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The discovery of *tertiary*-amine LXR agonists with potent cholesterol efflux activity in macrophages

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ABSTRACT

The liver X receptors (LXR) play a key role in cholesterol homeostasis and lipid metabolism. SAR studies around *tertiary*-amine lead molecule **2**, an LXR full agonist, revealed that steric and conformational changes to the acetic acid and propanolamine groups produce dramatic effects on agonist efficacy and potency. The new analogs possess good functional activity, demonstrating the ability to upregulate LXR target genes, as well as promote cholesterol efflux in macrophages.

Mammalian cells participate in a diverse set of functions, and central to this is the regulation of cellular cholesterol, which includes biosynthesis, internalization, transport, and metabolism. It is widely recognized that elevated systemic levels of low density lipoprotein cholesterol (LDLc) and reduced levels of high density lipoprotein cholesterol (HDLc) represent major risk factors for coronary heart disease. The liver X receptors (LXRα and β) of the nuclear hormone receptor super family serve as oxysterol activated transcription factors.[1] LXRα is expressed in high levels in the liver, but is also prevalent in macrophages, adipose, gut, and kidney tissue. LXRβ is ubiquitously expressed, widely occurring in most tissue. The LXR's operate as heterodimers with retinoid X receptors (RXR), which are activated by ligands of either receptor. The primary role of LXRs is to control the expression of genes which code for key enzymes and transporters responsible for cholesterol homeostasis and lipid metabolism in response to intracellular cholesterol.[2,3] LXR target proteins include ABC (adenosine triphosphate binding cassette) transporters A1 and G1 (which drive cellular cholesterol efflux),[4–6] A5 and G8 (which promote hepatocyte-biliary and enterocyte excretion of cholesterol),[7,8] adipocyte and macrophage ApoE (promote cholesterol efflux),[9] and cholesterol ester transfer protein CETP (transfer of cholesterol from HDL to non-HDL lipoproteins).[10] LXRs inhibit atherosclerosis through reciprocal regulation of macrophage genes involved in cellular cholesterol efflux and inflammation.[11] Furthermore, the upregulation of ABC transporters by LXR promotes the process of reverse cholesterol transport, whereby cholesterol from peripheral tissue including atherosclerotic plaque is transported back to the liver where it is metabolized and excreted into the bile. Indeed treatment of mice with synthetic ligand **2** (Fig. 1) promoted reverse cholesterol transport, as measured by transport of cholesterol from macrophages to feces.[13e] Therefore LXR agonists represent an attractive approach for the prevention and treatment of atherosclerosis.

Small molecule agonists that mimic the natural LXR steroid ligand, 24(*S*),25-epoxycholesterol **1**,[1,12] have been found to increase expression of target genes, promote cholesterol efflux, and as a

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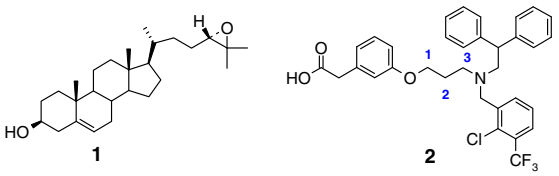


Figure 1. 24(*S*),25-Epoxycholesterol, **1**, and *tertiary*-amine GSK lead **2**.

result, elevate plasma lipids in multiple animal species.[13,14] However, the upregulation of lipogenic genes and elevation of LDL-c observed in CETP (cholesteryl ester transfer protein) species (hamster and monkey) is a major obstacle for the development of current LXR agonists such as **2** and **14b**.[13d]

Previously, GSK revealed the discovery of *tertiary*-amine **2**, a novel LXR agonist identified as a result of high through-put screening and parallel array synthesis.[14] Collins and co-workers demonstrated that the *tertiary*-amine **2**, was selective against a panel of nuclear hormone and steroid receptors. When administered to wild-type mice, compound **2** upregulated ABCA1 expression in macrophages and in the intestine, and increased HDL-c levels.[14] Further, **2**, inhibited atherosclerosis in both apoE−/− and LDLr−/− knockout mice models,[13b] as well as in double apoE−/− & LXRα−/− knockout mice.[15]

Early lead optimization goals around *tertiary*-amine **2** focused on improving functional activity as measured by greater upregulation of LXR target genes, and enhanced cholesterol efflux in macrophages. Towards this end exploratory chemistry efforts around *tertiary*-amine **2** were initiated in an attempt to probe the effects of (1) substitution on the propanolamine linker, and (2) steric and conformational constraints placed on the acetic acid moiety.

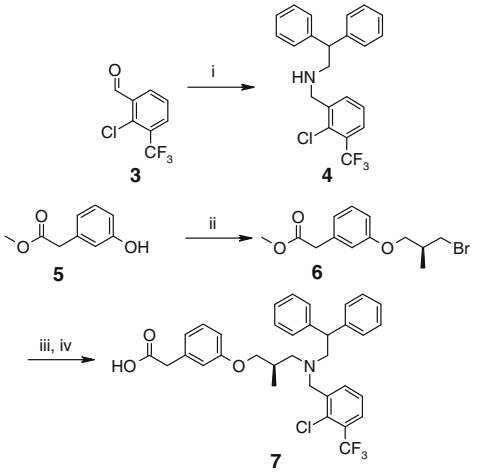
Initial SAR studies focused on the effects of methyl substitution on the propanolamine linker of **2**. The installation of a methyl group at the 2-position of the propanolamine linker is described in Scheme 1. Reductive amination of 2-chloro-3-trifluoromethylbenzaldehyde using 2,2,-diphenylethylamine afforded secondary amine **4**. Treatment of 3-hydroxy-phenyl acetic acid methyl ester with (*S*)-(−)-3-bromo-2-methyl-1-propanol under Mitsunobu conditions gave bromide **6**. Alkylation of bromide **6** with amine **4**, followed by ester hydrolysis afforded 3-(*R*)-methyl-analog **7**.[24]

Analogs with methyl substitution at the 1-position of the propanolamine chain were synthesized according to the procedure shown in Scheme 2. Conversion of the primary alcohol group of (*R*) or (*S*)-1,3-butanediol to the corresponding *p*-toluene-sulfonate ester, followed displacement by amine **4** (described in Scheme 1) afforded secondary alcohol **9**. Mitsunobu alkylation (inversion) of either enantiomer of alcohol **9** with phenol **5**, followed by ester hydrolysis, provided target molecules **10a** or **10b**. Incorporation of a methyl substituent at the 3-position of the propanolamine linker is described in Scheme 3. Mono-*p*-toluene-sulfonylation of either (*R*) or (*S*)-1,3-butanediol, and subsequent alkylation by phenol **5**, yielded intermediate **12**. Alcohol **12** was converted to the corresponding *p*-toluenesulfonate, and alkylated (inversion) with 2,2-diphenylethylamine to afford secondary amine **13**. Reductive amination of 2-chloro-3-trifluoromethylbenzaldehyde with either enantiomer of amine **13**, followed by ester hydrolysis afforded target molecules **14a** or **14b**.

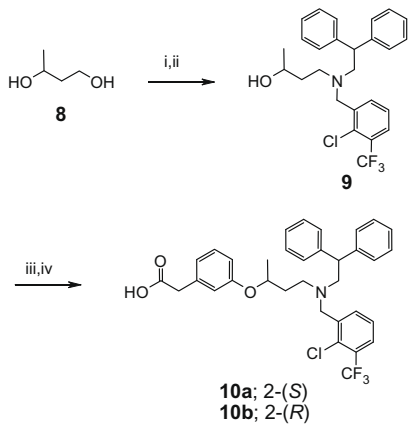
More severe conformational constraints on the propanolamine linker of **2** were also examined. This was accomplished by constructing a ring between the *ortho*-position of the aryl group (ether) and the ether methylene carbon. Based on this approach, two benzofuran isomers were synthesized according to Scheme 4. Iodination of 3-hydroxy and 4-hydroxy- phenylacetic acid **15** provided the corresponding *ortho*-iodophenol intermediate.[16] Sonogashira coupling followed by intramolecular cyclization afforded benzofurans **16a** and **16b**. The alcohol **16a** was converted to the corresponding amine. The amine was then subjected to two successive reductive aminations, and the resulting *tertiary*-amine-ester was hydrolyzed to yield the target 5-benzofuran acetic acid **17a**. Alcohol **16b** was first converted to the mesylate, and then reacted with (2,2-diphenylethyl)amine to form a secondary amine. Reductive amination of the secondary amine using 2-chloro-3-trifluoromethylbenzaldehyde followed by ester hydrolysis provided the target 6-benzofuran acetic acid **17b**.

The impact of steric and conformational changes to the acetic acid group of **2** was also studied. Substitution of the acetic acid methylene group, and cyclization to the *ortho*-position of the aryl group (ether) were examined. Mono-alkylation/dialkylation of **2**, and dimethylation of **14b** was accomplished following Scheme 5 below. Treatment of methyl esters **18a** and **18b** (from **2** and **14b**) with LDA (or sodium hydride), followed by addition of excess methyl-iodide or ethyl-iodide afforded the dimethyl ester intermediates. Hydrolysis using lithium chloride in DMF provided carboxylic acids **19a**, **19b**, **19c**, and **20**.

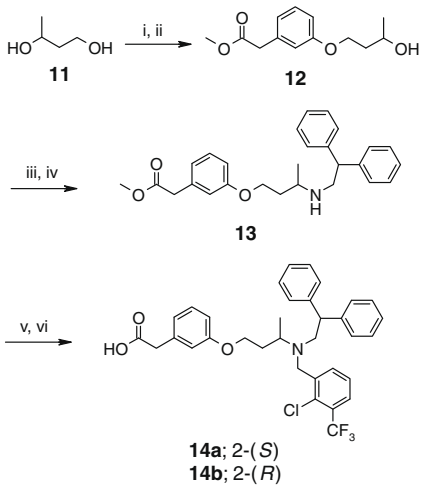
Synthesis of constrained tetrahydronaphthylene carboxylic acid **25** was carried out from commercially available 5-hydroxy-tetralone **23** (Scheme 6). The tetralone **23** was converted to **24** using straight-forward methodology. Alkylation of phenol **24** with bromide **22**, and subsequent saponification afforded the naphthylene ether **25**.



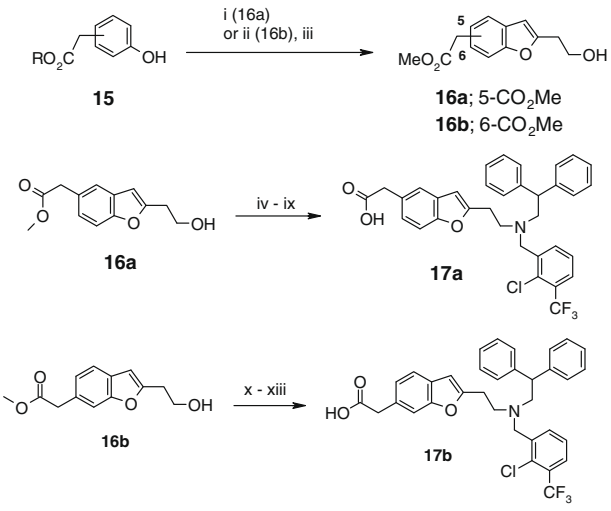
Scheme 1. Reagents and conditions: (i) $NaB(OAc)_3H$, (2,2-diphenylethyl)amine, AcOH, CH_2Cl_2, 76%; (ii) (*S*)-(−)-3-Br-2-methyl-1-propanol, polymer-$P(C_6H_5)_3$, DIAD, toluene, 63%; (iii) amine **4**, NaI, K_2CO_3, CH_3CN, reflux, 14%; (iv) LiOH, 3:1 THF/H_2O, 78%.



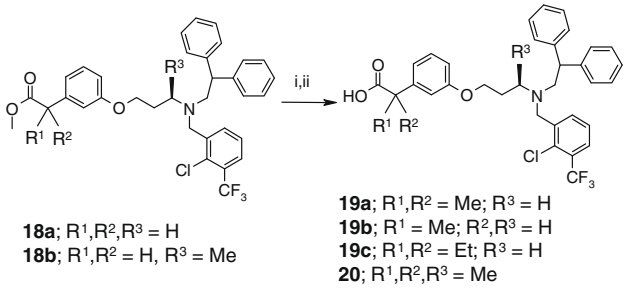
Scheme 2. Reagents and conditions: (i) TsCl, Et_3N, CH_2Cl_2, 89–96%; (ii) amine **4**, NaI, K_2CO_3, CH_3CN, reflux, 58–76%; (iii) phenol **5**, polymer-$P(C_6H_5)_3$, DIAD, toluene, 38–42%; (iv) LiOH, 3:1 THF/H_2O, 98%.



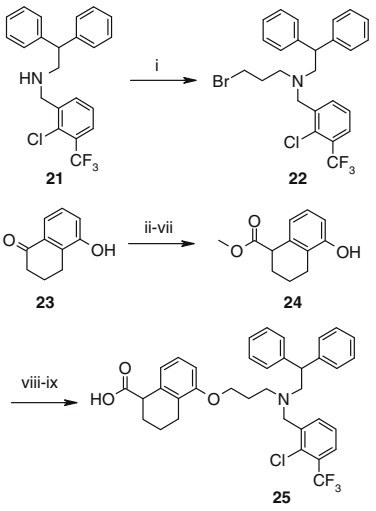
Scheme 3. Reagents and conditions: (i) TsCl, Et_3N, CH_2Cl_2, 82–89%; (ii) phenol **5**, Cs_2CO_3, DMF, reflux, 37–68%; (iii) TsCl, Et_3N, CH_2Cl_2, 63–67%; (iv) (2,2-diphenylethyl)amine, K_2CO_3, CH_3CN, reflux, 54–63%; (v) $NaB(OAc)_3H$, 2-Cl–3CF_3–benzaldehyde, AcOH, CH_2Cl_2, 44–64%; (vi) LiOH, 3:1 THF/H_2O, 89–98%.



Scheme 4. Reagents and conditions: (i) R = Me; chloroamine-T, NaI, DMF, 54%; (ii) R = H; (a) MeOH, cat. H_2SO_4, 100%; (b) I_2, KI, aq NH_2OH, 32%; (iii) 3-butyne-1-ol, CuI, $Pd(PPh_3)_2Cl_2$, PPh_3, 3:1 toluene/Et_3N; (iv) MsCl, Et_3N, CH_2Cl_2, 54–78%; (v) NaN_3, DMF, 75%; (vi) H_2, 10% Pd/C, MeOH; (vii) diphenylacetaldehyde, *p*-TsOH, $NaBH_4$, MeOH, 22% for two steps; (viii) 2-Cl–3-CF_3–benzaldehyde, $NaB(OAc)_3H$, AcOH, CH_2Cl_2, 100%; (ix) LiOH, 3:1 THF/H_2O, 43%; (x) MsCl, Et_3N, CH_2Cl_2, 100%; (xi) (2,2-diphenylethyl)amine, K_2CO_3, CH_3CN, reflux, 50%; (xii) 2-Cl–3-CF_3–benzaldehyde, $NaB(OAc)_3H$, AcOH, CH_2Cl_2, 74%; (xiii) LiOH, 3:1 THF/H_2O, 65%.



Scheme 5. Reagents and conditions: (i) LDA or NaH, MeI or EtI, THF, 2–42% (variable); (ii) LiCl, DMF, ~20%.



Scheme 6. Reagents and conditions: (i) 1,3-Br-propane, K_2CO_3, CH_3CN, reflux, 70%; (ii) benzylbromide, K_2CO_3, CH_3CN, 88%; (iii) $(PPh_3)_3PCH_2OMe$, *t*-Bu-O^-K^+, 1,4-dioxane, 23%; (iv) *p*-TsOH, H_2O, 1,4-dioxane, 58%; (v) CrO_3, H_2SO_4, H_2O, acetone, 57%; (vi) H_2SO_4, MeOH, 85%; (vii) H_2, 10% Pd/C, EtOAc, 97%; (viii) bromide **22**, NaI, K_2CO_3, acetone, 95%; (ix) LiCl, DMF, 15%.

A cell-free ligand sensing assay based on fluorescence resonance energy transfer (FRET) was used as a primary screen to determine LXR activity.[14,17] Ligand dependent recruitment of a 24-amino acid fragment of the steroid receptor coactivator 1 (SRC1) to the LXR binding domain was measured. Percent efficacy was also determined to compare efficacy relative to the maximal agonist response observed for compound **2** (previously determined to be similar to endogenous ligand, 24(*S*),25-epoxycholesterol[14]). All analogs showed no selectivity preference for LXRα versus LXRβ, and most compounds demonstrated comparable agonism or efficacy between the two receptor subtypes.

In the methyl-propanolamine linker series, methyl substitution at the 2-position, exemplified by compound **7**, maintained LXR potency however agonist efficacy dropped by 30% relative to lead **2**. Substitution at the 1-position of the linker, adjacent to oxygen, resulted in a slight decrease in potency for the *S*-enantiomer (**10a**) and a partial agonist response (39–59%) relative to lead **2**. In contrast to **2**, the *R*-enantiomer (**10b**) displayed comparable potency and almost a full agonist response. Placement of the methyl at the 3-position of the linker led to similar activity to lead **2** in the case of the *S*-enantiomer **14a**, however a slight improvement in potency was observed with *R*-enantiomer **14b**. Both stereoisomers exhibited comparable agonism to the lead **2**. The benzofurans **17a** and **17b** displayed partial agonist activity, and a small decrease in activity relative to the lead **2**. Given these observations it appears the flexibility of the linker group plays a key role in agonist response. In addition, methyl substitutions on the linker were also discovered to improve PXR selectivity[21] over lead **2**, and thus may be preferred in order to avoid blockade of PXR-mediated xenobiotic metabolism.[22] Substitution on the acetic acid moiety had a significant impact on potency. Mono-methyl and dimethyl analogs **19a, 19b,** and **20** demonstrated a 5–10-fold improvement in potency, with all three exhibiting full agonist activity compared to the lead **2**. This SAR is consistent with structural observations which can be made from the published co-crystal structure of LXRβ and **2**, suggesting improved affinity may be gained from more favorable hydrophobic contacts in the pocket where the methylene group resides.[20] However this pocket is finite in terms of the size of group which may be accommodated given the loss in potency and efficacy observed for diethyl analog **19c** relative to its methylated counterparts. The constrained acetic acid, tetrahydroquinoline **25** (*racemic*), was comparable in potency and agonism to the lead **2**.

The new *tertiary*-amine analogs were also evaluated in a functional cellular assay to gauge their effectiveness in promoting cholesterol efflux.[18,19] This experiment measures a compounds ability to efflux ^{3}H-cholesterol from mouse RAW 264.7 macrophage cells. All compounds in Table 1 demonstrated cholesterol efflux activity comparable or superior to the potency observed in the FRET assay. Similar to the trend observed in the FRET assay, analogs **2**, **14b**, **19a**, **19b**, **20**, and **25** displayed the greatest potency in the efflux assay, exhibiting a 2–10-fold increase in potency relative to lead **2**.

As further confirmation that the cholesterol efflux effects observed in mouse macrophages was due to LXR activity, real-time PCR gene expression studies[18] in primary human macrophages were conducted on **2** and three new analogs, **14b**, **19a**, and **25** (Table 2). All compounds increased expression of target genes for the ABCA1 and ABCG1 transporters which play integral roles in macrophage cholesterol efflux, in a concentration-dependent manner. However, expression of SREBP1c, a lipogenic target gene believed to play a role in triglyceride synthesis, was also increased. It is important to note that the cholesterol efflux assay discussed earlier measures cholesterol efflux to the apolipoprotein ApoA-I mediated by ABCA1, however ABCG1 mediates cholesterol efflux to HDL particles rather than apoA-I.[5] Since **19a** produced the greatest effect on ABCG1 expression, it will be of interest to examine the activity of this compound in cholesterol efflux assays to HDL particles.

Compound **14b** was found to exhibit excellent in vivo PK characteristics in the rat such as a reasonable half-life, low clearance, and good oral bioavailability (Table 3). Consistent with the in vitro gene expression studies in macrophages discussed above, **14b** (also known as SB742881) has previously demonstrated robust effects on LXR target genes in the hamster.[13d] Once again increases in ABCA1, ABCG1, and SREBP1c gene expression was observed in tissue and macrophages taken from these animals, however **14b** to failed to produce significant increases in plasma HDL-c in the hamster. In fact, significant increases in plasma triglycerides and VLDL-c were observed, which is also consistent with the increased expression of the lipogenic gene SREBP1c.[13d] Moving forward these data suggest that an LXR modulator capable of targeting selective genes (ex. ABCA1 vs SREBP1c) is essential in order to achieve the desirable effects on HDL-c without the undesirable effects on triglycerides. Although unproven, it has been postulated that modulation could be achieved through the differential recruitment of LXR transcription cofactor peptides.[13d,23]

In summary, substitutions which impart conformational changes around the linker group of *tertiary*-amine lead **2** had a significant effect on LXR agonist efficacy but not potency. Interestingly, conformational and/or hydrophobic effects imparted by acetic acid *gem*-dimethyl substitution had a profound effect on LXR potency. Several compounds also demonstrated potent cholesterol efflux activity in macrophages and robust effects on LXR target gene expression as confirmation of functional LXR agonist activity in cells. Analogs such as **14b** and **19a** represent potent molecular tools for benchmarking the potential therapeutic benefits as well as liabilities of LXRα/β full agonists.

Table 1
LXR activity and cell assay results for compounds **2**, **7**, **10**, **14**, **17**, **19**, **20** and **25**

Compd	LXRα EC_{50}[a] (nM)	% Efficacy	LXRβ EC_{50}[a] (nM)	% Efficacy	Cholesterol efflux, EC_{50}[b] (nM)
2	200	100	40	100	29
7	280	67	86	70	76
10a	365	39	145	59	NT
10b	200	78	41	76	34
14a	211	91	87	80	54
14b	74	100	25	89	17
17a	305	20	145	26	NT
17b	331	53	63	75	NT
19a	43	100	15	95	7
19b	46	100	15	90	15
19c	380	69	174	68	NT
20	9	100	11	100	<3
25	56	100	26	100	18

[a] Values are means of at least two experiments, standard deviation ⩽10%.
[b] Mouse 264.7 macrophages; (NT = not tested).

Table 2
Gene expression data from macrophages[a] for **2**, **14b**, **19a**, and **25**

Compd	Fold ↑ in ABCA1[b] (@ 30 nM)	Fold ↑ in ABCA1[b] (@ 100 nM)	Fold ↑ in ABCG1[b] (@ 30 nM)	Fold ↑ in ABCG1[b] (@ 100 nM)	Fold ↑ in SREBP1c[b] (@ 100 nM)
2	2.0	3.1	3.2	5.4	4.1
14b	3.6	6.3	4.2	8.0	5.5
19a	3.0	4.0	7.5	11.2	5.8
25	1.4	2.6	2.0	3.6	2.6

[a] Primary human macrophages.
[b] Values represent a single experiment.

Table 3
In vivo pharmacokinetic data for compound **14b**

14b	Parameter[a]
Dose (mg/kg), iv	2.6
Clp (ml/min/kg)	23.7 ± 2.5
*V*dss (L/kg)	1.9 ± 0.2
$T_{1/2}$ (min), iv	109 ± 15
Cmax (ng/mL), iv	2126 ± 152
Dose (mg/kg), po	4.5
$T_{1/2}$ (min), po	144 ± 39
Oral *F* (%)	71 ± 10

[a] Sprague-dawley rats (n = 3) were used in this experiment.

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24. *Preparation of example* **7**: (*R*)-2-(3-{3-[[2-Chloro-3-(trifluoromethyl)benzyl]-(2,2-diphenylethyl)amino]-2-methyl-propoxy}-phenyl)acetic acid
(a) *(3-Hydroxy-phenyl)-acetic acid methyl ester*: To a stirring solution of (3-hydroxy-phenyl)-acetic acid (4.3 g, 0.028 mol) in methanol (30 mL) was added H_2SO_4 (1 mL) and the mixture was heated to reflux for 2 h. The solvent was removed, the residue was washed with H_2O, and extracted three times with EtOAc (ethyl acetate). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated to give 4.7 g (99% yield) of the title compound as an oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.21 (1H, t, *J* = 7.8 Hz), 6.86 (1H, d, *J* = 7.6 Hz), 6.76 (m, 2H), 4.90 (1H, br s), 3.72 (3H, s), and 3.60 (s, 2H); MS (ESI) 167.0 ($M+H^+$).
(b) *(S)-[3-(2-Methyl-3-bromopropoxy)phenyl]acetic acid methyl ester*: To a stirring solution of (3-hydroxy-phenyl) acetic acid methyl ester (0.75 g, 0.0045 mol) in anhydrous toluene (30 mL) was added (*S*)-(+)-3-bromo-2-methyl-1-propanol (0.90 g, 0.0059 mol). Polymer bound triphenylphosphine (2.4 g, 0.0072 mol, 3 mmol/g, Fluka Chemie) was then added, and the mixture was stirred for 15 minutes. The reaction mixture was then cooled to 0 °C and diisopropylazodicarboxylate (1.1 g, 0.00560 mol) was added in a dropwise fashion. After stirring at room temperature overnight, the crude reaction mixture was filtered, and the solid washed with toluene. After concentration of the filtrate in vacuo, the crude product was purified by column chromatography over silica gel (silica gel 60, EM Science) using 15% EtOAc/hexane as eluent to afford 0.86 g (63% yield) of the title compound as an oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.23 (m, 1H), 6.87 (m, 3H), 3.92 (d, 2H, *J* = 6.0 Hz), 3.72 (s, 3H), 3.62 (s, 2H), 3.59 (d, 2H, *J* = 5.4 Hz), 2.33 (m, 1H), and 1.17 (d, 3H, *J* = 6.8 Hz); MS (ESI) 303.0 ($M+2H^+$).
(c) *N-(2,2-Diphenylethyl)-N-(2-chloro-trifluoromethylbenzyl)amine*: To a stirring solution of 2,2-diphenethylamine (2.0 g, 0.010 mol) and 2-chloro-3-trifluoromethylbenzaldehyde (2.33 g, 0.011 mol) in dichloromethane (20 mL) was added sodium triacetoxyboro hydride (2.36 g, 0.011 mol) and acetic acid (2.0 mL). The reaction mixture was stirred overnight. Solvent was removed, the residue was washed with saturated $NaHCO_3$, and extracted three times with EtOAc. The organic extracts were dried over Na_2SO_4, filtered, and concentrated. The crude mixture was subjected to column chromatography over silica gel (Silica Gel 60, EM Science) using 30% EtOAc/hexane as eluent to afford 3.0 g (76% yield) of the title compound as a yellow oil: MS (ESI) 390.0 ($M+H^+$).
(d) *(R)-2-(3-{3-[[2-Chloro-3-(trifluoromethyl)benzyl](2,2-diphenylethyl)amino]-2-methyl-propoxy}-phenyl)acetic acid methyl ester*: To a stirring solution of (*S*)-[3-(2-methyl-3-bromopropoxy)phenyl]acetic acid methyl ester (100 mg, 0.33 mmol) and *N*-(2,2-diphenylethyl)-*N*-(2-chloro-3-trifluoromethyl)amine (130 mg, 0.33 mmol) in acetonitrile (5 mL) was added solid K_2CO_3 (138 mg, 1.0 mmol) and NaI (149 mg, 1.0 mmol). The reaction was heated to reflux and stirred overnight. Upon cooling to room temperature, the reaction was filtered, washed with acetonitrile, and the filtrate was concentrated. The crude product was purified by preparative HPLC (TMC CombiPrep PDS, 75 × 30 mm, 25 mL/min, acetonitrile/H_2O, UV detection at 254 nm) to give 29 mg (14% yield) of title compound as a viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52 (d, 1H, *J* = 7.6 Hz), 7.30 (d, 1H, *J* = 7.2 Hz), 7.24–7.13 (m, 11H), 6.96 (m, 1H), 6.84 (d, 1H, *J* = 8 Hz), 6.61–6.57 (m, 2H), 4.11 (m, 1H), 3.92 (d, 1H, *J* = 14.8 Hz), 3.76 (d, 1H, *J* = 14.8 Hz), 3.70 (s, 3H), 3.62 (s, 2H), 3.57 (m, 1H), 3.51 (m, 1H), 3.32 (m, 1H), 3.02 (m, 1H), 2.63 (m, 1H), 2.37 (m, 1H), 2.10 (m, 1H), 0.94 (d, 3H, *J* = 6.8 Hz); MS(ESI) 610.2 (M^+).
(e) *(R)-2-(3-{3-[[2-Chloro-3-(trifluoromethyl)benzyl](2,2-diphenylethyl)amino]-2-methyl-propoxy}-phenyl)acetic acid hydrochloride salt*: A stirring solution of (*R*)-2-(3-{3-[[2-chloro-3-(trifluoromethyl)benzyl](2,2-diphenylethyl)amino]-2-methyl-propoxy}-phenyl)acetic acid methyl ester (22 mg, 0.0361 mmol) in THF (0.75 mL) and water (0.25 mL) was treated with LiOH (3.0 mg, 0.072 mmol). The reaction mixture was stirred overnight at rt. The reaction mixture was concentrated and 3 N HCl (aq) was added until the pH was less than two. The aqueous layer was extracted three times with EtOAc, the combined organic layers were dried over sodium sulfate, filtered, and concentrated. The resulting amine/carboxylic acid was dissolved in Et_2O (diethylether) and acidified with 1.0 M HCl/ Et_2O. The reaction mixture was concentrated in vacuo and dried under reduced pressure to give 18 mg (78% yield) of the title compound as a white solid: ^{1}H NMR (400 MHz, MeOD): δ 7.51 (d, 1H, *J* = 7.8 Hz), 7.30 (d, 1H, 7.1 Hz), 7.14–7.27 (m, 11 H), 6.96 (t, 1H, *J* = 7.7 Hz), 6.85 (d, 1H, *J* = 7.6 Hz), 6.61 (m, 1H), 6.57 (m, 1H), 4.19 (dd, 1H, *J* = 5.7 and 9.7 Hz), 3.92 (d, 1H, *J* = 14.9 Hz), 3.76 (d, 1H, *J* = 14.9 Hz), 3.58 (s, 2H), 3.56 (m, 1H), 3.48 (dd, 1H, *J* = 5.2 and 8.8 Hz), 3.33 (m, 1H), 3.01 (dd, 1H, *J* = 5.8 and 12.9 Hz), 2.64 (dd, 1H, *J* = 9.8 and 12.8 Hz), 2.35 (dd, 1H, *J* = 4.9 and 12.8 Hz), 2.02 (m, 1H), 0.94 (d, 3H, *J* = 6.8 Hz); (MS(ESI) 596.0 (M^+). Anal. Calcd for $C_{34}H_{33}ClF_3NO_3 \cdot 0.1$EtOAc: C, 68.31; H, 5.63; N, 2.32. Found: C, 67.96; H, 5.46; N, 2.37.