). This tighter packing of helices is less pronounced for the region around Ile 324, but mostly affects helices 6 and 7, showing amino acid displacements up to 2.8 Å, and the end of RXR $\alpha$  helix 11, and with that the loop between helix 11 and 12. The carboxy-terminal part of helix 11 has been identified to play a pivotal role in the dimerization of RXRs,<sup>42–44</sup> such as via polar contacts between the C-terminal carboxylic acid of PPAR Helix 12 and lysine 431 of RXR Helix 10/11.<sup>45</sup> NURR1 features an atypical, longer, helix 12<sup>27</sup> which, following a modeled RXR-NURR1 heterodimer<sup>46</sup> and published RXR-PPAR crystal structures,<sup>45,47</sup> probably points towards the RXR $\alpha$  LBD, notably RXR $\alpha$  helices 7 and 11. RXR $\alpha$ -NURR1 heterodimerization thus implies repositioning of RXR $\alpha$  structural elements in this region to accommodate binding of the NURR1 helix 12. Ligand **4** shows the strongest bias towards RXR $\alpha$ -NURR1 heterodimerization (Table 2). The repositioning of RXR $\alpha$  helices 7 and 11 by the compact ligand structure might therefore explain its strong selectivity for heterodimerization.

Terphenyl ligands **6** and **7** were provided with a methyl functionality at the *ortho*-position at either of the two biphenyl bonds. Compared to non-methylated **2**, ligands **6** and **7** featured



decreased affinity for RXR $\alpha$  and bias towards RXR $\alpha$ –RXR $\alpha$  homodimerization. The X-ray co-crystallization of ligands **6** and **7** with RXR $\alpha$  showed the canonical NR fold, bound to a co-regulator peptide (Figure 2b). The biphenyl core scaffold of **6** and **7** was more out-of-plane rotated in comparison to the other agonists. Ligands **6** and **7** fit the canonical L-shaped ligand binding pocket via the same hydrogen bonding network, but compared to, for example, ligand **4** induce significant changes in the positioning of several amino acids and helices 6, 7, and 11 and their connecting loops, which form the lipophilic region of the binding pocket (Figure 2b). More specifically, compared to ligand **4**, the sidechain residues of Val332, Ser336 and Val342 are displaced by 1.0 to 2.2 Å when either ligand **6** or **7** are bound (Supporting Figures 60-62). The *ortho*-methyl substituent on ligand **6** causes the phenyl ring to rotate more out of plane than the other ligands due to steric clash between the methyl and phenyl groups (Supporting Figure 61). This specific out-of-plane orientation of the third phenyl group is apparently unfavorable for the binding of ligand **6** as illustrated by the 10-fold lower RXR $\alpha$  activity compared to **7** (Table 1). The additional *ortho*-methyl substituent on ligand **7** points into the direction of helix 11, displacing Leu436 (Supporting Figure 61). This steric interaction is unfavorable in the context of the phenyl substitution pattern, as highlighted by the strong drop in RXR $\alpha$  activity of **7** compared to ligand **2**. Combined, the methyl substituents on **6** and **7** result in both cases in repositioning of RXR $\alpha$  structural elements, which may correlate with a lower bias towards RXR $\alpha$ –NURR1 heterodimerization as observed in the BRET2 assay.

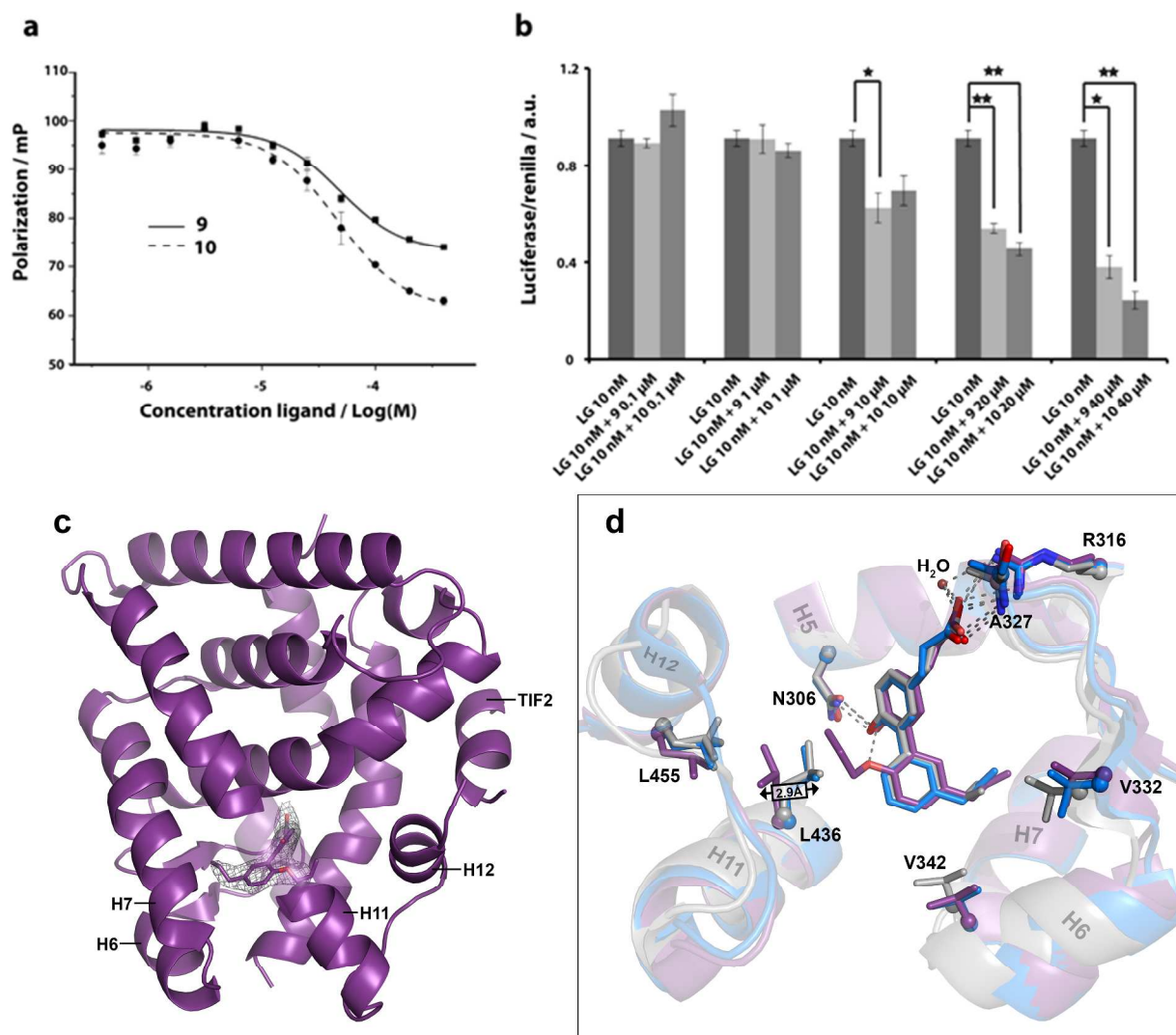


**Figure 2.** Crystal structures of RXR $\alpha$  with agonists 1, 3, 4, 6 and 7. a) Overlay of the X-ray co-crystal structures of ligands 1 (blue, PDB: 4OC7),<sup>35</sup> 3 (orange, PDB: 5MJ5, 1.9 Å resolution), and 4 (grey, PDB: 5MKU, 1.8 Å resolution) bound in the ligand binding pocket of RXR $\alpha$  in ribbon representation with the TIF2 derived co-regulator peptide. Zoom-in on the ligand binding

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3 pocket of RXR $\alpha$  with the amino acids represented as sticks showing the interactions and  
4 displacements. b) Overlay of the X-ray co-crystal structures of ligands **6** (light green, PDB:  
5 5MMW, 2.7 Å resolution) and **7** (dark green, PDB: 5MK4, 2.0 Å resolution) bound to the ligand  
6 binding pocket of RXR $\alpha$  in ribbon representation with the TIF2 derived co-regulator peptide.  
7  
8 Zoom-in on the ligand binding pocket of RXR with the amino acid represented as sticks showing  
9 the interactions between the ligands and the protein. c) Final 2F<sub>o</sub>-F<sub>c</sub> electron density maps  
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18 (contoured at 1 $\sigma$ ) of ligands **1**, **3**, **4**, **6** and **7**.  
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To gain structural information about the effects of the additional alkoxy group of **9** and **10** the X-ray structure of the RXR $\alpha$  ligand binding domain complexed with ligand **9** and the co-regulator peptide TIF2 was solved. The protein was crystallized in the agonist conformation, stabilized by interactions with the co-regulator peptide, and reflected the partial (ant)agonist character of **9**. Comparison of this structure with that of agonist **1** and its analogs (*vide supra*) revealed, as expected,<sup>36</sup> reorientations of amino acid residues related to helix 12 positioning (Figure 3c,d). The most dominant reorientation affected Leu436 in helix 11 induced by the *n*-propoxy chain, which rotated around 3.0 Å towards helix 12, notably L455. Leu436 has been described as a key residue for the communication between the ligand and helix 12 and the correlated activation function-2 (AF2).<sup>9,36,48</sup> The repulsive interaction between the L436 and L455 will lower the association strength between helix 12 and the ligand binding domain, shifting the equilibrium of conformations of the receptor in solution towards an antagonist conformation as demonstrated by the FP and M2H data (Figure 3a,b). The effect might be proportional to the chain length and provides a reasonable rationale for the full antagonism of ligand **10**.

The position of the biaryl scaffold of **9** in the RXR $\alpha$  ligand binding pocket is shifted compared to full agonists like **1** and **4** (Figure 3d). This results in concomitant shifts of RXR $\alpha$  helices 6 and 7, especially when compared to ligand **4** and analogous to ligands **6** and **7**. Similar to ligand **7**, the double substitution pattern on the biaryl scaffold allows partial agonist **9** to simultaneously address two parts of the ligand binding pocket, via helices 6 and 7 and via helices 11 and 12. The combined addressing of the different parts of the RXR $\alpha$  pocket via the hydrophobic substitution pattern leads to amino acid shifts that correlate with preferential affinity for RXR $\alpha$ -RXR $\alpha$  homodimers (Table 2).



**Figure 3.** Ligands **9** and **10** are antagonists at RXR $\alpha$ . a) Fluorescence polarization data showing antagonist activity for **9** and **10**. Ligand **9** displays partial antagonism, while **10** acts as a full antagonist in a competition assay against LG100268 (50 nM). b) Cellular antagonist activities of **9** and **10** measured in an M2H luciferase competition assay against LG100268 (10 nM). c) X-ray co-crystal structures of ligand **9** (purple, PDB: 5MKJ, 2.5 Å resolution) bound in the ligand binding pocket of RXR $\alpha$  in ribbon representation with the TIF2 derived co-regulator peptide. Ligand **9** is shown with final  $2F_o - F_c$  electron density maps (contoured at  $1\sigma$ ). Note that part of RXR $\alpha$  Helix 11 is omitted to allow visualization of the ligand. d) Zoom-in on an overlay of the

ligand binding pockets of RXR $\alpha$  co-crystallized with ligands **1** (blue), **4** (grey), and **9** (purple). Relevant helices and amino acids and their displacements are shown. Compared to **4**, ligand **1** induces an outward expansion of helices 6 and 7. Next to this, ligand **9** induces an additional outward displacement of leucines 436 and 455 on helices 11 and 12.

## Conclusions.

Despite the fact that RXR receptors play major roles in many biological processes through heterodimerization with other nuclear receptors, only a small number of small molecule RXR-heterodimer selective modulators are available, with limited chemical diversity and biophysical properties. This study has delivered a compact and focused selection of RXR $\alpha$ -NURR1 agonists based on a versatile biaryl scaffold, structurally different than previously reported molecules.<sup>10</sup> In earlier work using chiral dihydrobenzofuran acids, we demonstrated a 3-fold biased interaction with RXR $\alpha$ -NURR1.<sup>33</sup> The biaryl scaffold presented here provides a 25-fold selectivity bias for RXR $\alpha$ -NURR1 in the case of analog **4**, and a >100-fold switch in homo- vs. heterodimer selectivity when comparing analogs **4** & **9** in Table 2. The structural elucidation of five of these novel RXR $\alpha$  ligands, in our view, provides a first rationale towards understanding how to generate RXR $\alpha$ -NURR1 heterodimer selective ligands. Key ligand-protein interactions and correlated side-chain displacements were identified, modulating both selective dimerization and co-regulator recruitment. Interactions of the ligand with key amino acid chains such as Ile324, Val332, Ser336 and Val342 on helices 6 and 7 tune the size of the ligand binding pocket. These compact ligands bind RXR $\alpha$  in a manner that allows movement of helix 7 and 11 to generate a compact ligand binding pocket conformation which arguable is more suited for

heterodimerization with NURR1, potentially by enabling the accommodation of the long NURR1 helix 12. Interactions of substituents on the biaryl scaffold with RXR $\alpha$  amino acids involved in formation of the AF2, such as Leu436, induce helix 12 repositioning and translate into lower ligand affinities or, alternatively, into (partial) antagonist properties. These interactions with helices 11 and 12 are matched by the expansion of the RXR $\alpha$  ligand binding pocket via helices 6 and 7, leading to a selectivity of the biaryl scaffold for RXR $\alpha$ -RXR $\alpha$  heterodimers.

This novel series of ligands allows addressing a wide range of RXR $\alpha$  receptor conformations and associated functional outcomes via substitution patterns on the same biaryl scaffold, expanding the current RXR modulator repertoire with agonist as well as antagonist ligands. The data provide a rationale for the design of RXR ligands comprised of a unique hydrophilic region with a conserved hydrogen bonding network contributing to the binding affinity, and a hydrophobic region to probe the other parts of the receptor influencing dimerization properties or co-regulator recruitment. These findings justify further exploration of the ligand-controlled homo- vs. heterodimerization of RXR and its interaction partners, for activation of the NURR1:RXR $\alpha$  heterodimer as monotherapy for Parkinson's disease,<sup>2,4</sup> for delineating the resulting physiological effects on other RXR heterodimers, and also for potentially revealing conserved mechanisms for other nuclear receptors.

## Methods

**Synthesis.** All the solvents employed were commercially available and used without purification unless stated otherwise. Water was purified using a Millipore purification train. All the reagents are commercially available and used without purification. All the NMR data were recorded on a Varian Gemini 400 MHz NMR, a Bruker Cryomagnet 400 MHz a Bruker UltraShield Magnet 400 MHz or a Varian 200 MHz (400 or 200 MHz for  $^1\text{H}$ -NMR and 100 or 50 MHz for  $^{13}\text{C}$ -NMR). Proton experiments are reported in parts per million (ppm) downfield of TMS. All  $^{13}\text{C}$  spectra were reported in ppm relative to residual chloroform (77 ppm) Analytical LC-MS was performed on a C4, Jupiter SuC4300A, 150x2.00 mm column with a gradient 5% to 100% acetonitrile in  $\text{H}_2\text{O}$  supplemented with 0.1% v/v Formic Acid (F.A.) in 15 min. Silica column chromatography was performed manually using silica with particle size 60 – 200  $\mu\text{m}$ . Preparative HP-LC was performed on a Gemini S4 110A 150x21.20 mm column using  $\text{H}_2\text{O}$  and acetonitrile supplemented with 0.1% v/v F.A. Purity and exact mass of the compounds were determined using a High Resolution LC-MS system consisting of a Waters ACQUITY UPLC I-Class system coupled to a Xevo G2 Quadrupole Time of Flight (Q-tof). The system was comprised of a Binary Solvent Manager and a Sample Manager with Fixed-Loop (SM-FL). compounds were separated ( $0.3\text{ mL min}^{-1}$ ) by the column (Polaris C18A reverse phase column 2.0 x 100 mm, Agilent) using a 15% to 75% acetonitrile gradient in water supplemented with 0.1% v/v F.A. before analysis in positive mode in the mass spectrometer. On the basis of LC-UV data, all final compounds are  $\geq 95\%$  pure.

**General procedure for Suzuki couplings method A for the synthesis of compounds 13-16, 21, 22, 25 & 26**



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3 An oven dried Schlenk tube was charged with aryl halide (1.0 eq), boronic acid or boronic ester  
4 (1.2 eq), KF (5.0 eq), SPhos (0.30 eq) and Pd<sub>2</sub>(dba)<sub>3</sub> (0.010 eq). The Schlenk tube was evacuated  
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6 and backfilled with argon three times. Degassed dioxane/H<sub>2</sub>O (10:1 v/v, final aryl halide  
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8 concentration 0.2 M) was added under positive argon flow and the reaction was stirred at the  
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10 indicated temperature for the indicated time. The reaction mixture was then allowed to cool to  
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12 room temperature, passed through Celite with ethyl acetate and concentrated *in vacuo*. The crude  
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14 product was purified by flash silica gel chromatography using the indicated eluent and  
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16 concentrated *in vacuo*.  
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### 22 23 **General procedure for Suzuki couplings method B for the synthesis of 19 & 20**

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26 An oven dried Schlenk tube was charged with aryl halide (1.0 eq), boronic acid or boronic ester  
27 (2.0 eq), KOAc (3.0 eq) and Pd(dppf)Cl<sub>2</sub> (0.010 eq). The Schlenk tube was evacuated and  
28  
29 backfilled with argon three times. Degassed dioxane/H<sub>2</sub>O (5:1 v/v, final aryl halide concentration  
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31 0.4 M) was added under positive argon flow and the reaction was stirred at the indicated  
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33 temperature for the indicated time. The reaction mixture was then allowed to cool to room  
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35 temperature and was separated between CH<sub>2</sub>Cl<sub>2</sub> and H<sub>2</sub>O. The aqueous layer was extracted twice  
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37 with CH<sub>2</sub>Cl<sub>2</sub>. The combined organic layers were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered  
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39 and concentrated *in vacuo*. The crude product was purified by flash silica gel chromatography  
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41 using the indicated eluent and concentrated *in vacuo*.  
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### 49 **General procedure for deprotection, method A: methyl ethers and methyl esters**

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52 The compound was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> to a final concentration of ~0.25 M and cooled to -  
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54 78 °C. A solution of BBr<sub>3</sub> (1 M in CH<sub>2</sub>Cl<sub>2</sub>, 2.0 eq) was added dropwise and the reaction was  
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56 stirred at -78 °C for one hour. The temperature was raised to 0°C and the reaction was stirred for  
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another hour. The reaction was then allowed to warm to room temperature and quenched with H<sub>2</sub>O. The aqueous layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> three times and the combined organic layers washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated *in vacuo*.

The crude product was then dissolved in dioxane/MeOH (14/5 v/v) to a concentration of ~0.20 M. To this mixture was added NaOH (4 N in deionized water, 3.0 eq) was added and the resulting mixture was stirred overnight at either room temperature or 40 °C. The solvent was then removed *in vacuo* and the residue separated in H<sub>2</sub>O and CH<sub>2</sub>Cl<sub>2</sub>. The aqueous layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> three times and the combined organic layers were washed with brine, washed over Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated *in vacuo*. The products were then purified by preparative reversed-phase HPLC by UV detection.

#### General procedure for deprotection, method B: MOM ethers and methyl esters

The compound was dissolved in THF to a final concentration of ~0.2. A solution of HCl (6 N in H<sub>2</sub>O, 3.0 eq) was added and the reaction was stirred at room temperature overnight. The reaction was then diluted with H<sub>2</sub>O and extracted with Et<sub>2</sub>O three times. The combined organic layers washed with saturated aqueous NaHCO<sub>3</sub> and brine, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated *in vacuo*.

The crude product was then dissolved in dioxane/MeOH (14/5 v/v) to a concentration of ~0.20 M. To this mixture was added NaOH (4 N in deionized water, 3.0 eq) was added and the resulting mixture was stirred overnight at either room temperature or 40 °C. The solvent was then removed *in vacuo* and the residue separated in H<sub>2</sub>O and CH<sub>2</sub>Cl<sub>2</sub>. The aqueous layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> three times and the combined organic layers were washed with brine,

washed over Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated *in vacuo*. The products were then purified by preparative reversed-phase HPLC by UV detection and freeze-dried.

**(E)-methyl 3-(6-methoxy-[1,1':3',1''-terphenyl]-3-yl)acrylate (13)** The described procedure for Suzuki couplings method A was used with (E)-methyl 3-(3-chloro-4-methoxyphenyl)acrylate (120 mg, 0.529 mmol), [1,1'-biphenyl]-3-ylboronic acid (130 mg, 0.656 mmol), KF (155 mg, 2.67 mmol), SPhos (67 mg, 0.16 mmol) and Pd<sub>2</sub>(dba)<sub>3</sub> (48 mg, 0.052 mmol) at 110 °C for 18 h. The eluent used for purification was 17% v/v EtOAc in heptane to yield the title compound as a colorless oil, 180 mg, 0.52 mmol, 98% yield. Silica gel TLC *R<sub>f</sub>* = 0.25 (17% v/v EtOAc in heptane); LC-MS (ESI): calc. for C<sub>23</sub>H<sub>20</sub>O<sub>3</sub> [M+H]: 345.15 observed 345.08, LC Rt=8.52 min; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.74 – 7.66 (m, 2H), 7.66 – 7.60 (m, 2H), 7.60 – 7.53 (m, 2H), 7.52 – 7.47 (m, 3H), 7.47 – 7.41 (m, 2H), 7.40 – 7.31 (m, 1H), 6.98 (d, *J* = 8.5 Hz, 1H), 6.36 (d, *J* = 16.0 Hz, 1H), 3.83 (s, 3H), 3.78 (s, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 167.84, 158.44, 144.54, 141.26, 141.25, 138.25, 131.25, 130.64, 129.34, 128.86, 128.62, 128.50, 128.45, 127.42, 127.37, 127.33, 126.30, 115.76, 111.47, 55.87, 51.71..

**(E)-3-(6-hydroxy-[1,1':3',1''-terphenyl]-3-yl)acrylic acid (2)** The described procedure for deprotection, method A was used with (E)-methyl 3-(6-methoxy-[1,1':3',1''-terphenyl]-3-yl) (13) (100 mg, 0.29 mmol) to afford the title compound, **2**, as a white amorphous powder after preparative reverse-phase HPLC and subsequent freeze-drying (34 mg, 0.107 mmol, 37% over two steps). LC-MS (ESI): calc. for C<sub>21</sub>H<sub>16</sub>O<sub>3</sub> [M+H]: 317.12 observed 317.17, LC Rt=6.52 min; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.77 (d, *J* = 15.9 Hz, 1H), 7.71 – 7.55 (m, 5H), 7.54 – 7.42 (m, 5H), 7.42 – 7.36 (m, 1H), 7.04 (d, *J* = 8.7 Hz, 1H), 6.36 (d, *J* = 15.9 Hz, 1H); <sup>13</sup>C NMR (100

MHz, CDCl<sub>3</sub>)  $\delta$  171.82, 155.03, 146.63, 142.75, 140.50, 136.63, 130.84, 130.08, 129.81, 129.08, 128.83, 127.97, 127.92, 127.85, 127.35, 127.34, 127.31, 116.75, 115.09; HRMS (m/z): [M + H]<sup>+</sup> calcd 317.1178, found 317.1179.

**(E)-methyl 3-(3'-benzyl-6-methoxy-[1,1'-biphenyl]-3-yl)acrylate (14)** The described procedure for Suzuki couplings method A was used with (E)-methyl 3-(3-chloro-4-methoxyphenyl)acrylate (97 mg, 0.43 mmol), 2-(3-benzylphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (153 mg, 0.520 mmol), KF (125 mg, 2.15 mmol), SPhos (54 mg, 0.13 mmol) and Pd<sub>2</sub>(dba)<sub>3</sub> (39 mg, 0.040 mmol) at 110 °C for 18 h. The eluent used for purification was 17% v/v EtOAc in heptane to yield the title compound as a colorless oil, 146 mg, 0.41 mmol, 95% yield. Silica gel TLC *R<sub>f</sub>* = 0.28 (17% v/v EtOAc in heptane); LC-MS (ESI): calc. for C<sub>24</sub>H<sub>22</sub>O<sub>3</sub> [M+H]: 359.16 observed 359.08, LC, Rt=8.68 min; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.67 (d, *J* = 16.0 Hz, 1H), 7.48 – 7.42 (m, 2H), 7.37 – 7.25 (m, 5H), 7.24 – 7.14 (m, 4H), 6.92 (d, *J* = 8.3 Hz, 1H), 6.33 (d, *J* = 16.0 Hz, 1H), 4.02 (s, 2H), 3.77 (s, 3H), 3.77 (s, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  167.80, 158.38, 144.56, 141.07, 140.98, 137.83, 131.30, 130.61, 130.15, 129.08, 128.54, 128.28, 128.02, 127.31, 127.22, 126.17, 115.64, 111.41, 55.75, 51.67, 42.05.

**(E)-3-(3'-benzyl-6-hydroxy-[1,1'-biphenyl]-3-yl)acrylic acid (3)** The described procedure for deprotection, method A was used with (E)-methyl 3-(3'-benzyl-6-methoxy-[1,1'-biphenyl]-3-yl)acrylate (**14**) (60 mg, 0.17 mmol) to afford the title compound, **3**, as a white amorphous powder after preparative reverse-phase HPLC and subsequent freeze-drying (24 mg, 0.073 mmol, 43% over two steps). LC-MS (ESI): calc. for C<sub>22</sub>H<sub>18</sub>O<sub>3</sub> [M+H]: 331.14 observed 331.00, LC Rt=6.65 min; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.75 (d, *J* = 15.9 Hz, 1H), 7.49 – 7.40 (m, 3H), 7.33 – 7.19 (m, 8H), 6.99 (d, *J* = 8.4 Hz, 1H), 6.33 (d, *J* = 15.9 Hz, 1H), 4.05 (s, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  172.48, 154.98, 146.74, 142.96, 140.63, 136.23, 130.81, 129.80, 129.66,

129.57, 129.15, 129.05, 128.84, 128.78, 127.17, 126.75, 126.50, 116.63, 115.08, 42.09; HRMS (m/z): [M + H]<sup>+</sup> calcd 331.1334, found 331.1319.

**(E)-methyl 3-(3'-isopropyl-6-methoxy-[1,1'-biphenyl]-3-yl)acrylate (15)** The described procedure for Suzuki couplings method A was used with (E)-methyl 3-(3-chloro-4-methoxyphenyl)acrylate (145 mg, 0.640 mmol), (3-isopropylphenyl)boronic acid (128 mg, 0.780 mmol), KF (187 mg, 3.22 mmol), SPhos (81 mg, 0.20 mmol) and Pd<sub>2</sub>(dba)<sub>3</sub> (61 mg, 0.066 mmol) at 110 °C for 18 h. The eluent used for purification was 15% v/v EtOAc in heptane to yield the title compound as a colorless oil in a quantitative yield. Silica gel TLC *R<sub>f</sub>* = 0.35 (15% v/v EtOAc in heptane); LC-MS (ESI): calc. for C<sub>20</sub>H<sub>22</sub>O<sub>3</sub> [M+H]: 311.16 observed 311.08, LC, Rt=8.52 min; <sup>1</sup>H NMR (399 MHz, CDCl<sub>3</sub>) δ 7.70 (d, *J* = 16.0 Hz, 1H), 7.52 – 7.45 (m, 2H), 7.41 – 7.31 (m, 4H), 6.96 (d, *J* = 8.5 Hz, 1H), 6.35 (d, *J* = 16.0 Hz, 1H), 3.83 (s, 3H), 3.79 (s, 3H), 2.96 (hept, *J* = 6.9 Hz, 1H), 1.29 (d, *J* = 6.9 Hz, 6H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 167.86, 158.44, 148.73, 144.66, 137.65, 130.68, 129.06, 128.50, 128.09, 127.75, 127.24, 127.05, 125.56, 115.60, 111.42, 55.81, 51.69, 34.25, 24.14.

**(E)-3-(6-hydroxy-3'-isopropyl-[1,1'-biphenyl]-3-yl)acrylic acid (4)** The described procedure for deprotection, method A was used with (E)-methyl 3-(3'-isopropyl-6-methoxy-[1,1'-biphenyl]-3-yl)acrylate (**15**) (100 mg, 0.32 mmol) to afford the title compound, **4**, as a white amorphous powder after preparative reverse-phase HPLC and subsequent freeze-drying (45 mg, 0.16 mmol, 50%, over two steps). LC-MS (ESI): calc. for C<sub>18</sub>H<sub>18</sub>O<sub>3</sub> [M+H]: 283.13 observed 283.17, LC, Rt=6.43 min; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.77 (d, *J* = 15.9 Hz, 1H), 7.51 – 7.42 (m, 3H), 7.34 – 7.27 (m, 3H), 7.02 (d, *J* = 8.3 Hz, 1H), 6.34 (d, *J* = 15.9 Hz, 1H), 2.98 (d, *J* = 6.9 Hz, 1H), 1.30 (d, *J* = 6.9 Hz, 6H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 172.43, 155.05, 150.66, 146.82, 135.97,

130.79, 129.68, 129.65, 129.17, 127.26, 127.14, 126.80, 126.38, 116.57, 115.00, 34.34, 24.14;

HRMS (m/z): [M + H]<sup>+</sup> calcd 283.1334, found 283.1334.

**(E)-methyl 3-(4-methoxy-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)acrylate (12b)**<sup>35</sup>

**1-bromo-3-propylbenzene** To a solution of 3-bromopropiophenone (2.98 g, 14.0 mmol) in TFA (30 mL, 0.40 M) was added dropwise triethylsilane (11.5 mL, 72.0 mmol) at 0 °C in 5 minutes and the mixture was stirred for additional 20 minutes. The reaction mixture was heated to 80 °C and stirred overnight. The reaction mixture was allowed to cool to room temperature and concentrated *in vacuo*. Toluene was added and the mixture was again concentrated *in vacuo* to obtain the crude material. The product was purified via flash silica gel chromatography eluting with hexane to yield the title compound, 78 mg, 0.39 mmol, 3% yield. <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>): δ (ppm) 7.38 – 7.27 (m, 2H), 7.18 – 7.06 (m, 2H), 2.59 – 2.49 (m, 2H), 1.72 – 1.56 (m, 2H), 0.93 (td, *J* = 7.3, 0.9 Hz, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): 145.16, 131.65, 129.91, 128.87, 127.28, 122.45, 37.81, 24.49, 13.87.

**(E)-methyl 3-(6-methoxy-3'-propyl-[1,1'-biphenyl]-3-yl)acrylate (16)** The described procedure for Suzuki couplings method A was used with (E)-methyl 3-(4-methoxy-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)acrylate (152 mg, 0.478 mmol) 1-bromo-3-propylbenzene (78 mg, 0.39 mmol), KF (114 mg, 1.96 mmol), SPhos (49 mg, 0.12 mmol) Pd<sub>2</sub>(dba)<sub>3</sub> (37 mg, 0.040 mmol) at 100 °C for 7 h. The eluent used for purification was 5%-10% v/v EtOAc in hexane to yield the title compound as a dark yellow oil, 95 mg, 0.31 mmol, 78% yield. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): 7.69 (d, *J* = 16.0 Hz, 1H), 7.55 – 7.45 (m, 2H), 7.36 – 7.30 (m, 3H), 7.23 – 7.13 (m, 1H), 6.98 (d, *J* = 8.4 Hz, 1H), 6.35 (d, *J* = 16.0 Hz, 1H), 3.85 (s, 3H),

3.80 (s, 3H), 2.75 – 2.57 (m, 2H), 1.76 – 1.61 (m, 2H), 0.98 (t,  $J = 7.3$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ): 167.90, 158.50, 144.68, 142.66, 137.65, 130.69, 129.73, 129.12, 128.54, 128.08, 127.68, 127.30, 126.94, 115.68, 111.45, 55.89, 51.75, 38.27, 24.73, 14.07.

**(*E*)-3-(6-hydroxy-3'-propyl-[1,1'-biphenyl]-3-yl)acrylic acid (5)** The described procedure for deprotection, method A was used with (*E*)-methyl 3-(6-methoxy-3'-propyl-[1,1'-biphenyl]-3-yl)acrylate (**16**), with the adjustment that the intermediate after treatment with  $\text{BBr}_3$  was purified using silica gel chromatography eluting with 5%-10% v/v EtOAc in Hexane to yield the title compound, **5**, as a yellow oil, 25 mg, 0.085 mmol, 42% yield. On repeating the reaction a second time (25.1 mg, 0.084 mmol), the title compound was isolated in a 37% yield over two steps (8.8 mg, 0.03 mmol) as a white amorphous powder after preparative reverse-phase HPLC and subsequent freeze-drying. LC-MS (ESI): calc. for  $\text{C}_{18}\text{H}_{18}\text{O}_3$   $[\text{M}+\text{H}]$ : 283.13, observed 283.17, LC,  $R_t = 6.47$ .  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.76 (d,  $J = 15.9$  Hz, 1H), 7.53 – 7.36 (m, 3H), 7.27 – 7.23 (m, 3H), 7.01 (d,  $J = 8.2$  Hz, 1H), 6.33 (d,  $J = 15.9$  Hz, 1H), 2.72 – 2.62 (m, 2H), 1.75 – 1.66 (m, 2H), 0.98 (t,  $J = 7.3$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  172.22, 155.07, 146.83, 144.45, 135.93, 130.77, 129.64, 129.58, 129.19, 129.08, 128.78, 127.14, 126.26, 116.58, 114.95, 38.16, 24.69, 14.02.

**3-chloro-2-methyl-1,1'-biphenyl (19)** The described general Suzuki coupling conditions method B was used with 1-chloro-3-iodo-2-methylbenzene (275  $\mu\text{L}$ , 1.97 mmol), phenylboronic acid (364 mg, 2.99 mmol) KOAc (584 mg, 5.95 mmol) and  $\text{Pd}(\text{dppf})\text{Cl}_2$  (148 mg, 0.181 mmol). at 90  $^\circ\text{C}$  for 5.5 h. The eluent used for purification was heptane to yield the title compound as a colorless oil, 229 mg, 1.13 mmol, 57% yield. Silica gel TLC  $R_f = 0.56$  (heptane); GC-MS (EI)  $m/z$  calc. for  $\text{C}_{13}\text{H}_{11}\text{Cl}$ : 202.05, most abundant peaks observed: 202, 167;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.43 – 7.29 (m, 4H), 7.28 – 7.21 (m, 2H), 7.18 – 7.03 (m, 2H), 2.27 (s, 3H);  $^{13}\text{C}$  NMR

(100 MHz, CDCl<sub>3</sub>)  $\delta$  144.11, 141.57, 135.40, 133.84, 129.27, 128.42, 128.29, 127.28, 126.49, 17.99.

**(E)-methyl 3-(6-(methoxymethoxy)-2'-methyl-[1,1':3',1''-terphenyl]-3-yl)acrylate (21)** The described general Suzuki coupling conditions method A was used with (E)-methyl 3-(4-(methoxymethoxy)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)acrylate (104 mg, 0.300 mmol), 3-chloro-2-methyl-1,1'-biphenyl (52 mg, 0.26 mmol), KF (72 mg, 1.2 mmol), SPhos (32 mg, 0.078 mmol) and Pd<sub>2</sub>(dba)<sub>3</sub> (25 mg, 0.027 mmol) at 110 °C for 21 h. The eluent used for purification was 17 % v/v EtOAc in heptane to yield the title compound as a colorless oil in a quantitative yield. Silica gel TLC  $R_f$  = 0.31 (heptane/EtOAc 17% v/v); LC-MS (ESI): calc. for C<sub>25</sub>H<sub>24</sub>O<sub>4</sub> [M+H]: 389.18 observed 389.08, LC, Rt=8.63 min. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.68 (d,  $J$  = 16.0 Hz, 1H), 7.49 (dd,  $J$  = 8.6, 2.3 Hz, 1H), 7.45 – 7.32 (m, 6H), 7.31 – 7.20 (m, 3H), 7.18 (dd,  $J$  = 7.0, 2.1 Hz, 1H), 6.35 (d,  $J$  = 16.0 Hz, 1H), 5.14 (s, 2H), 3.79 (s, 3H), 3.37 (s, 3H), 2.02 (s, 3H).; <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  167.77, 156.38, 144.46, 142.45, 142.43, 138.75, 134.27, 132.90, 131.03, 129.50, 129.16, 129.10, 128.26, 128.19, 126.92, 125.33, 116.25, 115.08, 94.72, 56.33, 51.74, 18.18.

**(E)-3-(6-hydroxy-2'-methyl-[1,1':3',1''-terphenyl]-3-yl)acrylic acid (6)** The described procedure for deprotection, method B was used at 40 °C with (E)-methyl 3-(6-(methoxymethoxy)-2'-methyl-[1,1':3',1''-terphenyl]-3-yl)acrylate (**21**) LC-MS (ESI): calc. for C<sub>22</sub>H<sub>18</sub>O<sub>3</sub> [M+H]: 331.13 observed 331.17, LC, Rt=6.67 min. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.76 (d,  $J$  = 15.9 Hz, 1H), 7.51 (dd,  $J$  = 8.5, 2.2 Hz, 1H), 7.47 – 7.32 (m, 8H), 7.24 (dd,  $J$  = 6.7, 2.1 Hz, 1H), 7.04 (d,  $J$  = 8.4 Hz, 1H), 6.34 (d,  $J$  = 15.9 Hz, 1H), 2.04 (s, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  172.46, 155.20, 146.81, 143.84, 141.79, 135.45, 135.00, 130.90, 130.83, 129.85,



129.67, 129.37, 129.05, 128.37, 127.26, 127.03, 126.53, 116.25, 115.05, 17.94. HRMS ( $m/z$ ):  $[M + H]^+$  calcd for  $C_{22}H_{18}O_3$ , 331.1334, found 331.1331.

**3-chloro-4-methyl-1,1'-biphenyl (20)** The described general Suzuki coupling conditions method B was used with 2-chloro-4-iodo-1-methylbenzene (321 mg, 1.27 mmol), phenylboronic acid (226 mg, 1.85 mmol), KOAc (354 mg, 3.61 mmol) and  $Pd(dppf)Cl_2$  (95 mg, 0.12 mmol) at 90 °C for 3.5 h. The eluent used for purification was heptane to yield the title compound as a colorless oil, 124 mg, 0.61 mmol, 51% yield. Silica gel TLC  $R_f$  = 0.49 (heptane); GC-MS (ESI)  $m/z$  calc. for  $C_{13}H_{11}Cl$ : 202.05, most abundant peaks observed: 202, 167;  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  7.62 – 7.49 (m, 3H), 7.47 – 7.29 (m, 4H), 7.29 – 7.22 (m, 1H), 2.39 (s, 3H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  140.56, 139.88, 134.98, 134.87, 131.35, 128.96, 127.70, 127.67, 127.04, 125.37, 19.85.

**(E)-methyl 3-(6-(methoxymethoxy)-6'-methyl-[1,1':3',1''-terphenyl]-3-yl)acrylate (22)** The described general Suzuki coupling conditions method A was used with (E)-methyl 3-(4-(methoxymethoxy)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)acrylate (103 mg, 0.300 mmol), 3-chloro-4-methyl-1,1'-biphenyl (48 mg, 0.24 mmol), KF (72 mg, 1.2 mmol), SPhos (32 mg, 0.078 mmol) and  $Pd_2(dba)_3$  (23 mg, 0.025 mmol) at 110 °C for 21 h. The eluent used for purification was 17 % v/v EtOAc in heptane to yield the title compound 65 mg, 0.17 mmol, 70% yield. Silica gel TLC  $R_f$  = 0.29 (Heptane/EtOAc 17% v/v); LC-MS (ESI): calc. for  $C_{25}H_{24}O_4$   $[M+H]^+$ : 389.18 observed 389.08, LC,  $R_t$ =8.65 min.  $^1H$  NMR (400 MHz,  $CDCl_3$ ):  $\delta$  (ppm) 7.69 (d,  $J$  = 16.0 Hz, 1H), 7.63 – 7.59 (m, 2H), 7.57 – 7.32 (m, 9H), 6.36 (d,  $J$  = 16.0 Hz, 1H), 5.13 (s, 2H), 3.79 (s, 3H), 3.36 (s, 3H), 2.21 (s, 3H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  167.76, 156.42, 144.42, 140.90, 138.60, 138.39, 135.96, 132.41, 130.98, 130.30, 129.20, 128.87, 128.73, 128.30, 127.22, 127.08, 126.37, 116.30, 115.18, 94.80, 56.35, 51.75, 19.79.

**(E)-3-(6-hydroxy-6'-methyl-[1,1':3',1''-terphenyl]-3-yl)acrylic acid (7)** The described procedure for deprotection, method B was used at 40 °C with (E)-methyl 3-(6-(methoxymethoxy)-6'-methyl-[1,1':3',1''-terphenyl]-3-yl)acrylate (**22**) (32 mg, 0.082 mmol LC-MS (ESI): calc. for C<sub>22</sub>H<sub>18</sub>O<sub>3</sub> [M+H]: 331.13 observed 331.17, LC, Rt=6.67 min; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.76 (d, *J* = 15.9 Hz, 1H), 7.64 – 7.56 (m, 3H), 7.52 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.48 (d, *J* = 2.0 Hz, 1H), 7.47 – 7.38 (m, 4H), 7.38 – 7.31 (m, 1H), 7.04 (d, *J* = 8.5 Hz, 1H), 6.33 (d, *J* = 15.9 Hz, 1H), 2.21 (s, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 172.26, 155.19, 146.75, 140.26, 139.90, 136.44, 135.16, 131.56, 130.85, 129.92, 129.15, 129.02, 128.52, 127.73, 127.63, 127.10, 127.06, 116.30, 115.05, 19.54. HRMS (*m/z*): [M + H]<sup>+</sup> calcd for C<sub>22</sub>H<sub>18</sub>O<sub>3</sub>, 331.1334, found 331.1328.

**(E)-methyl 3-(5'-allyl-6-(methoxymethoxy)-2'-propoxy-[1,1'-biphenyl]-3-yl)acrylate (25)**

The described general Suzuki coupling conditions method A was used with (E)-methyl 3-(4-(methoxymethoxy)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)acrylate (72 mg, 0.21 mmol), 4-allyl-2-chloro-1-propoxybenzene (40 mg, 0.19 mmol), KF (52 mg, 0.90 mmol), SPhos (22.2 mg, 0.054 mmol) and Pd<sub>2</sub>(dba)<sub>3</sub> (17 mg, 0.020 mmol) at 110 °C for 21 h. The eluent used for purification was 17% v/v EtOAc in heptane to yield the title compound, 67 mg, 0.17 mmol, 94% yield. Silica gel TLC *R<sub>f</sub>* = 0.29 (heptane / EtOAc 17% v/v); LC-MS (ESI): calc. for C<sub>24</sub>H<sub>28</sub>O<sub>5</sub> [M+H]: 397.20, observed 397.00, LC, Rt = 8.80; <sup>1</sup>H NMR (400 MHz, Chloroform-*d*) δ 7.67 (d, *J* = 16.0 Hz, 1H), 7.49 – 7.38 (m, 2H), 7.19 (d, *J* = 9.1 Hz, 1H), 7.13 (dd, *J* = 8.3, 2.3 Hz, 1H), 7.06 (d, *J* = 2.4 Hz, 1H), 6.89 (d, *J* = 8.4 Hz, 1H), 6.33 (d, *J* = 16.0 Hz, 1H), 5.98 (ddt, *J* = 16.8, 10.0, 6.7 Hz, 1H), 5.17 – 4.95 (m, 4H), 3.85 (t, *J* = 6.5 Hz, 2H), 3.78 (s, 3H), 3.44 – 3.28 (m, 5H), 1.68 – 1.56 (m, 2H), 0.83 (t, *J* = 7.4 Hz, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 167.73,

156.79, 154.86, 144.64, 137.71, 131.64, 131.45, 131.44, 129.55, 128.77, 128.75, 127.73, 127.29, 115.67, 115.55, 115.02, 112.43, 94.78, 70.18, 56.00, 51.54, 39.36, 22.56, 10.40.

**(E)-3-(5'-allyl-6-hydroxy-2'-propoxy-[1,1'-biphenyl]-3-yl)acrylic acid (9)** The described procedure for deprotection, method B was used at room temperature with methyl (E)-3-(5'-allyl-6-(methoxymethoxy)-2'-propoxy-[1,1'-biphenyl]-3-yl)acrylate (**25**) (31 mg, 0.078 mmol) LC-MS (ESI): calc. for  $C_{21}H_{22}O_4$  [M+H]: 339.16 observed 339.0, LC, Rt=6.82 min.  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  7.79 (d,  $J$  = 15.9 Hz, 1H), 7.52 (dd,  $J$  = 8.4, 2.2 Hz, 1H), 7.47 (d,  $J$  = 2.2 Hz, 1H), 7.22 (dd,  $J$  = 8.4, 2.2 Hz, 1H), 7.16 (d,  $J$  = 2.2 Hz, 1H), 7.04 (d,  $J$  = 8.4 Hz, 1H), 7.00 (d,  $J$  = 8.4 Hz, 1H), 6.35 (d,  $J$  = 15.9 Hz, 1H), 5.98 (ddt,  $J$  = 16.8, 10.1, 6.7 Hz, 1H), 5.17 – 5.04 (m, 2H), 4.02 (t,  $J$  = 6.7 Hz, 2H), 3.44 – 3.37 (m, 2H), 1.76 (dtd,  $J$  = 13.8, 7.4, 6.5 Hz, 2H), 0.94 (t,  $J$  = 7.4 Hz, 3H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  171.78, 156.66, 153.39, 147.04, 137.37, 134.45, 132.68, 132.27, 129.84, 129.43, 127.28, 127.21, 126.82, 118.47, 116.25, 114.65, 113.69, 71.77, 39.50, 31.09, 22.61, 10.51; HRMS (m/z): [M + H] $^+$  calcd for  $C_{21}H_{22}O_4$ , 339.1596, found 339.1592.

**(E)-methyl 3-(5'-allyl-2'-(hexyloxy)-6-(methoxymethoxy)-[1,1'-biphenyl]-3-yl)acrylate (26)**

The described general Suzuki coupling conditions method A was used with (E)-methyl 3-(4-(methoxymethoxy)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)acrylate (66 mg, 0.19 mmol), 4-allyl-2-chloro-1-(hexyloxy)benzene (40 mg, 0.16 mmol), KF (49 mg, 0.84 mmol), SPhos (20 mg, 0.049 mmol) and  $Pd_2(dba)_3$  (16 mg, 0.017 mmol) at 110 °C for 21 h. The eluent used for purification was 17% v/v EtOAc in heptane to yield the title compound, 68 mg, 0.15 mmol, 98% yield. Silica gel TLC  $R_f$  = 0.39 (heptane / EtOAc 17% v/v); LC-MS (ESI): calc. for  $C_{27}H_{34}O_5$  [M+H]: 439.25, observed 439.08, LC, Rt = 9.63 min.;  $^1H$  NMR (399 MHz, Chloroform-d)  $\delta$  7.67 (d,  $J$  = 16.0 Hz, 1H), 7.48 – 7.39 (m, 3H), 7.19 (d,  $J$  = 9.1 Hz, 1H), 7.16 – 7.04 (m, 1H), 6.90 (d,  $J$  = 8.3 Hz, 1H), 6.33 (d,  $J$  = 16.0 Hz, 1H), 5.98 (ddt,  $J$  = 16.8, 10.0, 6.7

Hz, 1H), 5.17 – 4.97 (m, 4H), 3.88 (t,  $J = 6.5$  Hz, 2H), 3.78 (s, 3H), 3.37 (s, 5H), 1.65 – 1.48 (m, 2H), 1.37 – 1.08 (m, 6H), 0.85 – 0.76 (m, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{cdCl}_3$ )  $\delta$  167.88, 156.92, 155.02, 144.80, 137.86, 131.77, 131.61, 131.58, 129.10, 128.92, 128.53, 127.86, 127.41, 115.80, 115.72, 115.15, 112.61, 94.89, 68.87, 56.16, 51.70, 39.52, 31.58, 29.32, 25.77, 22.68, 14.11.

**(*E*)-3-(5'-allyl-2'-(hexyloxy)-6-hydroxy-[1,1'-biphenyl]-3-yl)acrylic acid (10)** The described procedure for deprotection, method B was used at room temperature with methyl (*E*)-3-(5'-allyl-2'-(hexyloxy)-6-(methoxymethoxy)-[1,1'-biphenyl]-3-yl)acrylate (**26**) (17 mg, 0.039 mmol) LC-MS (ESI): calc. for  $\text{C}_{24}\text{H}_{28}\text{O}_4$   $[\text{M}+\text{H}]$ : 381.21 observed 381.00, LC,  $\text{Rt}=7.83$  min;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.79 (d,  $J = 15.9$  Hz, 1H), 7.51 (dd,  $J = 8.4, 2.2$  Hz, 1H), 7.47 (d,  $J = 2.2$  Hz, 1H), 7.22 (dd,  $J = 8.3, 2.2$  Hz, 1H), 7.16 (d,  $J = 2.2$  Hz, 1H), 7.04 (d,  $J = 8.3$  Hz, 1H), 7.00 (d,  $J = 8.3$  Hz, 1H), 6.35 (d,  $J = 15.9$  Hz, 1H), 5.98 (ddt,  $J = 16.8, 10.1, 6.7$  Hz, 1H), 5.20 – 5.01 (m, 2H), 4.04 (t,  $J = 6.7$  Hz, 2H), 3.41 (d,  $J = 6.7$  Hz, 2H), 1.72 (p,  $J = 6.7$  Hz, 2H), 1.44 – 1.12 (m, 6H), 0.83 (t,  $J = 6.8$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  172.27, 156.68, 153.45, 147.06, 137.38, 134.46, 132.64, 132.26, 129.84, 129.44, 127.30, 127.22, 126.88, 118.49, 116.24, 114.76, 113.78, 70.35, 39.51, 31.48, 29.13, 25.57, 22.59, 14.05; HRMS ( $m/z$ ):  $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{24}\text{H}_{28}\text{O}_4$ , 381.2066, found 381.2063.

**4-bromo-2-chloro-1-(methoxymethoxy)benzene** To a solution of 4-bromo-2-chlorophenol (7.35 g, 35.4 mmol, 1.0 eq) in  $\text{CH}_2\text{Cl}_2$  (50 mL, 0.70 M) was added  $\text{N,N}$ -Diisopropylethylamine (18.5 mL, 106 mmol, 3.0 eq) and MOMCl (5.38 mL, 70.8 mmol, 2.0 eq). The reaction was stirred at room temperature for 17 h. The reaction mixture was separated between  $\text{CH}_2\text{Cl}_2$  and  $\text{H}_2\text{O}$  and the aqueous layer was extracted twice with  $\text{CH}_2\text{Cl}_2$ . The combined organic layers were washed with brine, dried over  $\text{Na}_2\text{SO}_4$ , filtered and concentrated *in vacuo*. The product was purified via flash silica gel chromatography eluting with 50 % v/v  $\text{CH}_2\text{Cl}_2$  in heptane to yield the

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2  
3 title compound as a colorless oil, 8.9 g, 35 mmol, 99% yield. Silica gel TLC  $R_f$  = 0.51 ( $\text{CH}_2\text{Cl}_2$  /  
4 Heptane 1/1 v/v);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.51 (d,  $J$  = 2.4 Hz, 1H), 7.30 (dd,  $J$  = 8.8, 2.4  
5 Hz, 1H), 7.05 (d,  $J$  = 8.8 Hz, 1H), 5.22 (s, 2H), 3.50 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$   
6 152.30, 132.84, 130.72, 124.88, 117.74, 114.19, 95.36, 56.54.  
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14 **(E)-methyl 3-(3-chloro-4-(methoxymethoxy)phenyl)acrylate (17)** An oven dried Schlenk tube  
15 was charged with 4-bromo-2-chloro-1-(methoxymethoxy)benzene (2.0 g, 8.0 mmol, 1.0 eq),  
16 methyl acrylate (3.0 mL, 33.1 mmol, 4.1 eq),  $\text{Pd}(\text{dppf})\text{Cl}_2$  (0.94 g, 1.2 mmol, 0.15 eq),  $\text{NEt}_3$  (35  
17 mL, 251 mmol, 31 eq) and DMF (20 mL, 0.40 M) and the reaction was stirred at 110 °C. After  
18 20 h the reaction mixture was poured into  $\text{H}_2\text{O}$  and extracted with  $\text{CH}_2\text{Cl}_2$  three times. The  
19 combined organic layers were washed with brine, dried over  $\text{Na}_2\text{SO}_4$ , filtered and concentrated  
20 *in vacuo*. The product was purified via flash silica gel chromatography, eluting with 20% v/v  
21 EtOAc in heptane to yield the title compound as a white solid, 1.6 g, 6.2 mmol, 78 % yield.  
22 Silica gel TLC  $R_f$  = 0.34 (heptane / EtOAc 20% v/v); GC-MS (ESI)  $m/z$  calc. for  $\text{C}_{12}\text{H}_{13}\text{ClO}_4$ :  
23 256.05, most abundant peaks observed: 256, 226, 195;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.61 – 7.55  
24 (m, 2H), 7.36 (dd,  $J$  = 8.6, 2.0 Hz, 1H), 7.18 (d,  $J$  = 8.6 Hz, 1H), 6.33 (d,  $J$  = 15.9 Hz, 1H), 5.28  
25 (s, 2H), 3.80 (s, 3H), 3.52 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  167.41, 154.45, 143.15,  
26 129.75, 129.27, 128.04, 124.24, 117.37, 116.14, 95.08, 56.63, 51.87.  
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46 **(E)-methyl 3-(4-(methoxymethoxy)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)acrylate (18)** An oven dried Schlenk tube was charged with (E)-methyl 3-(3-chloro-4-  
47 (methoxymethoxy)phenyl)acrylate (489 mg, 1.91 mmol, 1.0 eq) KOAc (573 mg, 5.84 mmol, 3.1  
48 eq), Bis(pinacolato)diboron (1.34 g, 5.28 mmol, 2.8 eq), Xphos (78 mg, 0.16 mmol, 0.080 eq)  
49 and  $\text{Pd}(\text{OAc})_2$  (22 mg, 0.098 mmol, 0.051 eq). The schlenk tube was evacuated and backfilled  
50 with argon three times. Dioxane (6.5 mL, aryl halide concentration 0.30 M) was added under a  
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positive argon flow and the reaction was stirred at 110 °C for 5 h. The reaction mixture was then allowed to cool to room temperature and passed through celite, eluting with EtOAc. The product was purified via flash silica gel chromatography eluting with 25% v/v EtOAc in heptane to yield the title compound, 524 mg, 1.51 mmol, 80% yield. Silica gel TLC  $R_f$  = 0.25 (heptane / EtOAc 25% v/v); LC-MS (ESI): calc. for  $C_{18}H_{25}BO_6$  [M+H]: 349.18, observed 348.92, LC,  $R_t$  = 7.42;  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  7.87 (d,  $J$  = 2.3 Hz, 1H), 7.65 (d,  $J$  = 16.0 Hz, 1H), 7.53 (dd,  $J$  = 8.6, 2.3 Hz, 1H), 7.03 (d,  $J$  = 8.6 Hz, 1H), 6.36 (d,  $J$  = 16.0 Hz, 1H), 5.22 (s, 2H), 3.78 (s, 3H), 3.49 (s, 3H), 1.35 (s, 12H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  167.82, 163.36, 144.49, 136.92, 132.38, 128.00, 116.05, 115.18, 94.78, 83.89, 56.35, 51.71, 24.97.

**4-bromo-2-chloro-1-propoxybenzene** 4-bromo-2-chlorophenol (4.0 g, 19 mmol, 1.0 eq) was dissolved in dry DMF (100 mL, concentration 0.20 M) in an oven dried round bottom flask. To this solution,  $K_2CO_3$  (8.0 g, 58 mmol, 3.1 eq) and 1-bromopropane (8.8 mL, 96 mmol, 5.1 eq) were added and the reaction was stirred at 70 °C for 22 hour. The reaction was then quenched with  $H_2O$  and extracted with  $CH_2Cl_2$  three times. The combined organic layers were washed with brine, dried over  $Na_2SO_4$ , filtered and concentrated *in vacuo*. The product was purified via flash silica gel chromatography, eluting with 5% v/v EtOAc in heptane to yield the title compound as a colorless oil, 4.7 g, 19 mmol, 98% yield. Silica gel TLC  $R_f$  = 0.46 (Heptane/EtOAc 5% v/v); GC-MS (ESI)  $m/z$  calc. for  $C_9H_{10}BrClO$ : 249.53, most abundant peaks observed: 250, 210, 208,  $R_t$  = 5.04;  $^1H$  NMR (400 MHz,  $CDCl_3$ ):  $\delta$  (ppm) 7.45 (d,  $J$  = 2.4 Hz, 1H), 7.25 (dd,  $J$  = 8.7, 2.4 Hz, 1H), 6.72 (d,  $J$  = 8.7 Hz, 1H), 3.94 (t,  $J$  = 6.6 Hz, 2H), 1.53 – 1.40 (m, 2H), 0.95 – 0.82 (m, 3H.);  $^{13}C$  NMR (50 MHz,  $CDCl_3$ )  $\delta$  153.99, 132.62, 130.48, 124.07, 114.49, 112.26, 70.84, 22.52, 10.54.

**4-allyl-2-chloro-1-propoxybenzene (23)** An over dried schlenk flask was charged with 4-bromo-2-chloro-1-propoxybenzene (1.02 g, 4.09 mmol, 1.0 eq), CsF (1.31 g, 8.62 mmol, 2.1 eq) and Pd(PPh<sub>3</sub>)<sub>4</sub> (467 mg, 0.404 mmol, 0.099 eq). The flask was then evacuated and backfilled with argon three times and THF (34 mL, aryl halide concentration 0.12 M) was added. The mixture was stirred for 30 minutes at room temperature before 2-allyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (1.35 mL, 7.21 mmol, 1.8 eq) was added and the reaction was stirred at 78 °C for 22 h. Another portion of CsF (1.26 g, 8.29 mmol, 2.0 eq), Pd(PPh<sub>3</sub>)<sub>4</sub> (467 mg, 0.404 mmol, 0.099 eq) and THF (30 mL) were added and the reaction was stirred at 78 °C for another 24 h. The reaction was then allowed to cool to room temperature and was separated between pentane and H<sub>2</sub>O. The aqueous layer was washed with pentane twice and the combined organic layers were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered twice and concentrated *in vacuo*. The product was purified via flash silica gel chromatography eluting with 5% v/v EtOAc in heptane to yield the title compound as colorless oil, 690 mg, 3.27 mmol, 82% yield. Silica gel TLC *R<sub>f</sub>* = 0.58 (Heptane/EtOAc 5% v/v); GC-MS (ESI) *m/z* calc. for C<sub>12</sub>H<sub>15</sub>ClO: 210.70, most abundant peaks observed: 210, 168, 133, *R<sub>t</sub>* = 5.02; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.18 (d, *J* = 2.2 Hz, 1H), 6.98 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.82 (d, *J* = 8.4 Hz, 1H), 6.04 – 5.70 (m, 1H), 5.10 – 5.06 (m, 1H), 5.06 – 5.01 (m, 1H), 3.95 (t, *J* = 6.5 Hz, 2H), 3.54 – 2.99 (m, 2H), 1.91 – 1.76 (m, 2H), 1.05 (t, *J* = 7.4 Hz, 3H); <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) δ 153.04, 137.21, 133.11, 130.36, 127.70, 122.87, 116.11, 113.56, 70.82, 39.14, 22.66, 10.61.

**4-bromo-2-chloro-1-(hexyloxy)benzene** 4-bromo-2-chlorophenol (4.0 g, 19mmol) was dissolved in dry DMF (100 mL, final concentration 0.19 M) in an oven dried round bottom flask. To this solution was added K<sub>2</sub>CO<sub>3</sub> (8.0 g, 58 mmol, 3.1 eq) and 1-bromohexane (14 mL, 1.0 · 10<sup>2</sup> mmol, 5.3 eq) and the reaction was stirred at 70 °C for 18 hours. The reaction was separated

between H<sub>2</sub>O and CH<sub>2</sub>Cl<sub>2</sub> and the aqueous layer was washed with CH<sub>2</sub>Cl<sub>2</sub> twice. The combined organic layers were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated *in vacuo*. The product was purified via flash silica gel chromatography eluting with 5% v/v EtOAc in hexane to yield the title compound as a colorless oil, 5.5 g, 19 mmol, 98% yield. Silica gel TLC  $R_f$  = 0.70 (Hexane/EtOAc 5% v/v). GC-MS (ESI)  $m/z$  calc. for C<sub>12</sub>H<sub>16</sub>BrClO: 291.6, most abundant peaks observed: 292, 210, 208,  $R_t$  = 6.25; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 7.45 (d,  $J$  = 2.5 Hz, 1H), 7.25 (dd,  $J$  = 8.7, 2.4 Hz, 1H), 6.72 (d,  $J$  = 8.8 Hz, 1H), 3.94 (t,  $J$  = 6.6 Hz, 2H), 1.87 – 1.71 (m, 2H), 1.53 – 1.40 (m, 2H), 1.40 – 1.24 (m, 4H), 0.95 – 0.82 (m, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  154.04, 132.68, 130.53, 124.10, 114.47, 112.26, 69.44, 31.60, 29.08, 25.69, 22.68, 14.11.

**4-allyl-2-chloro-1-(hexyloxy)benzene (24)** An oven dried schlenk flask was charged with 4-bromo-2-chloro-1-(hexyloxy)benzene (1.01 g, 3.47 mmol, 1.0 eq), CsF (1.12 g, 7.37 mmol, 2.1 eq) and Pd(PPh<sub>3</sub>)<sub>4</sub> (397 mg, 0.344 mmol, 0.099 eq). The flask was evacuated and backfilled with argon three times and THF (22 mL, aryl halide concentration 0.16 M) was added. The mixture was stirred for 30 minutes at room temperature, then 2-allyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (1.16 mL, 6.17 mmol, 1.8 eq) and THF (7.5 mL) were added. The reaction was stirred at 78 °C for 21 h. Another portion of CsF (1.12 g, 7.37 mmol, 2.1 eq), Pd(PPh<sub>3</sub>)<sub>4</sub> (401 mg, 0.347 mmol, 0.010 eq) and THF (30 mL) were added and the mixture was stirred at 78 °C for another 24 h. The mixture was allowed to cool to room temperature and was separated between pentane and H<sub>2</sub>O. The aqueous layer was washed with pentane twice and the combined organic layers were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated *in vacuo*. The product was purified via flash silica gel chromatography eluting with 3% v/v EtOAc in heptane to yield the title compound as a colorless oil, 789 mg, 3.12 mmol, 91% yield. Silica gel



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3 TLC  $R_f$  = 0.38 (Heptane/EtOAc 3% v/v); GC-MS (ESI)  $m/z$  calc. for  $C_{15}H_{21}ClO$ : 252.78, most  
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5 abundant peaks observed: 252, 168, 133,  $R_t$  = 6.21;  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  7.18 (d,  $J$  =  
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7 2.2 Hz, 1H), 7.00 (dd,  $J$  = 8.4, 2.2 Hz, 1H), 6.84 (d,  $J$  = 8.4 Hz, 1H), 5.92 (ddt,  $J$  = 17.6, 9.5, 6.7  
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9 Hz, 1H), 5.12 – 5.05 (m, 1H), 5.08 – 5.01 (m, 1H), 4.00 (t,  $J$  = 6.7 Hz, 2H), 3.30 (dt,  $J$  = 6.7, 1.5  
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11 Hz, 2H), 1.88 – 1.72 (m, 2H), 1.57 – 1.42 (m, 2H), 1.41 – 1.28 (m, 4H), 0.90 (td,  $J$  = 5.9, 4.7, 3.5  
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13 Hz, 3H));  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  153.11, 137.26, 133.16, 130.41, 127.71, 122.94,  
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15 116.15, 113.64, 69.47, 39.18, 31.69, 29.27, 25.79, 22.74, 14.17.  
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**Mammalian two-hybrid (M2H) assays.** Mammalian two-hybrid (M2H) assays were performed as previously described.<sup>35</sup>

**General considerations for protein expression and purification.** All solutions and equipment used in the handling of microbial cultures were autoclaved or sterile filtered. Media, plastic and glassware were autoclaved at 121 °C for 20 min prior to use. Bacterial cultures were incubated in a New Brunswick Series 25 shaker. Centrifugation was performed in a Beckman Coulter Avanti J-25 centrifuge. Microcentrifugation was performed in an Eppendorf Centrifuge 5415R or a Beckman Coulter Microfuge 18. All biological laboratory buffers and media were bought from common suppliers and used as purchased. BL21(DE3) and NovaBlue *E. coli* competent cells were purchased from Novagen, XL-10. DNA and protein concentration was determined using a NanoDrop 1000 spectrometer from Thermo Scientific using 260 nm and 280 nm wavelength respectively. Gel electrophoresis for proteins was performed using 12% SDS-PAGE gels in running buffer and visualized using InstantBlue stain. Protein concentration was determined using a NanoDrop 1000 spectrometer with a wavelength ratio of 280-260 nm. The fluorescent D22 coactivator peptide was purchased from Invitrogen life technologies.<sup>TM</sup> RXR $\alpha$ -NURR1 heterodimerization and RXR $\alpha$  homodimerization BRET2 assays were performed as described.<sup>2,33</sup> Briefly, RXR $\alpha$  and NURR1 receptors were tagged with GFP and renilla luciferase. pEC<sub>50</sub> is the negative logarithm of the EC<sub>50</sub> in molar, efficacies were compared to that of LG100268 (100%). Values represent mean (SD) of three or more independent experiments.

**Fluorescence Polarization Assay.** His<sub>6</sub>-RXR $\alpha$ -LBD (1  $\mu$ M), fluorescein-labeled D22 coactivator peptide (0.1  $\mu$ M), and the ligand at the indicated concentration in buffer containing 100 mM HEPES (pH 7.5), 100 mM NaCl, 5 mM DTT and 0.1% bovine serum albumin, were incubated for 60 minutes at 4 °C and protected from light. Conditions for the competition assay:

His<sub>6</sub>-RXR $\alpha$ -LBD (500 nM), fluorescein-labeled D22 co-activator peptide (50 nM), LG100268 (50 nM). Fluorescent polarization signals (mP) were measured with a Tecan Infinite F500 plate reader. Experiments were performed in triplicate and the data were analyzed using Origin software.

**Expression, purification and crystallization of the RXR $\alpha$  LBD.** The histidine-tagged LBD of human RXR $\alpha$  (in a pET15b vector) was expressed in *Escherichia coli* BL21(DE3). Cells were grown at 37 °C in LB medium supplemented with 100 mg mL<sup>-1</sup> ampicillin until OD<sub>600</sub> reached about 0.7. Expression of T7 polymerase was induced by addition of isopropyl-b-d-thiogalactoside (IPTG) to a final concentration of 0.1 mM. After an additional incubation for 15 h at 15°C, cell cultures were harvested by centrifugation at 8,000  $\times$  g for 20 min. The cell pellet from 2 liters of RXR $\alpha$  LBD was resuspended in 50 ml buffer A (20 mM Tris-HCl pH 8.0, 500 mM NaCl, 5 mM imidazole) supplemented with a protease inhibitor (PMSF) and DNase I. The suspension was then lysed by sonication and centrifuged at 35,000 g and 4 °C for 45 min. The supernatant was loaded onto a 5 ml Ni<sup>2+</sup>-affinity column, preequilibrated with buffer A. The column was washed with 10 volumes of buffer A and 10 volumes of buffer A supplemented with 50 mM imidazole. Bound proteins were eluted with buffer A containing 200 mM imidazole. The fractions containing RXR LBD were pooled, concentrated and desalted to buffer B (10 mM Tris-HCl pH 7.5, 150 mM NaCl, 5 mM DTT). To remove the histidine-tag the protein was incubated for 16 h at 4°C with thrombin (1 unit/mg RXR). The protein was passed through a Ni<sup>2+</sup> column and a superdex gel filtration column. The protein was concentrated and stored at -80°C until further use.

Before crystallization the protein was mixed with a 1.5-fold molar excess of ligand and a 3-fold excess of TIF2 NR2 cofactor peptide (686-KHKILHRLQLQDSS-698). The complex was

incubated for 1h at 4 °C. Drops with a size of 2 - 3  $\mu$ L using different reservoir to protein ratio were manually mixed and equilibrated against reservoirs with a volume of 500  $\mu$ L. Optimal crystals were grown in a week in 3  $\mu$ L drops with protein solution to reservoir ratio of 2:1 with 0.1 M PIPES, pH 7.0, 0.1 M NaCl, 22% PEG 2K MME. The crystals were cryo-cooled in liquid nitrogen using sucrose as cryo-protectant for X-ray data collection. Diffraction data for RXR were collected at the DESY beamline (Deutsches Elektronen-Synchrotron, Hamburg, Germany). The data set was indexed and integrated using iMosflm and scaled using SCALA. The structure was phased by molecular replacement using PDB ID 5EC9 as search model in Phaser. Coot and phenix.refine were used in alternating cycles of model building and refinement. All data collection, refinement and validation statistics are shown in Table S1.

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### Author Contributions

The manuscript was written through contributions of all authors. MS and FAM performed synthesis; MS, FAM, and JM performed biochemical and cellular studies; MS, SAA, RMJMV, and CO performed structural characterization and analysis; ESB, RO, LGM, and LB designed the studies; MS, LGM, and LB wrote the manuscript. All authors have given approval to the final version of the manuscript.

### Funding Sources

The authors thank the Michael J. Fox Foundation for Parkinson's Research, The Netherlands Organization for Scientific Research via Gravity program 024.001.035, ECHO grants 711011017 and 717014001, and VICI grant 016150366.

## ASSOCIATED CONTENT

### Supporting Information

Supporting table and figures,  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR and LC-MS analytical data of all new compounds, protein crystallography data.

### Accession Codes

Atomic coordinates and structure factors for the crystal structures of RXR $\alpha$  with ligands **3**, **4**, **6**, **7**, and **9** can be accessed using PDB codes 5MJ5, 5MKU, 5MMW, 5MK4, and 5MKJ respectively. Authors will release the atomic coordinates and experimental data upon article publication.

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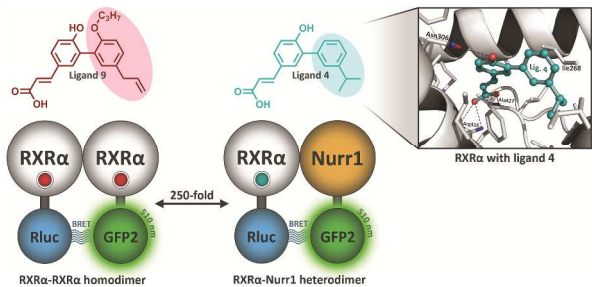


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Ligand dependent switch from RXR homo- to RXR-NURR1 heterodimerization

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A set of biaryl-based RXR-ligands show high binding affinity and allow addressing different parts of the ligand binding pocket to act as a ligand dependent switch for RXR homo- to RXR-NURR1 heterodimerization.