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Effects of modifications of the linker in a series of phenylpropanoic acid derivatives: Synthesis, evaluation as PPARα/γ dual agonists, and X-ray crystallographic studies

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Abstract—A new series of α-aryl or α-heteroarylphenyl propanoic acid derivatives was synthesized that incorporate acetylene-, ethylene-, propyl-, or nitrogen-derived linkers as a replacement of the commonly used ether moiety that joins the central phenyl ring with the lipophilic tail. The effect of these modifications in the binding and activation of PPARα and PPARγ was first evaluated in vitro. Compounds possessing suitable profiles were then evaluated in the *ob*/*ob* mouse model of type 2 diabetes. The propylene derivative **40** and the propyl derivative **53** demonstrated robust plasma glucose lowering activity in this model. Compound **53** was also evaluated in male Zucker diabetic fatty rats and was found to achieve normalization of glucose, triglycerides, and insulin levels. An X-ray crystal structure of the complex of **53** with the PPARγ-ligand-binding domain was obtained and discussed in this report.

1. Introduction

Type 2 diabetes (T2D) is a progressive disorder where insulin resistance and declining β-cell function are the core defects.[1–3] Unlike Type 1 diabetes, environmental factors such as diet and lack of exercise are thought to be major causative contributors to T2D. Additionally, T2D and insulin resistance are commonly associated with dyslipidemia and a markedly increased incidence in micro- and macrovascular disorders, including cardiovascular disease.[4] The world prevalence of diabetes is staggering: according to the World Health Organization (WHO) more than 171 million people or 2.8% of the population were afflicted with this disease in 2000.[5] In the United States the number of people with diabetes is estimated to be 20.8 million, reflecting 7% of the population.[6] Moreover, there are no signs of disease decline; on the contrary, the incidence of diabetes has increased 6% annually in the United States,[7] and the WHO estimates that by the year 2025 there will be at least 300 million people with this disease. T2D accounts for over 90% of the diabetic population. Currently

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approved agents for the treatment of diabetes are capable of providing glycemic control; however, they generally exhibit limited effects in attenuating cardiovascular risks. Therefore, novel therapeutic approaches that provide not only glucose control, but also demonstrate beneficial effects for reducing cardiovascular disease are needed.

The peroxisome-proliferator activated receptors (PPARs) are ligand-activated transcription factors that constitute a subfamily of nuclear receptors.[8,9] PPARs have a central role in regulating the storage and catabolism of lipids in both humans and animals. PPARα is a modulator of fatty acid catabolism that regulates the expression of genes involved in lipid and lipoprotein metabolism.[10] PPARα activation has been identified to mediate the lipid-lowering activity of the fibrate class of hypolipidemic drugs. These agents are effective in lowering serum triglycerides and raising high-density lipoprotein (HDL) cholesterol levels. In addition, activation of PPARα was reported to produce insulin sensitizing effects and to improve glucose tolerance in type 2 diabetic patients.[11–13]

PPARγ is a critical modulator of adipocyte differentiation and function.[8,9] PPARγ activation also leads to modulation of genes involved in lipid metabolism and hormones that affect whole body energy metabolism. PPARγ was identified as the primary molecular target responsible for the glucose lowering activity of the thiazolidinediones (TZDs) class of antihyperglycemic agents.[14,15] The TZDs have been shown to increase insulin sensitivity in target tissues and reduce glucose, lipid, and insulin levels in animal models of type 2 diabetes and in humans. The two TZDs currently in the market, rosiglitazone (**1**) and pioglitazone (**2**), shown in Figure 1, represent important agents in the treatment of type 2 diabetes. Unfortunately, along with their beneficial effects, they also produce undesirable side effects including weight gain, edema, and anemia. The possible beneficial effects of regulating both glucose and lipid levels through simultaneous activation of both PPARα and PPARγ have spurred a search for safe PPARα/γ dual agonists.[16–19] Studies with dual agonists that are more potent on PPARγ relative to PPARα have shown a profile in which the PPARγ-mediated undesired effects observed in humans were not mitigated by PPARα activation at clinically efficacious doses.[16] Therefore, a more balanced profile might be obtained with dual agonists possessing the PPARα activation needed to obtain the lipid-lowering effects and improvements in insulin sensitization at a lower level of PPARγ activation.

Among the different types of PPARα/γ dual agonists, phenylpropanoic acid-based agonists represent one of the most important classes,[16,17,19] and several of these compounds have reached clinical evaluation (Fig. 1), including farglitazar (**3**),[20] ragaglitazar (**4**),[21] tesaglitazar (**5**),[22] and muraglitzar (**6**).[23] The structure of these phenylpropanoic acid derivatives is comprised of four key regions: the carboxylic acid head group, a central phenyl ring, a linker, and a lipophilic tail (Fig. 2).[19] The lipophilic tail is generally attached to the rest of the molecule by an ether linker. Replacement of the ether moiety by acetylene-, ethylene-, propyl-, or nitrogen-derived linkers was identified as a potential source of nov-

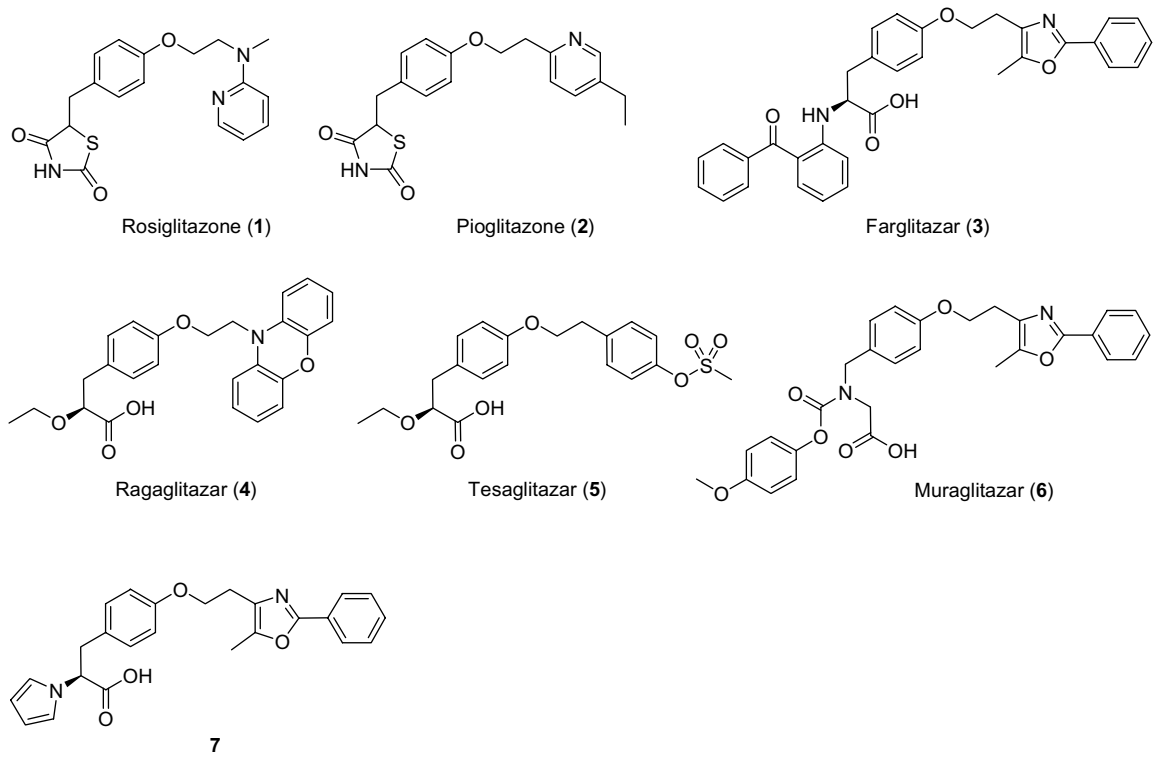


Figure 1. Chemical structures of marketed glitazones and selected PPARα/γ dual agonists.

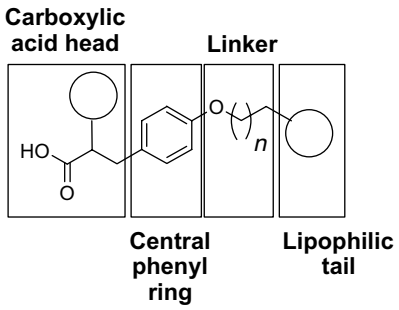
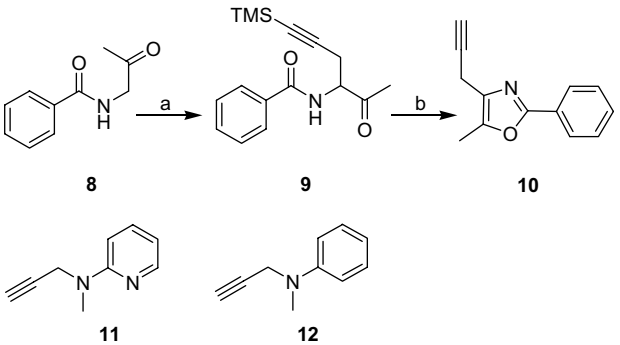


Figure 2. Schematic representation of the structure of phenylpropanoic acid-based dual PPARα/γ agonists.

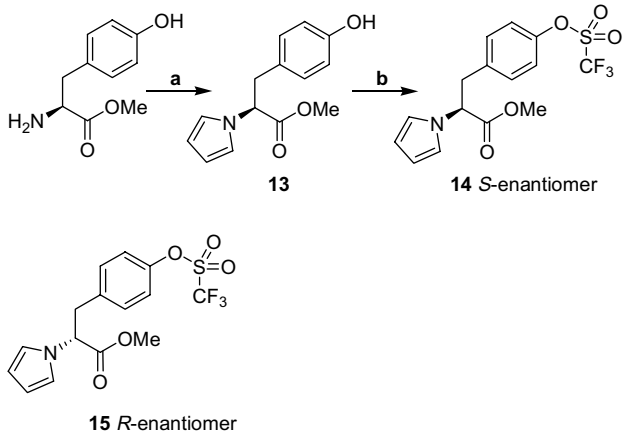
elty within this class of PPARα/γ dual agonists. The linker region is mainly involved in hydrophobic interactions with PPARα and γ, as observed from the reported crystal structures of PPARα or PPARγ ligand-binding domain (LBD) in complex with the phenylpropanoic acid derivatives farglitazar,[24] tesaglitazar,[25] GW 409554,[26] and ragaglitazar.[27] Based on this observation, the carbon-based linkers were not expected to cause noticeable changes to the binding affinity. However, the effects of modifications in the linker region on the subtype selectivity were unknown, and offered an interesting opportunity to modulate activity at each PPAR receptor subtype. The nitrogen-based linkers were expected to have a more significant effect in the binding affinity due to the reduced hydrophobic nature of these linkers. The substituent at the α-position of the carboxylic acid was recognized as an additional site to increase diversity within the phenylpropanoic acid class of dual agonists based on the observation that in the majority of the reported compounds an alkoxy group or an aryloxy group has been installed at this position.[16,18,19] The pyrrole analog **7** shown in Figure 1,[28] reported by the GlaxoSmithKline Group as an extension of their L-tyrosine-based series of PPARγ ligands,[20,29,30] was an interesting template that incorporated a heteroaryl group at the α-position. Pyrrole derivative **7** was reported with high selectivity for PPARγ over PPARα in both binding and functional assays (PPARγ: K_i = 6.9 nM, EC_{50} = 4.7 nM; PPARα: K_i = 560 nM, EC_{50} = 3600 nM).[28] Moreover, when evaluated orally at 10 mg/kg in male Zucker diabetic *falfa* rats, **7** was found to produce a reduction of 40% in plasma glucose and 24% decrease in fasting serum triglycerides. High-density lipoprotein (HDL) cholesterol was increased by 31%. These results indicated that heteroaryl groups at the α-position of the phenylpropanoic acid scaffold might provide new PPARα/γ dual agonists with suitable profiles for the treatment of hyperglycemia and cardiovascular disease. With these considerations in mind, and as the first step in the identification of novel PPARα/γ dual agonists with more balanced profiles for activation of each receptor subtype, modifications in the linker group were investigated with the synthesis of new derivatives incorporating acetylene-, ethylene-, propyl-, or nitrogen-derived linkers using the phenylpropanoic scaffold provided by **7** as a point of reference. In addition, efforts were directed at the identification of novel aryl or heteroaryl substituents at the α-position. The synthesis of these compounds, their evaluation as PPARα/γ dual agonists, in vivo testing of selected analogs, and X-ray crystallographic studies are described in this report.

2. Chemistry

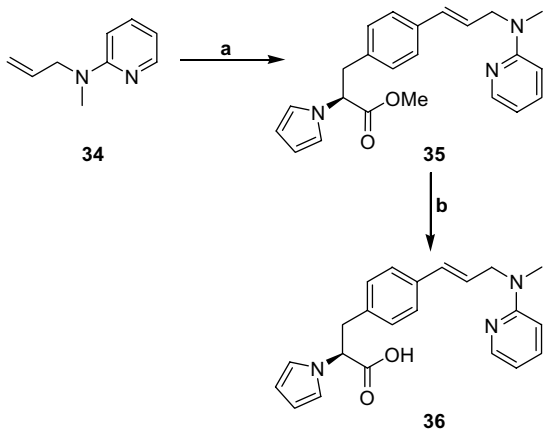
The synthesis of derivatives containing an acetylene linker was accomplished via palladium-catalyzed Sonogashira cross-coupling between acetylene derivatives and appropriate aryl halides or triflates.[31,32] The requisite alkyne **10** needed for the preparation of analogs incorporating the 5-methyl-2-phenyloxazol moiety present in **7** and several other PPARα/γ dual agonists as the lipophilic tail has been previously reported by Hulin et al.[33] A shorter and more efficient synthesis of this intermediate was developed and is described in Scheme 1. It is based on the *C*-selective alkylation of benzamidoacetone (**8**) originally explored by Hoye.[34] Deprotonation of **8** with lithium bis(trimethylsilyl)amide at low temperature, followed by treatment of the resulting enolate with trimethylsilyl-protected propargyl bromide, provided the *C*-alkylated product **9**. Removal of the trimethylsilyl group, followed by cyclization under the conditions reported by Hulin, provided alkyne **10**. Analogs containing the *N*-methyl-*N*-pyridinylamino tail present in rosiglitazone were also planned, as well as compounds containing a *N*-methyl-*N*-phenylamino moiety. The required propargyl derivatives **11** and **12**[35] were prepared from the corresponding amine and propargyl bromide. Triflate **14** was obtained in two steps from L-tyrosine methyl ester (Scheme 2). First, L-tyrosine methyl ester was reacted with 2,5-dimethoxytetrahydrofuran in acetic acid to provide pyrrole **13**. Triflate formation using phenyltriflimide afforded **14**.[36] Triflate **15**, the opposite enantiomer of **14**, was prepared using the same route, but starting from D-tyrosine methyl ester. In addition to the analogs incorporating pyrrole as the substituent at the α-position of the propanoic acid, selected compounds incorporating 3-pyridinyl, phenyl, 4-biphenyl, or 3-biphenyl at the α-position were also planned. The biphenyl derivatives were planned to achieve-binding interactions with the large lipophilic pocket formed by helices H3, H7, and H10



Scheme 1. Synthesis of alkyne **10**. Reagents and conditions: (a) i—LHMDS, THF, −78 °C; ii—TMS-propargyl bromide, 73%; (b) i—KOH, MeOH, 97%; ii—TFA, TFAA, 35–40 °C, 81%.



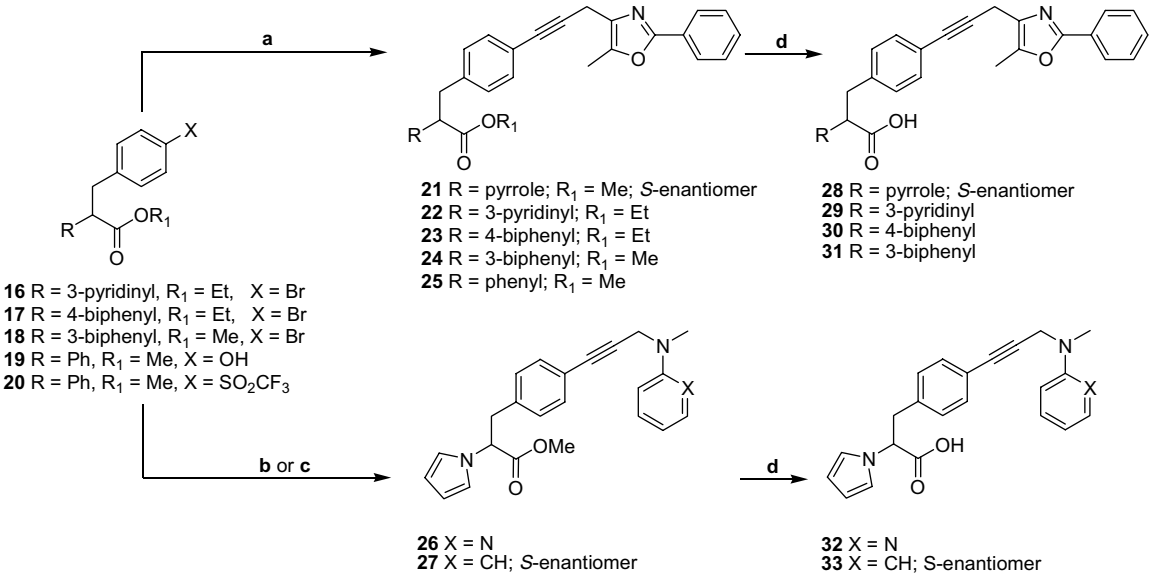
Scheme 2. Synthesis of triflates **14** and **15**. Reagents: (a) 2,5-dimethoxytetrahydrofuran, NaOAc, AcOH-H_2O, **13**: 80%; (b) $PhN(Tf)_2$, Et_3N, CH_2Cl_2, **14**: 93%.



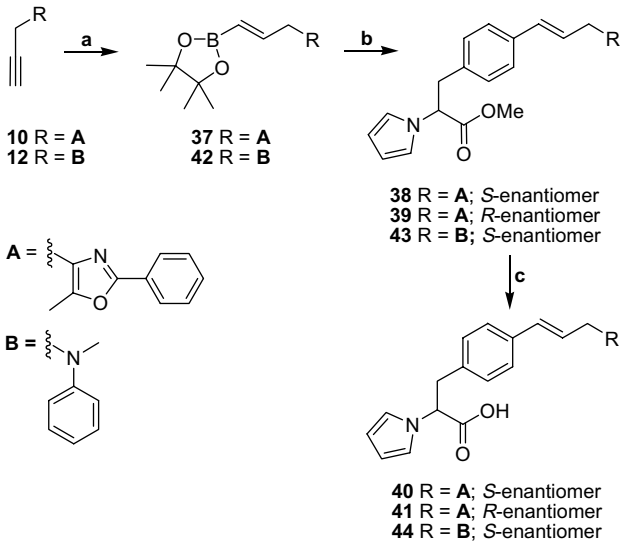
Scheme 4. Synthesis of ethylene derivative **36**. Reagents and conditions: (a) **14**, $Pd(OAc)_2$, PPh_3, Et_3N, DMF, 90 °C, 60%; (b) LiOH, THF–H_2O, 26%.

of the PPARγ ligand-binding site, in a similar manner as those interactions observed with the benzophenone group of farglitazar.[24] The required bromides **16**–**18** were obtained from deprotonation of properly substituted acetic acid ester derivatives with lithium bis(trimethylsilyl)amide at low temperature, followed by alkylation with benzyl bromide. Triflate **20** was prepared from commercially available phenol **19** using standard conditions. Palladium-catalyzed Sonogashira cross-coupling between alkyne **10** and triflate **14** was evaluated under a variety of conditions and was found to be more efficient in the presence of $Pd(PPh_3)_4$, copper(I) iodide, and triethylamine in DMF at 90 °C to give the desired cross-coupled product **21** in good yield (80%) and with retention of the optical purity (Scheme 3).[37] It is important to mention that when the cross-coupling reaction was performed in piperidine, a significantly lower yield of **21** was obtained (19%), and more importantly when the reaction was performed in the presence of pyrrolidine (3 equiv), racemization of the α-center occurred. The optimal conditions were used in the cross-coupling reaction of **10** with bromides **16**–**18** and triflate **20** to give alkyne esters **22**–**25**. Similarly, coupling of triflate **14** with alkyne **12** was performed under these conditions to afford ester **27**. Sonogashira coupling of triflate **14** with alkyne **11** was performed in the presence of piperidine to give racemic ester **26**. The hydrolysis of esters **21**-**24**, **26**, and **27** was accomplished using lithium hydroxide in tetrahydrofuran/water solution to provide the corresponding phenylpropanoic acids derivatives **28**–**33** (Scheme 3).

A limited number of analogs incorporating an ethylene-based linker were prepared and their synthesis was achieved using either one of the two palladium-catalyzed cross-coupling reactions. Allyl amine **34** was prepared



Scheme 3. Synthesis of acetylene derivatives **28**–**33**. Reagents and conditions: (a) **10**, CuI, $Pd(PPh_3)_4$, Et_3N, DMF, 90 °C; (b) **11**, CuI, $Pd(PPh_3)_4$, piperidine, 90 °C, 25%; (c) **12**, CuI, $Pd(PPh_3)_4$, Et_3N, DMF, 80 °C, 77%; (d) LiOH, THF–H_2O.



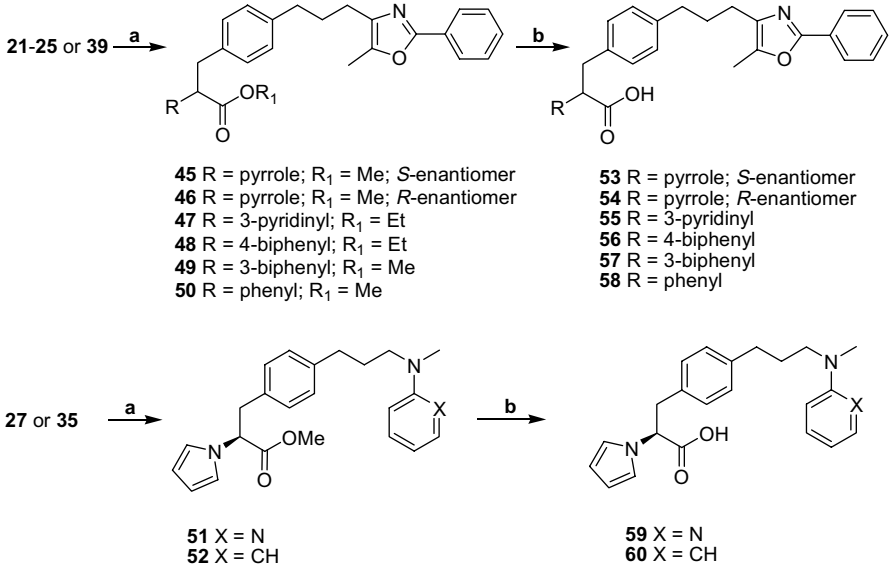
Scheme 5. Synthesis of propylene derivatives **40**, **41**, and **44**. Reagents and conditions: (a) Pinacolborane, Cp_2ZrClH, CH_2Cl_2; (b) **14** or **15**, $Pd(PPh_3)_4$, K_2CO_3, KBr, $PhCH_3$, 85–90 °C; (c) LiOH, THF–H_2O.

under similar conditions as those used for the corresponding propargyl derivative, but using allyl bromide as the alkylating reagent. Heck coupling between triflates **14** and **34** in the presence of $Pd(OAc)_2$ and triethylamine in DMF at 90 °C afforded ester **35** in modest yield (Scheme 4).[38] The Suzuki cross-coupling of 1-alkenylboronic esters with aryl triflates was used for the preparation of other analogs.[39] Hydroboration of alkynes **10** and **12** with pinacolborane catalyzed with $HZrCp_2Cl$ provided boronate esters **37** and **42** in moderate to excellent yield (Scheme 5).[40] Reaction of boronate **37** with triflate **14** in the presence of $Pd(PPh_3)_4$, potassium carbonate, and potassium bromide in toluene at 90 °C provided olefin ester **38** in moderate yield. In order to confirm that no epimerization at the α-center had occurred during this sequence, the chiral purity of **38** was determined by chiral HPLC and was found to be >99.6%. The *R*-enantiomer **39** was prepared from triflate **15** using similar conditions. Coupling of boronate ester **42** with triflate **14** gave ester **43** in good yield. Esters **35**, **38**, **39**, and **43** were hydrolyzed under the conditions described above to provide the corresponding phenylpropanoic acids derivatives **36**, **40**, **41**, and **44**.

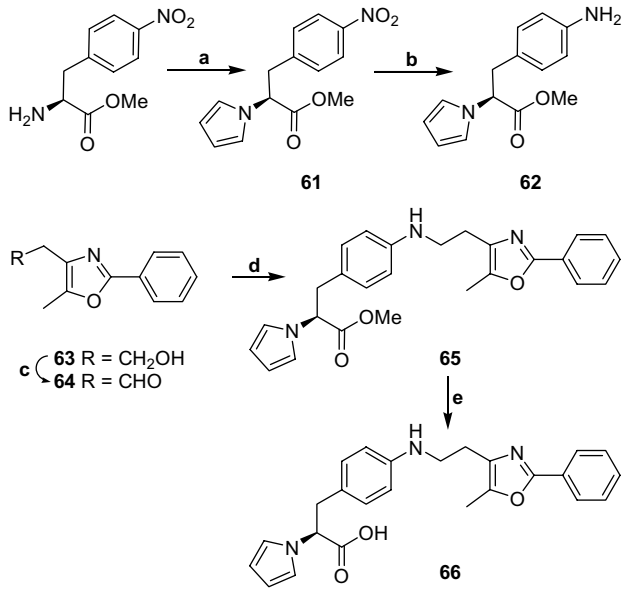
Analogs containing a propyl linker were prepared by hydrogenation of the corresponding acetylene or ethylene ester derivatives as depicted in Scheme 6. Hydrogenation of acetylene derivatives **21**–**25** and **27**, and ethylene derivatives **35** and **39** provided the corresponding reduced esters **45**–**52**, which were then hydrolyzed under the conditions previously used to provide derivatives **53**–**60**.

A limited set of compounds incorporating a nitrogen-based linker was prepared. The oxygen of the ether linker was replaced with an amino group as described in Scheme 7. This synthetic route departed from 4-nitro-L-tyrosine methyl ester. Pyrrole formation was accomplished using the conditions previously described and afforded **61**. Catalytic hydrogenation of the nitro group in the presence of Raney nickel gave **62**. Aldehyde **64** was obtained from the commercially available alcohol **63** using Dess Martin periodinane. Reductive amination of aldehyde **64** with amine **62** in the presence of sodium triacetoxyborohydride provided ester **65**. Ester hydrolysis of **65** under standard conditions gave **66**.

The internal methylene group in the linker was also replaced with an amino moiety as described in Scheme 8. Hydroformylation of triflate **14** was performed by reacting a DMF solution of this compound in a carbon monoxide atmosphere in the presence of $Pd(OAc)_2$, diphenylphosphinopropane (dppp), trioctylsilane, and triethylamine to provide benzaldehyde derivative **67**.[41]



Scheme 6. Synthesis of propyl derivatives **53**–**60**. Reagents: (a) H_2, 5% Pd/C, THF or H_2, Raney nickel, THF; (b) LiOH, THF–H_2O.



Scheme 7. Synthesis of derivative **66**. Reagents and conditions: (a) 2,5-dimethoxytetrahydrofuran, NaOAc, AcOH-H_2O; (b) H_2, Raney nickel, MeOH, 29% in two steps; (c) Dess–Martin periodinane, CH_2Cl_2, 87%; (d) **62**, $NaBH(OAc)_3$, 1,2-dichloroethane, Et_2O, AcOH, 35%; (e) LiOH, THF–H_20, 78%.

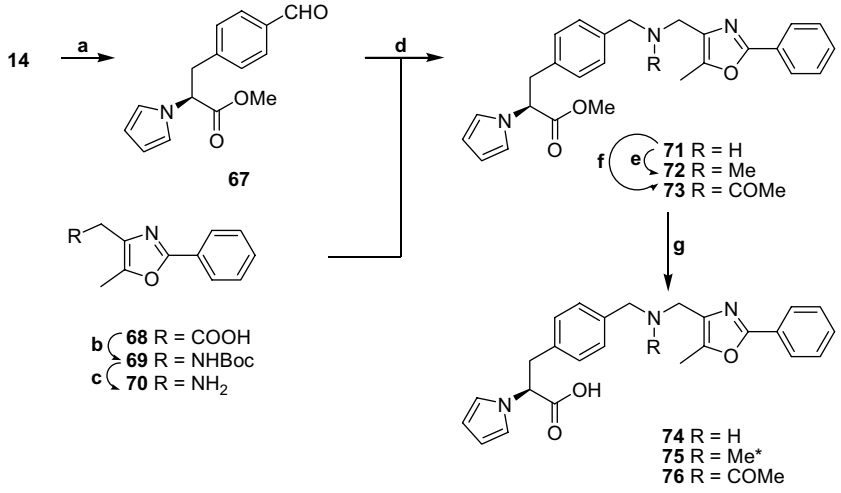
The amino-containing fragment was prepared in four steps from commercially available carboxylic acid **68**. Conversion of **68** to the corresponding acid chloride under standard conditions was followed by reaction with sodium azide in acetone/water. The crude acyl azide was then heated in toluene in the presence of *tert*-butyl alcohol to afford Boc-protected amine **69**. Cleavage of the Boc group with trifluoroacetic acid in dichloromethane was followed by reductive amination of amine **70** with **67** using sodium triacetoxyborohydride in the presence of 4 Å MS to provide ester **71**. The *N*-methyl and *N*-acetyl derivatives were prepared from **71** with the aim of determining the effects of substitution at this position and the impact of changing the basicity of the nitrogen. Reductive amination of **71** with formaldehyde in the presence of sodium triacetoxyborohydride gave the *N*-methyl derivative **72**. Treatment of **71** with acetic anhydride in the presence of pyridine gave ester **73**. Ester hydrolysis under the conditions previously described was performed with **71**–**73** to afford analogs **74**–**76**. A (+)-optical rotation was observed for **75**, which completely differed to the (−)-optical rotation recorded for all other optically active analogs derived from the same *S*-enantiomeric series (derived from L-tyrosine). The reasons for this switch in the optical rotation were unclear. The absolute stereochemistry of **75** is unknown.

In order to facilitate comparison of the new compounds with **7**, this compound was synthesized from **13** and alcohol **63** under Mitsunobu conditions, followed by ester hydrolysis.

3. Results and discussion

The new compounds were evaluated for their PPARα and γ agonist efficacy in PPAR-GAL4 chimeric transactivation assays and the results are expressed as EC_{50}, defined as the concentration of test compound that produces 50% of maximal reporter activity. Additionally, selected compounds were also evaluated for their affinity for PPARα and PPARγ using scintillation proximity assays (SPA) and the results are reported as IC_{50} for displacement of radiolabeled reference compound. The results of these assays are summarized in Table 1.

The results obtained from both the activation and the binding assays indicated that replacement of the ether moiety with an acetylene-, ethylene-, or propyl-based linker led to noticeable changes in the affinity and agonist activity for PPARα and γ. In the series of analogs



Scheme 8. Synthesis of analogs **74**–**76**. Reagents and conditions: (a) CO, $Pd(OAc)_2$, dppp, $(oct)_3SiH$, Et_3N, DMF, 53%; (b) i—$(COCl)_2$, cat. DMF, CH_2Cl_2; ii—NaN_3, acetone-H_2O, 0 °C; (ii) *tert*-BuOH, $PhCH_3$, reflux, 58%; (c) TFA, CH_2Cl_2; (d) **70**, $NaBH(OAc)_3$, 1,2-dichloroethane, Et_3N, 66%; (e) 37% formaldehyde, $NaBH(OAc)_3$, 1,2-dichloroethane, 93%; (f) Ac_2O, pyridine, 100%; (g) LiOH, THF–H_2O.* (+)-optical rotation observed for **75**. Absolute stereochemistry unknown.

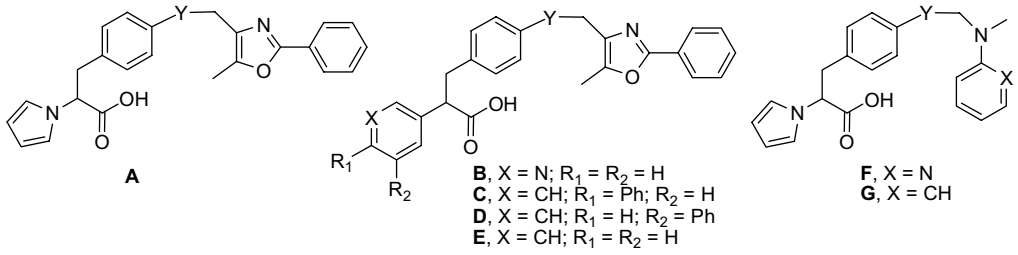
containing the 5-methyl-2-phenyloxazol moiety and the pyrrole ring as the substituent at the α-position, the acetylene derivative **28** (α, IC_{50} = 0.330 μM; γ, IC_{50} = 0.293 μM) showed no PPAR subtype selectivity in binding assays, while the ethylene derivative **40** (α, IC_{50} = > 3.30 μM; γ, IC_{50} = 0.255 μM) and propyl-based **53** (α, IC_{50} = 0.912 μM; γ, IC_{50} = 0.185 μM) exhibited a 13-fold and 5-fold binding selectivity for PPARγ over PPARα, respectively. Pyrrole derivative **7**, which contains an ether linker, was previously reported with high selectivity for PPARγ over PPARα in both binding and functional assays.[28] In this study, **7** (α, IC_{50} = 0.697 μM; γ, IC_{50} = 0.029 μM) demonstrated a 24-fold binding selectivity for PPARγ over PPARα. Clearly, modifications in the linker led to noticeable changes in the PPAR subtype binding selectivity. When compared to **7** (EC_{50} = 0.026 μM), compounds **28** (EC_{50} = 0.173 μM), **40** (EC_{50} = 0.102 μM), and **53** (EC_{50} = 0.140 μM) were moderately weaker agonists in the PPARγ transactivation assays. Also, a modest decrease in agonist activity for PPARα was observed with **28** (EC_{50} = 1.0 μM) and **40** (EC_{50} = 2.8 μM), while the propyl-based analog **53** (EC_{50} = 0.623 μM) was equipotent to **7** (EC_{50} = 0.684 μM) for activation of this PPAR subtype. The selectivity ratio of the agonist activity for PPARγ:PPARα (γ:α ratio) was calculated from the EC_{50} values and is displayed in Table 1. The selectivity ratio for **7** (γ:α ratio = 26:1) was consistent with the selectivity observed in binding assays. The modifications incorporated into both **28** (γ:α ratio = 6:1) and **53** (γ:α ratio = 4:1) clearly led to a shift in the PPAR subtype selectivity, while **40** (γ:α ratio = 27:1) retained a similar profile as that of **7**. The effect of the stereochemistry at the α-position of the phenylpropanoic scaffold was investigated within this set of compounds. For this purpose the *R*-enantiomer of the ethylene- and the propyl-based analogs, compounds **41** and **54**, was prepared and evaluated. Both compounds showed reduced agonist activity for both PPAR subtypes in transactivation assays when compared to their corresponding *S*-enantiomers. Specifically, **41** (α, EC_{50} = 11 μM; γ, EC_{50} = 2.6 μM) exhibited a 4-fold and 25-fold drop in the EC_{50} values for PPARα and PPARγ, respectively. Analog **54** (α, EC_{50} = 5 μM; γ, EC_{50} = 2.3 μM) was similarly less potent for activation of PPARα (7-fold) and PPARγ (16-fold) as determined from its EC_{50} values. The results with the *R*-enantiomers indicated that the binding and activation of both PPAR subtypes mainly resided within the *S*-enantiomer. The superior activity of the *S*-enantiomers **40** and **53** was consistent with the results previously reported for **7** and the first generation of tyrosine-based compounds.[20,28]

The replacement of the pyrrole moiety with a 3-pyridinyl, 4-biphenyl, 3-biphenyl, or phenyl group at the α-position of the phenylpropanoic acid scaffold greatly diminished the potency for both PPAR subtypes, regardless of the linker. This is demonstrated when comparing **28** to the other acetylene-based analogs **29**–**31**. Among these compounds, **30** (α, EC_{50} = 4 μM; γ, EC_{50} = 2.5 μM), which incorporates the 4-biphenyl moiety, was the most potent analog, but was approximately 15-fold less-potent when compared to the pyrrole derivative **28**. In the case of the propyl-based derivatives, the 4-biphenyl analog **56** (α, EC_{50} = > 30 μM; γ, EC_{50} = 1.8 μM) was the most potent compound, but was 13-fold less potent than the pyrrole-derived **53** for the activation of PPARγ. Most of these compounds exhibited weak PPARα agonist activity. An interesting compound from this set was **57** (α, IC_{50} = 0.529 μM, EC_{50} = 1.6 μM; γ, IC_{50} = 2.8 μM, EC_{50} = 4 μM) that incorporates a 3-biphenyl moiety as the substituent at the α-position of the carboxylic acid. This compound showed a reversal in the PPAR subtype selectivity and exhibited a 5-fold binding selectivity for α over γ. It also displayed the largest percent of maximum activation for PPARα (74%) from the compounds in this series. The selectivity change seems to be derived from the incorporation of the substituent at the *meta* position of the phenyl ring. The unsubstituted phenyl derivative **58** (α, IC_{50} = > 3.30 μM; γ, IC_{50} = 4 μM) showed no subtype binding selectivity, while the 4-biphenyl derivative **56** (EC_{50} = > 30 μM) displayed very weak PPARα activity in transactivation assays.

Replacement of the 5-methyl-2-phenyloxazol moiety with other lipophilic tails also led to detrimental effects against both PPAR subtypes. In the case of the analogs containing the *N*-methyl-*N*-pyridinylamino tail, the most potent compound for activation of PPARγ was the propylene-derived **36** (α, EC_{50} = 29 μM; γ, EC_{50} = 0.492 μM). This compound showed a 5-fold reduction in the PPARγ EC_{50} value when compared with **40**. The combination of the *N*-methyl-*N*-pyridinylamino tail and the acetylene linker, as in **32** (α, EC_{50} = > 100 μM; γ, EC_{50} = 9 μM), caused a 53-fold decrease in the EC_{50} value when compared with **28**. Potency against PPARα was greatly diminished by the incorporation of the *N*-methyl-*N*-pyridinylamino tail and all the compounds containing this tail exhibited PPARα EC_{50} values in the mid-micromolar range. The *N*-methyl-*N*-pyridinylamino tail was derived from rosiglitazone, which has been reported with weak activity for PPARα in binding and transactivation assays. The decreased activity of rosiglitazone and the TZDs for PPARα has been explained by taking into consideration steric interactions of the headgroup with Tyr314 in PPARα that lead to a disruption of the network of hydrogen bonds between the headgroup and the key amino acid residues.[16] However, the results of this study with compounds containing the rosiglitazone tail clearly indicated that the tail portion of the molecule has a profound effect in the interaction with PPARα (compare **53** EC_{50} = 0.623 μM vs. **59** EC_{50} = 25 μM). Compounds containing the *N*-methyl-*N*-phenylamino tail exhibited a similar profile as those with the rosiglitazone tail. These compounds exhibited PPARγ EC_{50} values in the low micromolar range and showed no PPARα activity as determined from transactivation assays.

The replacement of the ether moiety with a nitrogen-containing linker also led to substantial changes in the binding and agonist activity for PPAR α and γ. The replacement of the oxygen by an amino moiety as in **66** (α, EC_{50} = 2.3 μM; γ, EC_{50} = 0.480 μM) led to a 3-fold and 18-fold drop in the EC_{50} values for PPARα

Table 1. In vitro PPAR binding and transactivation activity of new propanoic acid derivatives



Compound	Scaffold (Stereo)[a]	Y[b]	TA EC_{50} (μM)[c]			Binding IC_{50} (μM)[d]	
			h-PPARγ (% max)[e]	h-PPARα (% max)[f]	γ:α ratio	h-PPARγ	h-PPARα
Rosi (**1**)			0.223 (84)	>12 (23)	>54:1	0.274	21%[g]
7	A (*S*)	OCH_2	0.026 (115)	0.684 (64)	26:1	0.029	0.697
28	A (*S*)	C≡C	0.173 (116)	1 (50)	6:1	0.293	0.330
29	B (rac)	C≡C	4.5 (34)	16 (16)	3:1	26	NT
30	C (rac)	C≡C	2.5 (44)	4 (14)	2:1	2.2	NT
31	D (rac)	C≡C	13 (51)	2.8 (66)	<1:1	2.8	NT
32	F (rac)	C≡C	9 (115)	>100 (NA)	>11:1	53	NT
33	G (*S*)	C≡C	2 (88)	>100 (NA)	>50:1	12	NT
36	F (*S*)	CH=CH	0.492 (111)	29 (26)	59:1	12	NT
40	A (*S*)	CH=CH	0.102 (87)	2.8 (44)	27:1	0.255	>3.30
41	A (*R*)	CH=CH	2.6 (100)	11 (19)	4:1	2.6	NT
44	G (*S*)	CH=CH	3.7 (71)	>30 (NA)	>8:1	2	NT
53	A (*S*)	CH_2CH_2	0.140 (94)	0.623 (52)	4:1	0.185	0.912
54	A (*R*)	CH_2CH_2	2.3 (64)	5 (28)	2:1	3.1	1.43
55	B (rac)	CH_2CH_2	3.9 (63)	12 (27)	3:1	22	NT
56	C (rac)	CH_2CH_2	1.8 (54)	>30 (NA)	>17:1	0.587	NT
57	D (rac)	CH_2CH_2	4 (39)	1.6 (74)	<1:1	2.8	0.529
58	E (rac)	CH_2CH_2	3.9 (57)	5 (28)	1:1	4	>3.30
59	F (*S*)	CH_2CH_2	1.2 (79)	25 (19)	21:1	28	NT
60	G (*S*)	CH_2CH_2	3.6 (57)	>100 (NA)	>28:1	11	NT
66	A (*S*)	$NHCH_2$	0.480 (103)	2.3 (39)	5:1	1.6	1.9
74	A (*S*)	CH_2NH	9 (13)	>30 (NA)	>3:1	>50	NT
75	A (*S*)	$CH_2N(CH_3)$	7 (77)	12 (72)	2:1	>100	NT
76	A (*S*)	$CH_2N(COCH_3)$	>30 (NA)	>30 (NA)	1:1	>100	NT

[a] Stereochemistry of the chiral center.
[b] (*E*)-isomer for **36**, **40**, **41**, and **44**.
[c] TA (transactivation assay). Mean value of two determinations.
[d] Mean value of three determinations using scintillation proximity assay (SPA).
[e] The maximum efficacy of PPARγ activation of darglitazone was defined as 100%.
[f] The maximum efficacy of PPARα activation of GW9578 was defined as 100%.
[g] Inhibition at 10 μM. NT = not tested. NA = no activation up to the concentration shown.

and PPARγ, respectively. The subtype selectivity was also affected by this modification. In transactivation assays, **66** showed a γ:α ratio of 5:1, while no selectivity was observed from binding assays. These results contrast with the 24-fold binding selectivity for γ over α of the ether-based **7**. The incorporation of an amino group in the middle of the propyl chain as in **74** (α, EC_{50} = > 30 μM; γ, EC_{50} = 9 μM) led to a greatly reduced activity against both PPAR subtypes. Compound **74** was 358-fold and 44-fold less potent than **7**, as determined from the PPARγ and the PPARα EC_{50} values. Rationalizing that the basic nature of the amino group could lead to the loss of activity, the amino group was capped as the *N*-acetyl derivative **76** (α, EC_{50} = > 30 μM; γ, EC_{50} = > 30 μM), but this modification was found to further decrease the potency. The *N*-methyl analog **75** (α, EC_{50} = 12 μM; γ, EC_{50} = 7 μM) provided only a modest improvement in the activity.

Selected compounds **40**, **41**, **53**, **56**, **66**, and **75** were also evaluated for their PPARδ agonist efficacy in chimeric transactivation assays. No activity at this PPAR subtype was observed with these compounds. These results confirmed this series as selective PPARα/γ dual agonists.

3.1. X-ray crystallography studies

In an effort to better understand the interactions of **53** with PPARγ, this compound was selected for crystallographic analysis with the PPARγ-LBD (residues Glu207 to Tyr477). Crystals of PPARγ-LBD were grown without the addition of ligand or co-activator peptide as previously described.[42] A 0.8 mM solution of **53** was soaked into the apo crystals overnight at room temperature prior to flash-cooling the crystals for crystallographic data collection at 100 K. The complex structure was determined to 2.25 Å resolution with an

R-factor of 21.3% and *R*-free of 27.4% (see Supplementary material for X-ray data collection and structure refinement statistics). The unbiased $F_o - F_c$ electron density map revealed clearly interpretable electron density for **53** in the ligand-binding pocket of one monomer of the sPPARγ homodimer. As observed in previous full agonist structures, the pyrrole derivative **53** binds to the activated PPARγ receptor with its carboxyl head group forming a hydrogen bonding network involving the 'charge clamp' residues His323 and Tyr327 from helix 6, His449 from helix 11, and Tyr473 from the activation helix 12 (Fig. 3). The central hydrophobic linker of the ligand bridges across a saddle formed by Cys285 from helix 3 and Met364 from helix 7, while the 5-methyl-2-phenyloxazole tail group extends into a deep hydrophobic pocket created by Val339 and Ile341 from helices 6 and 7.

In order to better understand the interactions of the linker region of **53**, the complex was superimposed onto the co-crystal structure of the potent, full agonists farglitazar and a close structural analog GW409544 complexed with both the PPARγ- LBD and the PPARα-LBD, respectively.[26,42] All three agonists form a network of hydrogen bond interactions extending from the carboxyl group of the agonists to the charge clamp residues, as depicted for **53** and GW409544 in Figure 4. In addition, all the compounds appear to position their 5-methyl-2-phenyloxazole tails in the same hydrophobic region. However, both farglitazar and GW409544 contain an ether moiety in the linker segment, while **53** contains a hydrophobic methylene. The differences in potency observed between **53** and the ether linker containing agonists are likely due in part to the loss of a hydrogen-bond interaction between the ether oxygen and the conserved Cys thiol (3.9 Å).

The structural information from the complex of **53** bound to PPARγ-LBD was used to better understand the impact in the binding and selectivity of PPARγ and PPARα of altering the linker with an acetylene (**28**), ethylene (**40**), or amino (**66**) moiety. The bound structure of **53** was modified to incorporate the acetylene, ethylene, or amino linker. It was observed that in general, the incorporation of these linkers led the 5-methyl-2-phenyloxazole tail into potential steric crashes

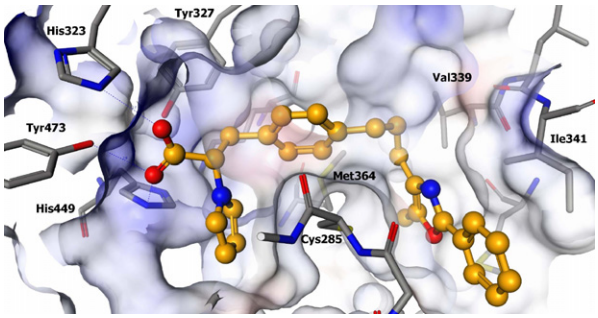


Figure 3. Three-dimensional surface representation of **53** (gold) bound in the ligand-binding pocket of PPARγ (gray). The figure shows the carboxylate head group of **53** forming a bridging H-bond interaction with the charge clamp residues.

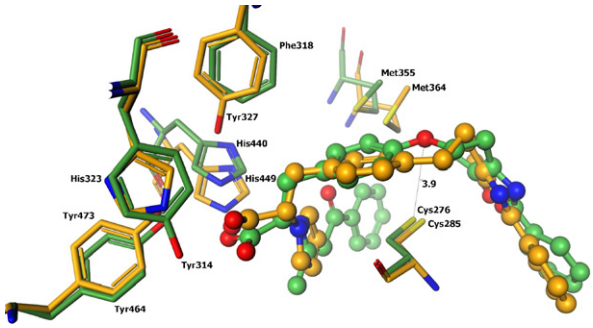


Figure 4. Three-dimensional representation of **53** (gold ball and stick) bound to PPARγ (gold stick) docked onto the crystal structure of GW409544 (green ball and stick) bound to PPARα (green stick).[26] The figure shows the carboxylate head groups of both agonists form an H-bond interaction with the charge clamp residues. However, **53** cannot form an interaction with the conserved cysteine thiol found in the linker region that can be formed by ether containing agonists like farglitazar and GW409544.

with residues of the hydrophobic pocket. In order to relieve these interactions, it is likely that **28**, **40**, and **66** might be shifted in the direction of the acidic headgroup binding region. As these compounds may come into closer interaction with this site, residue differences between PPARγ and PPARα in this region could then have a greater impact in subtype selectivity. The substitution of Tyr314 in PPARα for His323 in PPARγ has already been referred as a key determinant for subtype selectivity.[16,26] PPARγ with the smaller His323 might accommodate more easily the changes in binding mode produced by linker modifications and this is consistent with the PPARγ EC_{50} values observed for **28**, **40**, **53**, and **66** (Table 1). On the other hand, PPARα with the larger Tyr314 might be less tolerant to changes in the binding mode as the carboxylate group of **28**, **40**, and **66** may encounter more steric interaction with the larger Tyr314. This could lead to a diminished potency for activation of PPARα, which is consistent with the results observed when comparing the EC_{50} value for **53** to those of **28**, **40**, and **66** (Table 1).

3.2. In vivo and pharmacokinetic evaluation

In an effort to understand the impact of the linker modifications on the pharmacokinetics and PPAR-mediated in vivo effects, **28**, **40**, **53**, and **66** (Fig. 5) were selected for initial evaluation in the *ob/ob* mouse, an obese rodent model of type 2 diabetes.[43] This model was used as a screening tool to identify and characterize compounds, which were then studied in the Zucker diabetic fatty (ZDF) male rat for a definitive measure of efficacy. The mice were treated with **28**, **40**, **53**, **66**, or rosiglitazone at 20 mg/kg for 14 days (Fig. 6). Compounds **40** and **53** demonstrated robust antihyperglycemic activity with a 71% and 70% reduction in blood glucose levels, respectively. These results are comparable to the effects observed with rosiglitazone at the same dose. The amino derivative **66** failed to produce a glucose lowering effect. The acetylene derivative **28** was not able to sustain robust plasma glucose lowering effects during the complete length of the study. The lack of robust effect of **28** could

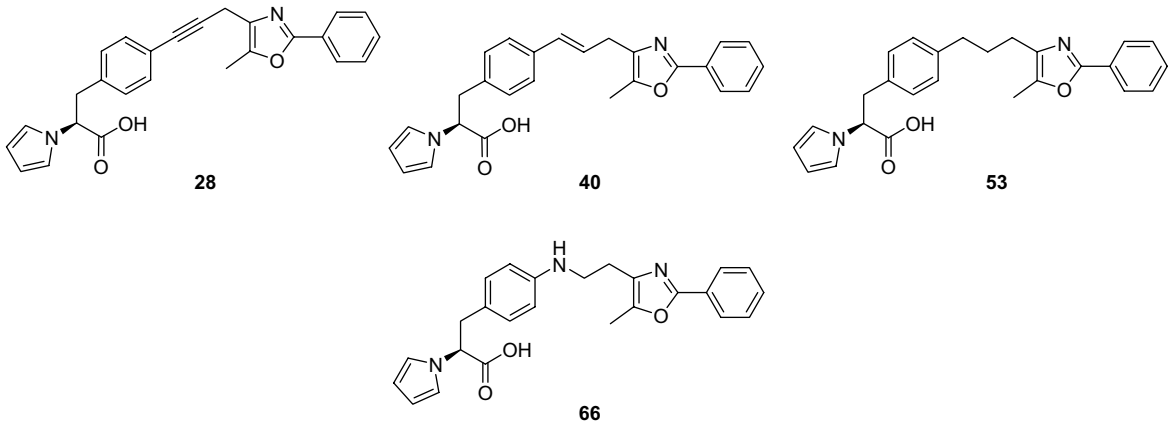


Figure 5. Chemical structures of analogs **28**, **40**, **53**, and **66**.

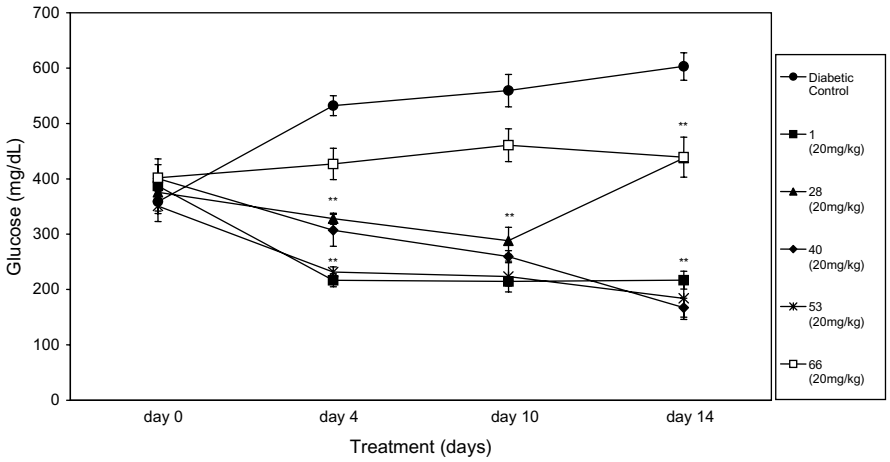


Figure 6. Effects of **28**, **40**, **53**, **66**, and rosiglitazone after 14 days of oral administration at 20 mg/kg in *ob*/*ob* mouse. All data represented as Mean ± SEM, $N = 5$–10 mice/group. $^{**}p < 0.01$ highly significant difference from corresponding treatment day control (Student's *t*-test).

not be explained based on ADME data or in the degree of agonism. The 2 h plasma concentrations post final dose in the mouse were similar for **28**, **40**, and **53** as well as the in vitro ADME data (data not shown). In addition, all three compounds achieved full activation of PPARγ. Compounds **40** and **53** were also evaluated in a dose response study in the *ob*/*ob* mouse and the results are depicted in Figure 7. Although both compounds exhibited similar effects on plasma glucose at the same dose, **53** was more potent and efficacious than **40** in regard to lowering plasma triglycerides.

Compound **53** was chosen for further evaluation in the male Zucker diabetic fatty (ZDF) rat, a model that develops massive obesity with hyperglycemia, hyperinsulinemia, and hypertriglyceridemia.[44] Compound **53** and rosiglitazone were administered at 10 mg/kg daily by oral gavage for 4 weeks. The effects on glucose, insu-

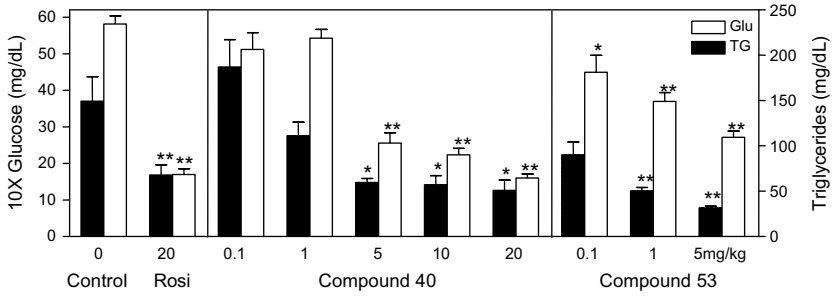


Figure 7. Dose-dependent effects of **40**, **53**, and rosiglitazone in 14 day treated *ob*/*ob* mouse. Triglyceride data represented as Mean ± SEM, Glucose data as 10× Mean ± SEM, $N = 5$–10 mice/group. $^{*}p < 0.05$ significant or $^{**}p < 0.01$ highly significant difference from corresponding treatment day control (Student's *t*-test).

Table 2. Evaluation of **53** in male ZDF rats[a]

Parameter	Compound		
	Vehicle control	Rosiglitazone	**53**
Glucose (mg/dL)	467.2 ± 27.5[b]	121.6 ± 5.6[b]	119.0 ± 3.9[b]
Insulin (ng/mL)	4.4 ± 1.6	4.4 ± 0.9	2.8 ± 0.7
Triglyceride (mg/dL)	983.0 ± 146.1	217.6 ± 15.3	114.0 ± 5.9
Free fatty acids (mEq/L)	4.0 ± 0.2	1.3 ± 0.1	0.9 ± 0.1
Total cholesterol (mg/dL)	145.2 ± 4.0	130.6 ± 2.1	131.6 ± 2.6
Body weight (g)	375.3 ± 8.3	471.7 ± 12.0	490.8 ± 18.7

[a] Tested compounds were administered at 10 mpk daily for 4 weeks.
[b] (±SE).

lin, triglycerides, free fatty acids, and total cholesterol at the end of this study are summarized in Table 2. In agreement with the results observed in *ob*/*ob* mouse, compound **53** demonstrated plasma glucose lowering effects comparable to rosiglitazone. Furthermore, **53** produced a significant reduction of triglycerides, insulin, and free fatty acid levels that were superior to those observed for rosiglitazone. No significant effects on total cholesterol levels were observed. Compound **53** was also observed to produce a body weight increase similar to that observed for rosiglitazone.

Species-specific differences for transactivation of PPARα with a variety of agonists have been described.[45–48] Among them, reports of phenylpropanoic acid derivatives possessing high selectivity for human PPARα over rat PPARα have been documented.[49–51] In order to investigate differences for transactivation of PPARα and PPARγ with **28**, **40**, **66**, and **53**, these compounds were evaluated in chimeric transactivation assays using rat PPARα and mouse PPARγ. Interestingly, no activation for rat PPARα was observed with **28**, **40**, and **66**, while **53** only demonstrated very weak activation (EC_{50} > 10 μM). These results differed notably with the results obtained with hPPARα and presented in Table 1 (**28**, EC_{50} = 1 μM; **40**, EC_{50} = 2.8 μM; **66**, EC_{50} = 2.3 μM; **53**, EC_{50} = 0.623 μM). In contrast, results obtained for transactivation of mouse PPARγ (**28**, EC_{50} = 0.272 μM; **40**, EC_{50} = 0.151 μM; **66**, EC_{50} = 0.536 μM; **53**, EC_{50} = 0.102 μM) were comparable to the values reported in Table 1 for hPPARγ (**28**, EC_{50} = 0.173 μM; **40**, EC_{50} = 0.102 μM; **66**, EC_{50} = 0.480 μM; **53**, EC_{50} = 0.140 μM). These results indicated that this series of compounds exhibited almost complete selectivity for human PPARα over rat PPARα. Also, in vivo effects observed with **40** and **53** were likely primarily driven by activation of PPARγ. In the case of **53**, the weak activation of PPARα might contribute to the triglycerides lowering effect observed in *ob*/*ob* mice, which was superior to that observed with **40**. Body weight reduction in rodents has been reported with the PPARα agonists oleylethanolamide and its derivatives.[52,53] If this effect was obtained with **53** in the ZDF rat, it was likely not sufficient to counterbalance the PPARγ-mediated weight gain effect due to the significantly higher transactivation of PPARγ over PPARα.

Table 3. Pharmacokinetic properties of **40** and **53** in male SD rats

Parameter	Compound	
	40	**53**
AUC (ng h/mL)	55,400[a]	8650[a]
$t_{1/2}$ (h)	5.6[b]	8.4[c]
Cl (mL/min/kg)	0.9	5.3
Vss (mL/kg)	408	1060
F (%)	62	50

[a] Dosed at 5 mg/kg po.
[b] Dosed at 1 mg/kg iv.
[c] Dosed at 5 mg/kg iv.

The pharmacokinetic parameters of the ethylene-derivative **40** and the propyl-based **53** in rat are summarized in Table 3. Both compounds exhibited a long half-life and good bioavailability. Low systemic clearance was also observed for both compounds. The data presented in Table 3 show that **40** had an approximately 7-fold better exposure than **53** in the rat. However, as stated earlier, in the *ob*/*ob* mice study both compounds exhibited similar 2 h plasma concentrations post final dose. The discrepancy between exposure data in the rat and the *ob*/*ob* models may be explained by distribution differences due to the higher percentage of fat in *ob*/*ob* mice or an artifact of the sparse PK sampling in the *ob*/*ob* model.

4. Conclusion

This study demonstrated that replacement of the ether moiety with acetylene-, ethylene-, propyl-, or nitrogen-derived linkers in a series of phenylpropanoic acid derivatives led to changes in the binding and activation of PPARα and PPARγ. The most notable effects were observed for PPARγ when the ether linker was replaced with carbon-based linkers. The fact that PPARα was less affected by these modifications was interesting because compounds with more balanced profiles for the two PPAR subtypes might be obtained using this strategy. Increasing the PPARα activity in this series may also improve the weight gain problem observed with **53**. Results derived from in vitro, in vivo, and X-ray crystallography studies demonstrated the superiority of the propyl linker over the other moeities evaluated in this study. Future reports will describe the utilization of this element for the design of new phenylpropanoic acid derivatives as PPARα/γ dual agonists.

5. Experimental

5.1. General chemistry

All chemicals, reagents, and solvents purchased from commercial sources (e.g., Aldrich Chemical Co., Inc., Milwaukee, WI; Mallinckrodt Baker, Inc., Paris, KY, etc.) were available and used without further purification. All intermediates were characterized by proton nu-

clear magnetic spectroscopy (^{1}H NMR) and mass spectrometry (MS) using atmospheric pressure chemical ionization (CI) sources. All final compounds were determined to be consistent with the proposed structure by ^{1}H NMR and MS. Elemental analysis was obtained for all final compounds, and values were within ±0.4% of the calculated composition. Melting points were determined in capillary tubes and are uncorrected. Final compounds were analyzed by chiral HPLC. When a pair of enantiomers was available, percent enantiomer excess (% ee) was calculated and reported. In all other cases, percent purity obtained from chiral HPLC was reported.

5.1.1. *N*-[1-Acetyl-4-(trimethylsilyl)but-3-ynyl]benzamide (9). Benzamidoacetone (**8**)[54] (2.55 g, 14.4 mmol) was dissolved in THF (150 mL) and cooled to −78 °C under nitrogen. A 1.0 M solution of LHMDS in THF (14.4 mL, 14.4 mmol) was added and the mixture stirred for 0.5 h. A solution of 3-bromo-1-(trimethylsilyl)-1-propyne (2.6 mL, 18.7 mmol) in THF (15 mL) was added. The mixture was allowed to warm to room temperature and stirred overnight. The mixture was diluted with water and the phases were separated. The aqueous phase was extracted with ethyl acetate (3 × 50 mL) and the combined organic extracts were dried over $MgSO_4$, filtered, and the solvent removed. Purification by flash chromatography on silica gel eluting with hexanes:ethyl acetate (3:1) gave amide **9** as a white solid (3.01 g, 73%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.68 (d, J = 7.2 Hz, 2H), 7.40 (t, J = 7.2 Hz, 1H), 7.32 (t, J = 7.2 Hz, 2H), 7.02 (bd, J = 6.4 Hz, 1H), 4.71 (q, J = 5.2 Hz, 1H), 2.78 (d, J = 5.6 Hz, 2H), 2.20 (s, 3H), 0.01 (s, 9H); CIMS m/z 288 $(M+H)^+$. Anal. calcd for $C_{16}H_{21}NO_2Si$: C, 66.86; H, 7.36; N, 4.87. Found: C, 67.15; H, 7.49; N, 4.72.

5.1.2. 5-Methyl-2-phenyl-4-prop-2-ynyloxazole (10).[33] Amide **9** (3.01 g, 10.472 mmol) was dissolved in MeOH (150 mL) and treated with 10% KOH (10 mL). The mixture was stirred at room temperature for 4.5 h. At this time the solvents were removed and the residue was diluted with water and acidified to pH 2 with 6 M HCl. The solid that precipitated was separated by vacuum filtration and dried. The filtrate was extracted with ethyl acetate (3 × 40 mL) and the combined organic extracts were dried over $MgSO_4$, filtered, and the solvent was removed. The combined solids (2.19 g) were treated with trifluoroacetic acid (16 mL) and trifluoroacetic anhydride (8 mL) at 35–40 °C overnight. Concentration and purification by flash chromatography over silica gel eluting with hexanes:ethyl acetate (10:1 to 9:1) gave oxazole **10** as an off-white solid (1.89 g, 94%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.96–7.99 (m, 2 H), 7.37–7.44 (m, 3H), 3.50 (d, J = 2.8 Hz, 2H), 2.41 (s, 3H), 2.12 (t, J = 2.8 Hz, 1H); CIMS m/z 198 $(M+H)^+$.

5.1.3. 2-(*N*-Methyl-*N*-prop-2-ynyl)pyridine (11). Sodium hydride (0.467 g, 11.673 mmol) was suspended in DMF (10 mL) under nitrogen and stirred in an ice bath. 2-(Methylamino)pyridine (1 mL, 9.728 mmol) was added and the mixture stirred at 0 °C for 45 min. An 80% solution of propargyl bromide in toluene (1.19 mL, 10.7 mmol) was then incorporated. The mixture was allowed to reach room temperature and stirred overnight. The mixture was diluted with water and extracted with diethyl ether. The combined organic extracts were washed with water and brine, dried over $MgSO_4$, filtered, and the solvent was removed. Purification by flash chromatography on silica gel eluting with hexanes:ethyl acetate (8:1) afforded **11** as an oil (0.867 g, 61%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.17 (dd, JJ = 4.6, 1.7 Hz, 1H), 7.46 (dt, J = 8.5, 2.0 Hz, 1 H), 6.58 (m, 2H), 4.35 (d, J = 2.4 Hz, 2H), 3.04 (s, 3 H), 2.11 (t, J = 2.4 Hz, 1H); CIMS m/z 147 $(M+H)^+$.

5.1.4. (*S*)-3-(4-Hydroxy-phenyl)-2-pyrrol-1-yl-propionic acid methyl ester (13). A mixture of L-tyrosine methyl ester (15.0 g, 76.8 mmol), 2,5-dimethoxytetrahydrofuran (17 mL, 130.6 mmol), and sodium acetate (12.6 g, 153.6 mmol) in 1:1 water:acetic acid (120 mL) was stirred at room temperature until a clear solution was obtained. The mixture was then heated at 100 °C for 25 min. A dark brown solution was obtained. The mixture was cooled and diluted with water (150 mL) and extracted with ethyl acetate (3 × 150 mL). The combined organic extracts were washed with water and brine, dried over $MgSO_4$, filtered, and the solvent removed. Purification by flash chromatography on silica gel eluting with hexanes:ethyl acetate (3:1) afforded **13** as a white solid (15.09 g, 80%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.85 (d, J = 8.8 Hz, 2H), 6.69 (t, J = 2.2 Hz, 2H), 6.67 (d, J = 8.8 Hz, 2H), 6.13 (t, J = 2.2 Hz, 2H), 4.67 (dd, J = 8.8, 6.6 Hz, 1H), 3.68 (s, 3H), 3.32 (d, J = 14.0, 6.6 Hz, 1H), 3.16 (dd, J = 14.0, 8.8 Hz, 1H); CIMS m/z 246.1 $(M+H)^+$.

5.1.5. (*S*)-2-Pyrrol-1-yl-3-[(4-trifluoromethanesulfonyl-oxy)phenyl]-propionic acid methyl ester (14). A mixture of phenol **13** (6.64 g, 27.088 mmol) and *N*-phenyltrifluoromethanesulfonimide (10.47 g, 27.9 mmol) in dichloromethane (70 mL) under a nitrogen atmosphere was cooled at 0 °C. Triethylamine (4.15 mL, 29.8 mmol) was added slowly. The mixture was stirred at 0 °C for 1 h. Then the temperature was allowed to reach room temperature slowly and stirred under these conditions for 2.5 h. The mixture was diluted with diethyl ether (70 mL) and washed with water, 1 N NaOH, and brine. The organic phase was dried over $MgSO_4$, filtered, and the solvent removed. Purification by flash chromatography on silica gel eluting with hexanes:ethyl acetate (5:1) afforded **14** as a thick oil (9.55 g, 93%), which solidified upon cooling and trituration to give a white solid: $[\alpha]_D^{25} = -57.2$ (c = 0.51, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.10 (d, J = 8.9 Hz, 2H), 6.99 (d, J = 8.8 Hz, 2H), 6.63 (t, J = 2.1 Hz, 2H), 6.12 (t, J = 2.2 Hz, 2H), 4.66 (dd, J = 9.3, 5.9 Hz, 1 H), 3.70 (s, 3H), 3.39 (dd, J = 13.9, 5.9 Hz, 1H), 3.25 (dd, J = 13.9, 9.2 Hz, 1H); CIMS m/z 378 $(M+H)^+$. Chiral HPLC conditions: chiralcel OD-H, 4.6 mm × 250 mm; mobile phase A: hexanes; mobile phase B: ethanol; gradient: 95% A to 40% A in 15 min; flow rate: 0.8 mL/min; injection volume: 15 μL; detection: DAD 254 nm: t_R (major enantiomer) = 9.550 min, t_R (minor enantiomer) = 10.686 min; >99% ee. Anal. calcd for $C_{15}H_{14}F_3NO_5S$: C, 47.75; H, 3.74; N, 3.71. Found: C, 47.83; H, 3.64; N, 3.54.

5.1.6. (*R*)-2-Pyrrol-1-yl-3-[(4-trifluoromethanesulfonyl-oxy)phenyl]-propionic acid methyl ester (15)

5.1.6.1. Step 1: (*R*)-3-(4-Hydroxy-phenyl)-2-pyrrol-1-yl-propionic acid methyl ester. Prepared from D-tyrosine methyl ester following the procedure described for **13**. This compound was obtained as a white solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.86 (d, J = 8.8 Hz, 2H), 6.70 (t, J = 2.2 Hz, 2H), 6.68 (d, J = 8.8 Hz, 2 H), 6.14 (t, J = 2.2 Hz, 2H), 4.68 (dd, J = 8.8, 6.6 Hz, 1H), 3.69 (s, 3H), 3.33 (d, J = 14.0, 6.6 Hz, 1H), 3.17 (dd, J = 14.0, 8.8 Hz, 1H); CIMS m/z 246.1 $(M+H)^+$.

5.1.6.2. Step 2: (*R*)-2-Pyrrol-1-yl-3-[(4-trifluoro-methanesulfonyloxy)phenyl]-propionic acid methyl ester (15). Prepared from (*R*)-3-(4-hydroxy-phenyl)-2-pyrrol-1-yl-propionic acid methyl ester following the procedure described for **14** . Compound **15** was obtained as a white solid: $[\alpha]_D^{25} = +58.8°$ (c = 0.51, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.10 (d, J = 8.8 Hz, 2H), 6.99 (d, J = 8.8 Hz, 2H), 6.63 (t, J = 2.1 Hz, 2H), 6.11 (t, J = 2.1 Hz, 2H), 4.66 (dd, J = 9.3, 5.9 Hz, 1H), 3.69 (s, 3H), 3.38 (dd, J = 13.9, 5.9 Hz, 1H), 3.25 (dd, J = 13.9, 9.2 Hz, 1H); CIMS m/z 378 $(M+H)^+$. Chiral HPLC conditions: chiralcel OD-H, 4.6 mm × 250 mm; mobile phase A: hexanes; mobile phase B: ethanol; gradient: 95% A to 40% A in 15 min; flow rate: 0.8 mL/min; injection volume: 15 μL; detection: DAD 254 nm: t_R (major enantiomer) = 10.686 min, t_R (minor enantiomer) = 9.550 min; >99% ee. Anal. calcd for $C_{15}H_{14}F_3NO_5S$: C, 47.75; H, 3.74; N, 3.71. Found: C, 47.53; H, 3.50; N, 3.77.

5.1.7. 3-(4-Bromo-phenyl)-2-pyridin-3-yl-propionic acid ethyl ester (16). Pyridin-3-yl-acetic acid ethyl ester (1.630 g, 9.861 mmol) was dissolved in dry THF (20 mL) and cooled to −78 °C under a nitrogen atmosphere. A 1.0 M solution of LHMDS in THF (10.8 mL, 10.8 mmol) was added. The mixture was stirred at −78 to −40 °C for 1.25 h. A solution of 4-chlorobenzyl bromide (2.43 g, 11.833 mmol) in THF (5 mL) was added. The reaction was allowed to reach room temperature overnight. The mixture was quenched with water (40 mL) and diluted with ethyl acetate (30 mL). The phases were separated and the aqueous phase was extracted with ethyl acetate (3 × 30 mL). The combined organic extracts were washed with brine, dried over magnesium sulfate, and the solvent was removed. Purification by column chromatography on silica gel eluting with ethyl acetate in hexanes (0 to 45%) afforded bromide **16** as an oil (1.37 g, 42%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.50 (dd, J = 4.8, 1.5 Hz, 1H), 8.47 (d, J = 2.2 Hz, 1H), 7.64 (d, J = 8.0 Hz, 1H), 7.34 (d, J = 8.3 Hz, 2H), 7.24 (m, 1H), 6.95 (d, J = 8.3 Hz, 2H), 4.08 (m, 2H), 3.79 (t, J = 7.8 Hz, 1H), 3.35 (dd, J = 13.8, 8.3 Hz, 1H), 2.96 (dd, J = 13.7, 7.3 Hz, 1H), 1.14 (t, J = 7.3 Hz, 3H); CIMS m/z 334.0 $(M)^+$.

5.1.8. 2-Biphenyl-3-(4-bromo-phenyl)-propionic acid ethyl ester (17). This compound was prepared from biphenyl-4-yl-acetic acid ethyl ester (1.900 g, 7.906 mmol) following the procedure described for **16**. Compound **17** was obtained as a white solid (1.140 g, 35%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.43–7.37 (m, 4 H), 7.27 (t, J = 6.0 Hz, 2H), 7.21–7.09 (m, 5H), 6.86 (d, J = 8.3 Hz, 2H), 3.99–3.85 (m, 2H), 3.67 (dd, J = 8.8, 6.8 Hz, 1H), 3.22 (dd, J = 13.9, 8.8 Hz, 1H), 2.84 (dd, J = 13.9, 6.8 Hz, 1H), 0.99 (t, J = 7.3 Hz, 3H); CIMS m/z 409 $(M)^+$.

5.1.9. 2-Biphenyl-3-yl-3-(4-bromo-phenyl)-propionic acid methyl ester (18). This compound was prepared from biphenyl-3-yl-acetic acid methyl ester (0.692 g, 3.058 mmol) following the procedure described for **16**. Compound **18** was obtained as a thick colorless oil (1.218 g, 99%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.53 (d, J = 8.1 Hz, 2H), 7.50–7.27 (m, 9H), 7.00 (d, J = 8.3 Hz, 2 H), 3.86 (dd, J = 8.8, 6.8 Hz, 1H), 3.62 (s, 3H), 3.40 (dd, J = 13.7, 8.8 Hz, 1H), 3.02 (dd, J = 13.7, 6.8 Hz, 1 H); CIMS m/z 395.1 $(M)^+$.

5.1.10. 2-Phenyl-3-(4-trifluoromethanesulfonyloxyphenyl)-propionic acid methyl ester (20). A solution of **19** (2.0 g, 7.8 mmol) and triethylamine (1.2 mL, 8.6 mmol) in dichloromethane (25 mL) was cooled to 0 °C. Trifluoromethanesulfonic anhydride (1.38 mL, 8.2 mmol) was added and the reaction was allowed to slowly warm to room temperature overnight. The reaction mixture was diluted with ethyl acetate, washed with 5% $NaHCO_3$, water and brine, dried over anhydrous Na_2SO_4, and evaporated to dryness. The residue was purified by column chromatography on silica gel eluting with ethyl acetate in hexanes (10%) to give **20** as a white solid (2.84 g, 95%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.29 (m, 5H), 7.15 (m, 4H), 3.81 (dd, J = 8.7, 7.0 Hz, 1H), 3.62 (s, 3H), 3.43 (dd,J = 8.7, 6.8 Hz, 1H), 3.04 (dd, J = 13.9, 6.8 Hz, 1H); CIMS: m/z 387 $(M-1)$.

5.1.11. (*S*)-3-{4-[3-(5-Methyl-2-phenyl-oxazol-4-yl)-prop-1-ynyl]-phenyl}-2-pyrrol-1-yl-propionic acid (28)

5.1.11.1. Step 1: General method I: (*S*)-3-{4-[3-(5-Methyl-2-phenyl-oxazol-4-yl)-prop-1-ynyl]-phenyl}-2-pyrrol-1-yl-propionic acid methyl ester (21). A mixture of triflate **14** (1.47 g, 3.9 mmol) and 5-methyl-2-phenyl-4-prop-2-ynyloxazole (**10**) (1.0 g, 5.070 mmol) in DMF (15 mL) was degassed by bubbling nitrogen for 15 min. CuI (0.148 g, 0.78 mmol) was added and the mixture heated at 90 °C for 20 h. The mixture was allowed to cool to room temperature and water was added (100 mL). The mixture was extracted with diethyl ether (5 × 50 mL). The combined organic extracts were washed with water, brine, dried over magnesium sulfate, and the solvent was removed. Purification by chromatography eluting with hexanes:ethyl acetate (5:1 to 4:1) gave pure **21** as a thick oil (1.20 g, 73%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.99 (d, J = 8.4 Hz, 1H), 7.98 (d, J = 7.2 Hz, 1H), 7.45–7.39 (m, 3 H), 7.29 (d, J = 8.0 Hz, 2H), 6.92 (d, J = 8.4 Hz, 2 H), 6.68 (t, J = 2.0 Hz, 2H), 6.13 (t, J = 2.0 Hz, 2 H), 4.70 (dd, J = 8.8, 6.4 Hz, 1H), 3.71 (s, 2H), 3.69 (s, 3 H), 3.37 (dd, J = 13.9, 6.3 Hz, 1H), 3.23 (dd, J = 13.9, 8.9 Hz, 1H) (m, 2H), 2.46 (s, 3H); CIMS m/z 425 $(M+H)^+$.

5.1.11.2. Step 2: General method II: (*S*)-3-{4-[3-(5-Methyl-2-phenyl-oxazol-4-yl)-prop-1-ynyl]-phenyl}-2-pyrrol-1-yl-propionic acid (28). Ester **21** (0.337 g, 0.794 mmol) was dissolved in THF (13 mL) and water was added (5 mL) followed by LiOH monohydrate (0.050 g, 1.191 mmol). The mixture was stirred for 4 h

at room temperature. The solvent was removed and the residue diluted with water and acidified with 10% HCl. The product was extracted with ethyl acetate (4×30 mL). The combined organic extracts were washed, dried over magnesium sulfate, and the solvent was removed. Purification by chromatography on silica gel eluting with ethyl acetate in hexanes (40%) containing 0.2% formic acid gave acid **28** as a pale yellow solid (0.231 g, 71%): mp 178–180 °C; $[\alpha]_D^{25} = -75$ ($c = 0.48$, THF); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.78–7.76 (m, 2H), 7.22 (m, 3H), 7.05 (d, $J = 8.0$ Hz, 2H), 6.72 (d, $J = 8.4$ Hz, 2H), 6.46 (t, $J = 2.0$ Hz, 2H), 5.86 (t, $J = 2.0$ Hz, 2H), 4.48 (dd, $J = 9.6$, 5.6 Hz, 1H), 3.50 (s, 2H), 3.19 (dd, $J = 13.9$, 5.6 Hz, 1H), 3.01 (dd, $J = 13.9$, 9.4 Hz, 1H), 2.26 (s, 3H); CIMS m/z 409 $(M - H)^+$. Chiral HPLC (SFC conditions): chiralpak AS-H, 4.6 mm × 250 mm; mobile phase A: CO_2; mobile phase B: methanol + 0.5% isopropylamine; gradient: 95% A to 40% A in 15 min; flow rate: 4.0 mL/min; injection volume: 15 μL; detection: DAD 254 nm: $t_R = 2.30$ min; >99% purity. Anal. calcd for $C_{26}H_{22}N_2O_3$: C, 76.08; H, 5.40; N, 6.82. Found: C, 75.77; H, 5.45; N, 6.70.

5.1.12. 3-{4-[3-(5-Methyl-2-phenyl-oxazol-4-yl)-prop-1-ynyl]-phenyl}-2-pyridin-3-yl-propionic acid (29)

5.1.12.1. Step 1: 3-{4-[3-(5-Methyl-2-phenyl-oxazol-4-yl)-prop-1-ynyl]-phenyl}-2-pyridin-3-yl-propionic acid methyl ester (22). Prepared from bromide **16** (1.354 g, 4.051 mmol) and alkyne **10** (1.04 g, 5.266 mmol) following the general method I. Purification by flash chromatography on silica gel eluting with ethyl acetate in hexanes (0% to 30%) gave pure **22** as a thick oil (0.838 g, 46%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.49 (dd, $J = 4.9$, 1.7 Hz, 1H), 8.47 (d, $J = 1.9$ Hz, 1H), 7.98 (d, $J = 8.0$ Hz, 2H), 7.64 (dt, $J = 8.0$, 1.9 Hz, 1H), 7.44–7.37 (m, 3H), 7.29 (d, $J = 6.6$ Hz, 2H), 7.24–7.21 (m, 1H), 7.01 (d, $J = 8.0$ Hz, 2H), 4.13–4.00 (m, 2H), 3.81 (t, $J = 8.0$ Hz, 1H), 3.71 (s, 2H), 3.38 (dd, $J = 13.8$, 8.3 Hz, 1H), 2.99 (dd, $J = 13.9$, 7.3 Hz, 1H), 2.46 (s, 3H), 1.13 (t, $J = 7.1$ Hz, 3H); CIMS m/z 451 $(M+H)^+$.

5.1.12.2. Step 2. 3-{4-[3-(5-Methyl-2-phenyl-oxazol-4-yl)-prop-1-ynyl]-phenyl}-2-pyridin-3-yl-propionic acid (29). Prepared from ester **22** (0.284 g, 0.630 mmol) following the general method II. Purification by column chromatography on silica gel eluting with methanol in chloroform (0% to 10%) afforded **29** as an orange solid (0.100 g, 37%): mp 115–117 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.53 (s, 1H), 8.43 (d, $J = 4.1$ Hz, 1H), 7.95 (d, $J = 7.8$ Hz, 2H), 7.69 (d, $J = 7.8$ Hz, 1H), 7.41–7.38 (m, 3H), 7.26 (d, $J = 6.8$ Hz, 3H), 7.02 (d, $J = 8.0$ Hz, 2H), 3.86 (t, $J = 7.8$ Hz, 1H), 3.68 (s, 2H), 3.44 (dd, $J = 13.8$, 7.9 Hz, 1H), 3.00 (dd, $J = 13.7$, 7.6 Hz, 1H), 2.43 (s, 3H); CIMS m/z 423 (M+1). Anal. calcd for $C_{27}H_{22}N_2O_3 \cdot 0.4\ CH_2Cl_2$: C, 72.10; H, 5.03; N, 6.14. Found: C, 71.78; H, 5.41; N, 5.82.

5.1.13. 2-Biphenyl-4-yl-3-{4-[3-(5-methyl-2-phenyl-oxazol-4-yl)-prop-1-ynyl]-phenyl}-propionic acid (30)

5.1.13.1. Step 1: 2-Biphenyl-4-yl-3-{4-[3-(5-methyl-2-phenyl-oxazol-4-yl)-prop-1-ynyl]-phenyl}-propionic acid ethyl ester (23). This compound was obtained from bromide **17** (1.130 g, 2.761 mmol) and alkyne **10** (0.708 g, 3.589 mmol) following the general method I. Ester **23** was obtained as a thick orange oil (0.539 g, 37%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.99 (d, $J = 8.1$ Hz, 2H), 7.56 (d, $J = 8.3$ Hz, 2H), 7.53 (d, $J = 8.3$ Hz, 2H), 7.45–7.30 (m, 6 H), 7.35 (d, $J = 8.3$ Hz, 2H), 7.31 (d, $J = 8.3$ Hz, 2H), 7.07 (d, $J = 8.3$ Hz, 2H), 4.12–4.01 (m, 2H), 3.84 (dd, $J = 8.8$, 6.6 Hz, 1H), 3.72 (s, 2H), 3.40 (dd, $J = 13.7$, 8.8 Hz, 1H), 3.03 (dd, $J = 13.7$, 6.6 Hz, 1H), 2.47 (s, 3H), 1.13 (t, $J = 7.1$ Hz, 3H); CIMS m/z 526 (M+1).

5.1.13.2. Step 2: 2-Biphenyl-4-yl-3-{4-[3-(5-methyl-2-phenyl-oxazol-4-yl)-prop-1-ynyl]-phenyl}-propionic acid (30). This compound was prepared from **23** (0.210 g, 0.399 mmol) following the general method II. Purification by flash chromatography on silica gel eluting with methanol in chloroform (0% to 6%) afforded **30** as an orange solid (0.107 g, 54%): mp 163–165 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.99–7.96 (m, 2 H), 7.54–7.48 (m, 4H), 7.46–7.26 (m, 10H), 7.04 (d, $J = 8.2$ Hz, 2H), 3.87 (dd, $J = 8.4$, 7.0 Hz, 1H), 3.71 (s, 2 H), 3.39 (dd, $J = 13.9$, 8.6 Hz, 1H), 3.03 (dd, $J = 13.9$, 7.0 Hz, 1H), 2.44 (s, 3H); CIMS m/z 498 (M+1). Anal. calcd for $C_{34}H_{27}NO_3 \cdot 0.4\ H_2O$: C, 80.90; H, 5.55; N, 2.77. Found: C, 80.50; H, 5.29; N, 2.44.

5.1.14. 2-Biphenyl-3-yl-3-{4-[3-(5-methyl-2-phenyl-oxazol-4-yl)-prop-1-ynyl]-phenyl}-propionic acid (31)

5.1.14.1. Step 1: 2-Biphenyl-3-yl-3-{4-[3-(5-methyl-2-phenyl-oxazol-4-yl)-prop-1-ynyl]-phenyl}-propionic acid methyl ester (24). This compound was obtained from bromide **18** (0.300 g, 0.759 mmol) and alkyne **10** (0.299 g, 1.52 mmol) following the general method I. Ester **24** was obtained as a thick orange oil (0.228 g, 59%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.99 (d, $J = 8.1$ Hz, 2H), 7.53 (d, $J = 8.1$ Hz, 2H), 7.52–7.27 (m, 12H), 7.05 (d, $J = 8.1$ Hz, 2 H), 3.87 (dd, $J = 8.8$, 6.8 Hz, 1 H), 3.72 (s, 2 H), 3.60 (s, 3H), 3.43 (dd, $J = 13.7$, 8.8 Hz, 1H), 3.04 (dd, $J = 13.7$, 6.8 Hz, 1H), 2.46 (s, 3H); CIMS m/z 512 (M+1).

5.1.14.2. Step 2: 2-Biphenyl-3-yl-3-{4-[3-(5-methyl-2-phenyl-oxazol-4-yl)-prop-1-ynyl]-phenyl}-propionic acid (31). This compound was prepared from ester **24** (0.199 g, 0.325 mmol) following the general method II. The purification was carried out by chromatography on silica gel eluting with ethyl acetate in hexanes (0 to 45%) to provide **31** as a solid (0.156 g, 96%): mp 105–107 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.89 (br s, 2H), 7.46–7.18 (m, 14H), 6.99 (d, $J = 7.6$ Hz, 2H), 3.84 (dd, $J = 8.5$, 6.8 Hz, 1H), 3.62 (s, 2 H), 3.36 (dd, $J = 13.4$, 8.5 Hz, 1H), 2.98 (dd, $J = 13.4$, 6.8 Hz, 1H), 2.38 (s, 3H); CIMS m/z 498.2 (M+1). Anal. calcd for $C_{34}H_{27}NO_3 \cdot 1.3\ H_2O$: C, 78.38; H, 5.73; N, 2.69. Found: C, 78.22; H, 5.57; N, 2.59.

5.1.15. 3-{4-[3-(Methyl-pyridin-2-yl-amino)-prop-1-ynyl]-phenyl}-2-pyrrol-1-yl-propionic acid (32)

5.1.15.1. Step 1: 3-{4-[3-(Methyl-pyridin-2-yl-amino)-prop-1-ynyl]-phenyl}-2-pyrrol-1-yl-propionic acid methyl ester (26). This compound was prepared from triflate **14** (0.526 g, 1.394 mmol) and 2-(*N*-methyl-*N*-prop-2-ynyl)pyridine (**11**) (0.407 g, 2.788 mmol) following the

general method I, with the exception that piperidine was used as solvent instead of DMF. Under these conditions, racemization was observed. Purification by chromatography on silica gel eluting with hexanes:ethyl acetate (4:1) gave **26** as a yellowish thick oil (0.132 g, 25%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.19–8.17 (m, 1H), 7.48–7.43 (m, 1H), 7.22 (d, J = 8.0 Hz, 2H), 6.87 (d, J = 8.0 Hz, 2H), 6.64 (t, J = 2.0 Hz, 2H), 6.59 (d, J = 7.6 Hz, 1H), 6.58 (d, J = 6.4 Hz, 1H), 6.09 (t, J = 2.0 Hz, 2H), 4.66 (dd, J = 9.0, 6.4 Hz, 1H), 4.54 (s, 2 H), 3.65 (s, 3H), 3.33 (dd, J = 13.8 and 6.2 Hz, 1H), 3.19 (dd, J = 13.8 and 9.0 Hz, 1H), 3.09 (s, 3H); CIMS m/z 374 $(M+H)^+$.

5.1.15.2. Step 2: 3-{4-[3-(Methyl-pyridin-2-yl-amino)-prop-1-ynyl]-phenyl}-2-pyrrol-1-yl-propionic acid (32). Prepared from ester **26** (0.110 g, 0.294 mmol) by the general method II. Compound **32** was obtained as a yellow solid (0.098 g, 93%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.02 (m, 1H), 7.51–7.46 (m, 1H), 7.14 (d, J = 8.3 Hz, 2H), 6.82 (d, J = 8.0 Hz, 2H), 6.62–6.54 (m, 2H), 6.58 (t, J = 2.0 Hz, 2H), 6.00 (t, J = 2.2 Hz, 2H), 4.61 (dd, J = 9.4, 5.6 Hz, 1H), 4.37 (s, 2H), 3.33 (dd, J = 14.0 and 5.7 Hz, 1H), 3.13 (dd, J = 14.0 and 9.4 Hz, 1 H), 3.05 (s, 3H); CIMS m/z 360 $(M+H)^+$. Anal. calcd for $C_{22}H_{21}N_3O_2 \cdot 0.5C_4H_8O_2$: C, 71.44; H, 6.25; N, 10.41. Found: C, 71.14; H, 6.07; N, 10.20.

5.1.16. (*S*)-3-{4-[3-(Methyl-phenyl-amino)-prop-1-ynyl]-phenyl}-2-pyrrol-1-yl-propionic acid (33)

5.1.16.1. Step 1: (*S*)-3-{4-[3-(Methyl-phenyl-amino)-prop-1-ynyl]-phenyl}-2-pyrrol-1-yl-propionic acid methyl ester (27). Prepared from triflate **14** (1.660 g, 4.399 mmol) and *N*-methyl-*N*-prop-2-ynyl aniline (**12**) (1.27 g, 8.798 mmol) following the general method I. Purification by flash chromatography on silica gel eluting with petroleum ether in dichloromethane (60–0%) followed by a second chromatographic purification eluting with hexanes:ethyl acetate (7:1 to 5:1) gave pure **27** as a thick oil (1.256 g, 77%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.29–7.21 (m, 4H), 7.23 (d, J = 8.0 Hz, 2H), 6.92–6.66 (m, 1H), 6.89 (d, J = 8.4 Hz, 2 H), 6.66 (t, J = 2.4 Hz, 2H), 6.12 (t, J = 2.4 Hz, 2 H), 6.68 (dd, J = 9.2, 6.4 Hz, 1H), 4.24 (s, 2H), 3.67 (s, 3 H), 3.33 (dd, J = 13.9 and 6.3 Hz, 1H), 3.19 (dd, J = 13.9 and 9.0 Hz, 1H), 3.02 (s, 3H); CIMS m/z 373 $(M+H)^+$.

5.1.16.2. Step 2: (*S*)-3-{4-[3-(Methyl-phenyl-amino)-prop-1-ynyl]-phenyl}-2-pyrrol-1-yl-propionic acid (33). Prepared from ester **27** (0.820 g, 2.201 mmol) following the general method II. Purification by chromatography on silica gel eluting with methanol in chloroform (0% to 2%) containing formic acid (0% to 0.1%) gave pure **33** as a light brown solid (0.727 g, 92%): mp 55–60 °C; $[\alpha]_D^{25} = -79.2$ (c = 0.54, MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.29–7.25 (m, 2H), 7.21 (d, J = 8.0 Hz, 2H), 6.92 (d, J = 8.0 Hz, 2H), 6.88 (d, J = 8.4 Hz, 2H), 6.83 (t, J = 7.4 Hz, 1H), 6.65 (t, J = 2.0 Hz, 2H), 6.13 (t, J = 2.0 Hz, 2H), 4.72 (dd, J = 9.2, 6.0 Hz, 1H), 4.23 (s, 2H), 3.38 (dd, J = 13.9, 5.6 Hz, 1H), 3.23 (dd, J = 13.9, 9.5 Hz, 1H), 3.00 (s, 3H); CIMS m/z 359 $(M+H)^+$. Chiral HPLC (SFC conditions): chiralpak AS-H, 4.6 mm × 250 mm; mobile phase A: CO_2; mobile phase B: methanol + 0.5% isopropylamine; gradient: 95% A to 40% A in 15 min; flow rate: 4.0 mL/min; injection volume: 15 μL; detection: DAD 254 nm: t_R = 2.46 min; >99% purity. Anal. calcd for $C_{23}H_{22}N_2O_2 \cdot 0.3H_2O$: C, 75.93; H, 6.26; N, 7.74. Found: C, 75.73; H, 6.19; N, 7.53.

5.1.17. (*S*)-3-{4-[(*E*)-3-(Methyl-pyridin-2-yl-amino)-propenyl]-phenyl}-2-pyrrol-1-yl-propionic acid (36)

5.1.17.1. Step 1: 2-(*N*-Methyl-*N*-prop-2-enyl)pyridine (34). Sodium hydride (0.82 g, 20.4 mmol) was suspended in DMF (10 mL) under nitrogen and stirred in an ice bath. 2-(Methylamino)pyridine (1.5 mL, 14.6 mmol) was added. The ice bath was removed and the mixture stirred at room temperature for 0.5 h. The mixture was cooled back in an ice bath and allyl bromide (1.9 mL, 21.9 mmol) was added. The mixture was allowed to reach room temperature and stirred overnight. The mixture was diluted with water and extracted with diethyl ether. The combined organic extracts were washed with water and brine, dried over $MgSO_4$, filtered, and the solvent removed. Purification by flash chromatography on silica gel eluting with ethyl acetate in hexanes (5%) afforded **34** as an oil (1.74 g, 80%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.15 (m, 1H), 7.42 (m, 1H), 6.53 (dd, J = 6.8, 4.8 Hz, 1H), 6.48 (d, J = 8.4 Hz, 1H), 5.89–5.79 (m, 1H), 5.14 (m, 1H), 4.14 (d, J = 5.1 Hz, 2H), 3.03 (s, 3H); CIMS m/z 149 $(M+H)^+$.

5.1.17.2. Step 2: (*S*)-3-{4-[(*E*)-3-(Methyl-pyridin-2-yl-amino)-propenyl]-phenyl}-2-pyrrol-1-yl-propionic acid methyl ester (35). A mixture of $Pd(OAc)_2$ (0.030 g, 0.132 mmol) and PPh_3 (0.076 g, 0.291 mmol) was stirred in dry DMF (8 mL) and nitrogen bubbled through the mixture for 10 min. Triflate **14** (1.000 g, 5.30 mmol), a solution of 2-(*N*-methyl-*N*-prop-2-enyl)pyridine (**34**) (0.785 g, 5.300 mmol) in dry DMF (2 mL) and triethylamine (0.74 mL, 5.30 mmol) were then added. The mixture was heated at 90 °C for 20 h. More $Pd(OAc)_2$ (0.030 g, 0.132 mmol) was added and heating continued for 8 h. Mixture was allowed to cool, diluted with diethyl ether, and filtered through a celite pad. The filtrate was diluted with water (100 mL) and the phases were separated. The aqueous phase was extracted with diethyl ether (4 × 50 mL) and the combined organic extracts were washed with brine, dried over $MgSO_4$, filtered, and the solvent was removed. Purification by flash chromatography eluting with ethyl acetate in hexanes (20% to 25%) gave **35** as a thick oil (0.60 g, 60%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.18–8.16 (m, 1H), 7.47–7.42 (m, 1H), 7.21 (d, J = 8.4 Hz, 2H), 6.92 (d, J = 8.0 Hz, 2 H), 6.69 (t, J = 2.0 Hz, 2H), 6.67–6.51 (m, 2H), 6.42 (d, J = 15.6 Hz, 1H), 6.18 (dt, J = 16.0, 5.6 Hz, 1H), 6.13 (t, J = 2.0 Hz, 2H), 4.71 (dd, J = 8.6, 6.0 Hz, 1H), 4.30 (dd, J = 5.6, 1.2 Hz, 2H), 3.66 (s, 3 H), 3.36 (dd, J = 13.9, 6.1 Hz, 1H), 3.22 (dd, J = 13.9, 9.0 Hz, 1H), 3.05 (s, 3H); CIMS m/z 376 $(M+H)^+$.

5.1.17.3. Step 3: (*S*)-3-{4-[(*E*)-3-(Methyl-pyridin-2-yl-amino)-propenyl]-phenyl}-2-pyrrol-1-yl-propionic acid (36). Prepared from ester **35** (0.481 g, 1.281 mmol) following the general method II. Purification by chromatography on silica gel eluting with methanol in chloroform (0%

to15%) followed by THF gave pure **36** (0.123 g, 26%): mp 155–165 °C; $[\alpha]_D^{25} = -67.2$ ($c = 0.53$, THF); ^{1}H NMR (CD_3OD, 400 MHz) δ 7.98 (dd, $J = 5.2$, 1.0 Hz, 1 H), 7.52–7.47 (m, 1H), 7.13 (d, $J = 8.4$ Hz, 2H), 6.91 (d, $J = 8.4$ Hz, 2H), 6.66 (t, $J = 2.0$ Hz, 2H), 6.56 (dd, $J = 6.4$, 5.2 Hz, 1H), 6.38 (d, $J = 15.6$ Hz, 1H), 6.14 (dt, $J = 16.0$, 5.6 Hz, 1H), 5.92 (t, $J = 2.0$ Hz, 2H), 4.58 (dd, $J = 9.6$, 5.6 Hz, 1H), 4.22 (dd, $J = 5.6$, 1.2 Hz, 2 H), 3.31 (dd, $J = 14.0$, 5.5 Hz, 1H), 3.11 (dd, $J = 14.0$, 9.5 Hz, 1H), 3.02 (s, 3H); CIMS *m/z* 362 $(M+H)^+$. Chiral HPLC (SFC conditions): chiralcel AS-H, 4.6 mm × 250 mm; mobile phase A: CO_2; mobile phase B: methanol; gradient: 95% A to 40% A in 15 min; flow rate: 4.0 mL/min; injection volume: 15 μL; detection: DAD 254 nm: $t_R = 2.37$ min; purity: 92.20%. Anal. calcd for $C_{22}H_{23}N_3O_2 \cdot 0.5\ H_2O$: C, 71.33; H, 6.53; N, 11.34. Found: C, 71.10; H, 6.22; N, 11.18.

5.1.18. (*S*)-3-{4-[(*E*)-3-(5-Methyl-2-phenyl-oxazol-4-yl)-propenyl]-phenyl}-2-pyrrol-1-yl-propionic acid (40)

5.1.18.1. Step 1: 5-Methyl-2-phenyl-4-[3-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-allyl]-oxazole (37). A mixture of alkyne **10** (3.000 g, 15.207 mmol) and pinacolborane (3.3 mL, 22.810 mmol) in dichloromethane (16 mL) was added to Cp_2ZrHCl (0.392 g, 1.520 mmol) at 0 °C under a nitrogen atmosphere. The mixture was stirred at 0 °C for 30 min and then allowed to warm to room temperature and stirred for 2 days. The mixture was quenched with water, diluted with diethyl ether (100 mL), and the phases were separated. The aqueous phase was extracted with diethyl ether (2 × 40 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and the solvent was removed. Purification by chromatography on silica gel eluting with hexanes: ethyl acetate (100:0 to 14:1) afforded **37** as a pale yellow solid (3.29 g, 66%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.96 (d, $J = 5.9$ Hz, 2H), 7.39 (m, 3H), 6.74 (dt, $J = 17.8$, 5.9 Hz, 1H), 5.48 (dt, $J = 17.8$, 1.7 Hz, 1H), 3.37 (dd, $J = 5.9$, 1.5 Hz, 2H), 2.29 (s, 3H), 1.23 (s, 12H); CIMS *m/z* 326 $(M+H)^+$. Anal. calcd for $C_{19}H_{24}BNO_3$: C, 70.17; H, 7.44; N, 4.31. Found: C, 70.00; H, 7.55; N, 4.21.

5.1.18.2. Step 2: (*S*)-3-{4-[(*E*)-3-(5-Methyl-2-phenyl-oxazol-4-yl)-propenyl]-phenyl}-2-pyrrol-1-yl-propionic acid methyl ester (38). A mixture of triflate **14** (0.966 g, 2.561 mmol), boronate **37** (1.000 g, 3.074 mmol), $Pd(PPh_3)_4$ (0.148 g, 0.128 mmol), K_2CO_3 (0.708 g, 5.122 mmol), KBr (0.335 g, 2.817 mmol) was stirred in dry toluene (25 mL). Nitrogen was passed through the mixture for 30 min. The reaction mixture was heated at 85 °C under a nitrogen atmosphere for 20 h. The mixture was cooled to 0 °C and 3 M NaOAc (8 mL) was added followed by 30% hydrogen peroxide (4 mL). Mixture was stirred for 2 h, then diluted with ethyl acetate (40 mL) and water (20 mL). The phases were separated and the aqueous phase was extracted with ethyl acetate (2 × 50 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered, and the solvent removed. Purification by flash chromatography on silica gel eluting with ethyl acetate in hexanes (0% to 17%) afforded **38** as a thick oil (0.675 g, 62%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.00–7.97 (m, 2H), 7.44–7.39 (m, 3H), 7.22 (d, $J = 8.0$ Hz, 2H), 6.91 (d, $J = 8.0$ Hz, 2H), 6.69 (t, $J = 2.0$ Hz, 2H), 6.42 (d, $J = 14.8$ Hz, 1H), 6.32 (dt, $J = 15.6$, 6.4 Hz, 1H), 6.13 (t, $J = 2.0$ Hz, 2H), 4.71 (dd, $J = 8.8$, 6.4 Hz, 1 H), 3.68 (s, 3H), 3.42 (d, $J = 6.4$ Hz, 2H), 3.37 (dd, $J = 13.9$, 6.3 Hz, 1H), 3.21 (dd, $J = 13.9$, 8.8 Hz, 1H), 2.34 (s, 3H); CIMS *m/z* 427 $(M+H)^+$. Chiral HPLC conditions: chiralpak AD, 250 mm × 4.6 mm; hexanes/ethanol 85:15 v/v, 1.0 mL/min: t_R (major enantiomer) = 11.042 min, t_R (minor enantiomer) = 9.337 min; 99.64% ee.

5.1.18.3. Step 3: (*S*)-3-{4-[(*E*)-3-(5-Methyl-2-phenyl-oxazol-4-yl)-propenyl]-phenyl}-2-pyrrol-1-yl-propionic acid (40). Prepared from ester **38** (0.140 g, 0.328 mmol) following the general method II. Purification by chromatography on silica gel eluting with hexanes: ethyl acetate (2:1) containing formic acid (0% to 0.2%) gave **40** as an off-white solid (0.085 g, 63%): mp 132–133 °C; $[\alpha]_D^{25} = -61.6$ ($c = 0.5$, THF); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.95–7.92 (m, 2 H), 7.41-7.39 (m, 3H), 7.19 (d, $J = 8.0$ Hz, 2H), 6.90 (d, $J = 8.4$ Hz, 2H), 6.65 (t, $J = 2.0$ Hz, 2H), 6.37 (d, $J = 15.6$ Hz, 1H), 6.25 (dt, $J = 16.0$, 6.4 Hz, 1H), 6.13 (t, $J = 2.0$ Hz, 2H), 4.57 (dd, $J = 8.8$, 6.0 Hz, 1H), 3.39 (d, $J = 6.8$ Hz, 2H), 3.32 (dd, $J = 14.0$, 5.9 Hz, 1H), 3.15 (dd, $J = 14.0$, 9.2 Hz, 1H), 2.33 (s, 3H); CIMS *m/z* 413 $(M+H)^+$. Chiral HPLC (SFC conditions): chiralcel OJ-H, 4.6 mm × 250 mm; mobile phase A: CO_2; mobile phase B: butanol + 0.5% isopropylamine; gradient: 95% A to 40% A in 15 min; flow rate: 4.0 mL/min; injection volume: 15 μL; detection: DAD 254 nm: t_R (major enantiomer) = 2.95 min, t_R (minor enantiomer) = 2.17 min; >99% ee. Anal. calcd for $C_{26}H_{24}N_2O_3 \cdot 0.1\ H_2O$: C, 75.38; H, 5.89; N, 6.76. Found: C, 75.58; H, 6.21; N, 6.37.

5.1.19. (*R*)-3-{4-[(*E*)-3-(5-Methyl-2-phenyl-oxazol-4-yl)-propenyl]-phenyl}-2-pyrrol-1-yl-propionic acid (41)

5.1.19.1. Step 1: (*R*)-3-{4-[(*E*)-3-(5-Methyl-2-phenyl-oxazol-4-yl)-propenyl]-phenyl}-2-pyrrol-1-yl-propionic acid methyl ester (39). Prepared from triflate **15** (0.300 g, 0.795 mmol) following the conditions reported for the synthesis of **38**. Ester **39** was obtained as a clear oil (0.057 g, 17%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.01–7.98 (m, 2H), 7.45-7.39 (m, 3H), 7.22 (d, $J = 8.0$ Hz, 2H), 6.92 (d, $J = 8.3$ Hz, 2H), 6.69 (t, $J = 2.2$ Hz, 2H), 6.42 (d, $J = 15.9$ Hz, 1H), 6.32 (dt, $J = 15.9$, 6.3 Hz, 1H), 6.13 (t, $J = 2.2$ Hz, 2H), 4.70 (dd, $J = 8.8$, 6.3 Hz, 1 H), 3.68 (s, 3H), 3.43 (d, $J = 6.1$ Hz, 2H), 3.33 (dd, $J = 13.8$, 6.4 Hz, 1H), 3.18 (dd, $J = 13.8$, 8.8 Hz, 1H), 2.34 (s, 3 H); CIMS *m/z* 427 $(M+H)^+$.

5.1.19.2. Step 2: (*R*)-3-{4-[(*E*)-3-(5-Methyl-2-phenyl-oxazol-4-yl)-propenyl]-phenyl}-2-pyrrol-1-yl-propionic acid (41). Prepared from ester **39** (1.100 g, 2.579 mmol) following the general method II. Purification by chromatography on silica gel eluting with methanol in chloroform (0% to 5%) gave **41** as a yellow solid (0.650 g, 61%): mp 132–134 °C; $[\alpha]_D^{25} = +67.2$ ($c = 0.50$, THF); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.95–7.91 (m, 2 H), 7.42–7.38 (m, 3 H), 7.19 (d, $J = 8.3$ Hz, 2H), 6.90 (d, $J = 8.3$ Hz, 2 H), 6.64 (t, $J = 2.1$ Hz, 2H), 6.37 (d, $J = 15.9$ Hz, 1 H), 6.24 (dt, $J = 15.9$, 6.3 Hz, 1H), 6.13 (t, $J = 2.1$ Hz, 2H), 4.56 (dd, $J = 9.0$, 5.9 Hz, 1H), 3.39 (d, $J = 6.3$ Hz, 2H), 3.36 (dd, $J = 14.0$, 6.1 Hz, 1H),

3.18 (dd, J = 14.0, 8.9 Hz, 1H), 2.33 (s, 3H); CIMS m/z 413 $(M+H)^+$. Chiral HPLC (SFC conditions): chiralcel OJ-H, 4.6 mm × 250 mm; mobile phase A: CO_2; mobile phase B: butanol + 0.5% isopropylamine; gradient: 95% A to 40% A in 15 min; flow rate: 4.0 mL/min; injection volume: 15 μL; detection: DAD 254 nm: t_R (major enantiomer) = 2.15 min, t_R (minor enantiomer) = 3.04 min; 82.47% ee. Anal. calcd for $C_{26}H_{24}N_2O_3 \cdot 0.6$ - H_2O: C, 73.77; H, 6.00; N, 6.62. Found: C, 73.50; H, 5.83; N, 6.53.

5.1.20. (*S*)-3-{4-[(*E*)-3-(Methyl-phenyl-amino)-propenyl]-phenyl}-2-pyrrol-1-yl-propionic acid (44)

5.1.20.1. Step 1: Methyl-phenyl-[2-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-vinyl]-amine (42). A mixture of alkyne **12** (1.0 mL, 6.873 mmol) and pinacolborane (1.2 mL, 8.247 mmol) in dichloromethane (5 mL) was added to Cp_2ZrHCl (0.088 g, 0.343 mmol) at 0 °C. The mixture was allowed to warm at room temperature and stirred for 8 days under nitrogen. At this time, the mixture was diluted with diethyl ether (50 mL) and washed with water (30 mL). The phases were separated and the aqueous phase was extracted with diethyl ether (2 × 30 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and the solvent was removed. Purification by chromatography on silica gel eluting with hexanes: ethyl acetate (20:1 to 10:1) afforded **42** as an off-white solid (0.990 g, 53%): ^1H NMR ($CDCl_3$, 400 MHz) δ 7.21–7.17 (m, 2H), 6.69–6.64 (m, 3H), 6.60 (dt, J = 18.0, 4.4 Hz, 1H), 5.55 (dt, J = 18.0, 1.6 Hz, 1H), 3.99 (dd, J = 4.4, 1.6 Hz, 2H), 2.94 (s, 3H), 1.25 (s, 12H); CIMS m/z 274 $(M+H)^+$.

5.1.20.2. Step 2: (*S*)-3-{4-[(*E*)-3-(Methyl-phenyl-amino)-propenyl]-phenyl}-2-pyrrol-1-yl-propionic acid methyl ester (43). Triflate **14** (0.98 g, 2.592 mmol), boronate ester **42** (0.850 g, 3.111 mmol), K_2CO_3 (0.716 g, 5.184 mmol) were stirred in dry toluene (25 mL). Nitrogen was passed through the mixture for 0.5 h. $Pd(PPh_3)_4$ (0.149 g, 0.129 mmol) was added and the reaction mixture was heated at 85–90 °C for 24 h. The mixture was allowed to cool, diluted with ethyl acetate (70 mL), and washed with sat $NaHCO_3$, water and brine. The organic extracts were dried over $MgSO_4$, filtered, and the solvent was removed. Purification by flash chromatography on silica gel eluting with petroleum ether in dichloromethane (50% to 0%) followed by a second chromatographic purification eluting with hexanes: ethyl acetate (8:1 to 5:1) gave ester **43** as a 94:6 mixture of *E*:*Z* isomers (0.819 g, 84%). *E-isomer*: ^1H NMR ($CDCl_3$, 400 MHz) δ 7.23–7.20 (m, 2 H), 7.21 (d, J = 8.0 Hz, 2 H), 6.92 (d, J = 8.0 Hz, 2H), 6.77–6.70 (m, 3H), 6.69 (t, J = 2.0 Hz, 2H), 6.44 (d, J = 16.0 Hz, 1H), 6.19 (dt, J = 15.6, 5.6 Hz, 1H), 6.13 (t, J = 2.0 Hz, 2H), 4.71 (dd, J = 9.2, 6.4 Hz, 1H), 4.05 (dd, J = 5.6, 1.2 Hz, 2H), 3.69 (s, 3H), 3.37 (dd, J = 13.8, 6.4 Hz, 1H), 3.21 (dd, J = 13.8, 9.0 Hz, 1H), 2.95 (s, 3H); CIMS m/z 375 $(M+H)^+$.

5.1.20.3. Step 3: (*S*)-3-{4-[(*E*)-3-(Methyl-phenyl-amino)-propenyl]-phenyl}-2-pyrrol-1-yl-propionic acid (44). Prepared from ester **43** (0.877 g, 2.342 mmol) following the general method II. Purification by chromatography on silica gel eluting with methanol in chloroform (0–3%) containing formic acid (0.1%) gave pure **44** as a brownish solid (0.280 g, 33%): mp 85–90 °C; $[\alpha]_D^{25} = -67.9$ (c = 0.53, THF); ^1H NMR ($CDCl_3$, 400 MHz) δ *E-isomer*: 7.26–7.18 (m, 3H), 6.92 (d, J = 8.4 Hz, 2H), 6.81–6.69 (m, 4 H), 6.68 (t, J = 2 Hz, 2H), 6.44 (d, J = 15.6 Hz, 1H), 6.14 (t, J = 2.0 Hz, 2H), 4.73 (dd, J = 9.2, 6.0 Hz, 1H), 4.03 (dd, J = 5.6, 1.2 Hz, 2H), 3.40 (dd, J = 14.0, 5.8 Hz, 1H), 3.24 (dd, J = 14.0, 9.5 Hz, 1H), 2.97 (s, 3H); CIMS m/z 361 $(M+H)^+$. Anal. calcd for $C_{23}H_{24}N_2O_2 \cdot 0.9$ H_2O: C, 74.41; H, 6.84; N, 7.55. Found: C, 74.03; H, 6.62; N, 7.36.

5.1.21. (*S*)-3-{4-[3-(5-Methyl-2-phenyl-oxazol-4-yl)-propyl]-phenyl}-2-pyrrol-1-yl-propionic acid (53)

5.1.21.1. Step 1: general method III: (*S*)-3-{4-[3-(5-Methyl-2-phenyl-oxazol-4-yl)-propyl]-phenyl}-2-pyrrol-1-yl-propionic acid methyl ester (45). A solution of alkyne **21** (0.249 g, 0.586 mmol) was dissolved in THF (16 mL) and the mixture was hydrogenated over 20% Pd/C (0.045 g) for 16 h. The catalyst was separated by filtration through a celite pad and washed with ethyl acetate. The filtrate was concentrated and the residue dried under high vacuum. Ester **45** was obtained as an oil (0.243, 97%): ^1H NMR ($CDCl_3$, 400 MHz) δ 7.97 (dd, J = 8.0, 1.6 Hz, 2H), 7.44–7.38 (m, 3H), 6.99 (d, J = 8.4 Hz, 2H), 6.91 (d, J = 8.0 Hz, 2H), 6.70 (t, J = 2.0 Hz, 2H), 6.12 (t, J = 2.0 Hz, 2H), 4.72 (dd, J = 8.8, 6.8 Hz, 1H), 3.68 (s, 3H), 3.37 (dd, J = 13.8, 6.4 Hz, 1H), 3.21 (dd, J = 13.8, 8.5 Hz, 1H), 2.61 (t, J = 7.6 Hz, 2H), 2.47 (t, J = 7.6 Hz, 2H), 2.26 (s, 3H), 1.96 (qn, J = 7.6 Hz, 2 H); CIMS m/z 429 $(M+H)^+$.

5.1.21.2. Step 2: (*S*)-3-{4-[3-(5-Methyl-2-phenyl-oxazol-4-yl)-propyl]-phenyl}-2-pyrrol-1-yl-propionic acid (53). Prepared from ester **45** (0.865 g, 2.018 mmol) following the general method II. Compound **53** was obtained as an off-white solid (0.808 g, 97%). An analytical sample was obtained by recrystallization from ethyl acetate and treatment with activated charcoal: mp 167–168 °C; $[\alpha]_D^{25} = -41.6$ (c = 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.94 (m, 2 H), 7.40 (m, 3 H), 7.04 (d, J = 7.8 Hz, 2 H), 6.93 (d, J = 7.8 Hz, 2H), 6.71 (br s, 2H), 6.14 (br s, 2H), 4.72 (m, 1H), 3.36 (dd, J = 13.9, 6.1 Hz, 1H), 3.18 (dd, J = 13.9, 8.8 Hz, 1H), 2.58 (t, J = 7.3 Hz, 2H), 2.47 (t, J = 7.3 Hz, 2H), 2.26 (s, 3H), 1.92 (qn, J = 7.3 Hz, 2 H); CIMS m/z 415 $(M+H)^+$. Chiral HPLC (SFC conditions): chiralpak AS-H, 250 mm × 4.6 mm; mobile phase A: CO_2; mobile phase B: 50:50 methanol:ethanol; gradient: 95% A to 40% A in 15 min; flow rate: 4.0 mL/min; injection volume: 15 μL; detection: (DAD 254 nm): t_R (major enantiomer) = 3.17 min, t_R (minor enantiomer) = 3.37 min; >99% ee. Anal. calcd for $C_{26}H_{26}N_2O_3 \cdot 0.4$ H_2O: C, 74.05; H, 6.41; N, 6.64. Found: C, 73.93; H, 6.22; N, 6.53.

5.1.22. (*R*)-3-{4-[3-(5-Methyl-2-phenyl-oxazol-4-yl)-propyl]-phenyl}-2-pyrrol-1-yl-propionic acid (54)

5.1.22.1. Step 1: (*R*)-3-{4-[3-(5-Methyl-2-phenyl-oxazol-4-yl)-propyl]-phenyl}-2-pyrrol-1-yl-propionic acid methyl ester (46). This compound was prepared from alkene **39** (0.057 g, 0.133 mmol) following the general method III. Ester **46** was obtained as an amber oil

(0.057, 99%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.97 (d, J = 8.0 Hz, 2 H), 7.44–7.38 (m, 3 H), 6.99 (d, J = 8.0 Hz, 2 H), 6.91 (d, J = 8.0 Hz, 2 H), 6.71 (t, J = 2.2 Hz, 2H), 6.13 (t, J = 2.2 Hz, 2H), 4.72 (dd, J = 8.8, 6.6 Hz, 1H), 3.68 (s, 3H), 3.37 (dd, J = 13.9, 6.6 Hz, 1H), 3.20 (dd, J = 13.9, 8.8 Hz, 1H), 2.61 (t, J = 7.6 Hz, 2H), 2.47 (t, J = 7.6 Hz, 2H), 2.26 (s, 3H), 1.96 (qn, J = 7.6 Hz, 2 H); CIMS m/z 429.2 $(M+H)^+$.

5.1.22.2. Step 2: (*R*)-3-{4-[3-(5-Methyl-2-phenyl-oxazol-4-yl)-propyl]-phenyl}-2-pyrrol-1-yl-propionic acid (54). Prepared from ester **46** (0.879 g, 2.051 mmol) following the general method II. Compound **54** was obtained as an off-white solid (0.724 g, 85%). An analytical sample was obtained by recrystallization from ethyl acetate: hexanes: mp 168–169.5 °C; $[\alpha]_D^{25} = +32$ (c = 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.94–7.91 (m, 2H), 7.40–7.38 (m, 3H), 7.04 (d, J = 8.0 Hz, 2H), 6.93 (d, J = 8.0 Hz, 2H), 6.71 (t, J = 2.2 Hz, 2H), 6.14 (t, J = 2.2 Hz, 2H), 4.72 (dd, J = 8.8, 6.3 Hz, 1H), 3.36 (dd, J = 14.0, 6.3 Hz, 1H), 3.18 (dd, J = 14.0, 8.8 Hz, 1 H), 2.58 (t, J = 7.3 Hz, 2H), 2.47 (t, J = 7.3 Hz, 2 H), 2.26 (s, 3H), 1.91 (qn, J = 7.3 Hz, 2H); CIMS m/z 415.2 $(M+H)^+$. Chiral HPLC (SFC conditions): chiralpak AS-H, 250 mm × 4.6 mm; mobile phase A: CO_2; mobile phase B: 50:50 methanol:ethanol; gradient: 95% A to 40% A in 15 min; flow rate: 4.0 mL/min; injection volume: 15 μL; detection: (DAD 254 nm): t_R (major enantiomer) = 3.39 min, t_R (minor enantiomer) = 3.20 min; 95.02% ee. Anal. calcd for $C_{26}H_{26}N_2O_3 \cdot 0.1\ H_2O$: C, 75.01; H, 6.34; N, 6.73. Found: C, 74.72; H, 6.39; N, 6.62.

5.1.23. 3-{4-[3-(5-Methyl-2-phenyl-oxazol-4-yl)-propyl]-phenyl}-2-pyridin-3-yl-propionic acid (55)

5.1.23.1. Step 1: 3-{4-[3-(5-Methyl-2-phenyl-oxazol-4-yl)-propyl]-phenyl}-2-pyridin-3-yl-propionic acid ethyl ester (47). Prepared from alkyne **22** (0.540 g, 1.198 mmol) following the general method III. Compound **47** was obtained as a thick oil (0.449 g, 82%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.48 (br s, 2H), 7.97 (d, J = 8.0 Hz, 2H), 7.69 (dt, J = 8.0, 1.8 Hz, 1H), 7.43–7.36 (m, 3H), 7.25 (t, J = 7.6 Hz, 1H), 7.05 (d, J = 8.0 Hz, 2H), 6.99 (d, J = 8.0 Hz, 2H), 4.14–4.01 (m, 2H), 3.83 (t, J = 7.6 Hz, 1H), 3.37 (dd, J = 13.6, 8.3 Hz, 1H), 2.98 (dd, J = 13.6, 7.3 Hz, 1H), 2.60 (t, J = 7.6 Hz, 2H), 2.47 (t, J = 7.6 Hz, 2H), 2.26 (s, 3H), 1.95 (qn, J = 7.6 Hz, 2 H); CIMS m/z 455 (M+1).

5.1.23.2. Step 2: 3-{4-[3-(5-Methyl-2-phenyl-oxazol-4-yl)-propyl]-phenyl}-2-pyridin-3-yl-propionic acid (55). Prepared from ester **47** (0.449 g, 0.988 mmol) following the general method II. Compound **55** was obtained as a solid (0.348 g, 58%): mp 138–141 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.54 (br s, 1H), 8.43 (br s, 1H), 7.91 (d, J = 4.1 Hz, 2H), 7.73 (m, 1H), 7.35–7.22 (m, 4H), 6.98 (m, 4H), 3.87 (m, 1H), 3.39 (dd, J = 14.0, 7.6 Hz, 1H), 2.97 (dd, J = 14.0, 6.2 Hz, 1H), 2.52 (t, J = 7.2 Hz, 2H), 2.43 (t, J = 6.8 Hz, 2H), 2.22 (s, 3H), 1.87 (qn, J = 7.6 Hz, 2 H); CIMS m/z 427 (M+1). Anal. calcd for $C_{27}H_{26}N_2O_3 \cdot 1.0\ H_2O$: C, 72.95; H, 6.35; N, 6.30. Found: C, 72.64; H, 6.32; N, 5.97.

5.1.24. 2-Biphenyl-4-yl-3-{4-[3-(5-methyl-2-phenyl-oxazol-4-yl)-propyl]-phenyl}-propionic acid (56)

5.1.24.1. Step 1: 2-Biphenyl-4-yl-3-{4-[3-(5-methyl-2-phenyl-oxazol-4-yl)-propyl]-phenyl}-propionic acid ethyl ester (48). Prepared from alkyne **23** (0.325 g, 0.618 mmol) following the general method III. Purification by chromatography on silica gel eluting with ethyl acetate in hexanes gave **48** as a clear oil (0.254 g, 77%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.98 (d, J = 7.9 Hz, 2H), 7.58–7.52 (m, 4 H), 7.44–7.31 (m, 8H), 7.07 (s, 4H), 4.12–4.02 (m, 2H), 3.86 (dd, J = 9.0, 6.6 Hz, 1H), 3.40 (dd, J = 13.8, 9.0 Hz, 1 H), 3.02 (dd, J = 13.7, 6.6 Hz, 1 H), 2.62 (t, J = 7.6 Hz, 2 H), 2.48 (t, J = 7.6 Hz, 2 H), 2.26 (s, 3 H), 1.97 (qn, J = 7.6 Hz, 2 H); CIMS m/z 530.2 (M+1).

5.1.24.2. Step 2: 2-Biphenyl-4-yl-3-{4-[3-(5-methyl-2-phenyl-oxazol-4-yl)-propyl]-phenyl}-propionic acid (56). Prepared from ester **48** (0.254 g, 0.479 mmol) following the general method II. Purification by chromatography on silica gel eluting with ethyl acetate in hexanes (0% to 30%) gave **56** as a solid (0.231 g, 96%): mp 158–160 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.94–7.92 (m, 2 H), 7.54–7.49 (m, 4 H), 7.40–7.22 (m, 8H), 7.03 (d, J = 8.6 Hz, 2H), 7.00 (d, J = 8.4 Hz, 2H), 3.87 (dd, J = 8.8, 6.6 Hz, 1H), 3.38 (dd, J = 13.9, 8.8 Hz, 1H), 3.00 (dd, J = 13.9, 6.6 Hz, 1 H), 2.55 (t, J = 7.6 Hz, 2H), 2.44 (t, J = 7.4 Hz, 2 H), 2.20 (s, 3 H), 1.91 (qn, J = 7.6 Hz, 2H); CIMS m/z 502.3 (M+1). Anal. calcd for $C_{34}H_{31}NO_3$: C, 81.41; H, 6.23; N, 2.79. Found: C, 81.05; H, 6.42; N, 2.63.

5.1.25. 2-Biphenyl-3-yl-3-{4-[3-(5-methyl-2-phenyl-oxazol-4-yl)-propyl]-phenyl}-propionic acid (57)

5.1.25.1. Step 1: 2-Biphenyl-3-yl-3-{4-[3-(5-methyl-2-phenyl-oxazol-4-yl)-propyl]-phenyl}-propionic acid methyl ester (49). This compound was prepared from alkyne **24** (0.284 g, 0.555 mmol) following the general method III. Ester **49** was obtained as a yellow oil (0.223, 91%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.98 (d, J = 8.3 Hz, 2H), 7.53 (d, J = 8.0 Hz, 2H), 7.48–7.27 (m, 10 H), 7.44–7.31 (m, 8H), 7.07 (d, J = 8.3 Hz, 2H), 7.03 (d, J = 8.3 Hz, 2H), 3.89 (dd, J = 8.8, 6.6 Hz, 1H), 3,61 (s, 3 H), 3.42 (dd, J = 13.7, 8.8 Hz, 1H), 3.02 (dd, J = 13.7, 6.6 Hz, 1H), 2.62 (t, J = 7.6 Hz, 2H), 2.48 (t, J = 7.6 Hz, 2H), 2.25 (s, 3H), 1.97 (qn, J = 7.6 Hz, 2 H); CIMS m/z 516.3 (M+1).

5.1.25.2. Step 2: 2-Biphenyl-3-yl-3-{4-[3-(5-methyl-2-phenyl-oxazol-4-yl)-propyl]-phenyl}-propionic acid (57). Prepared from ester **49** (0.427 g, 0.828 mmol) following the general method II. The purification was carried out by flash chromatography on silica gel eluting with ethyl acetate in hexanes (0% to 30%) to afford **57** as a solid (0.288 g, 69%): mp 75–80 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.94–7.92 (m, 2H), 7.50–7.43 (m, 4H), 7.38–7.27 (m, 8H), 7.01 (s, 4 H), 3.89 (dd, J = 8.6, 6.8 Hz, 1H), 3.39 (dd, J = 13.9, 8.6 Hz, 1H), 3.00 (dd, J = 13.9, 6.8 Hz, 1H), 2.54 (t, J = 7.6 Hz, 2H), 2.44 (t, J = 7.4 Hz, 2H), 2.19 (s, 3H), 1.90 (qn, J = 7.6 Hz, 2H); CIMS m/z 502 (M+1). Anal. calcd for $C_{34}H_{31}NO_3 \cdot 0.1\ H_2O$: C, 81.12; H, 6.25; N, 2.78. Found: C, 80.80; H, 6.33; N, 2.69.

5.1.26. 3-{4-[3-(5-Methyl-2-phenyl-oxazol-4-yl)-propyl]-phenyl}-2-phenyl-propionic acid (58)

5.1.26.1. Step 1: 3-{4-[3-(5-Methyl-2-phenyloxazol-4-yl)prop-1-ynyl]phenyl}-2-phenylpropionic acid methyl ester (25). Prepared from triflate **20** (1.52 g, 3.9 mmol) and 5-methyl-2-phenyl-4-prop-2-ynyloxazole (**10**) (1.0 g, 5.07 mmol) following the general method I. Purification by flash chromatography on silica gel eluting with ethyl acetate in hexanes (5 to 50%) provided **25** as a yellow oil (0.89 g, 52%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.00 (m, 2H), 7.43 (m, 3H), 7.30 (m, 5H), 7.04 (m, 4H), 3.81 (m, 1H), 3.73 (s, 2H), 3.60 (s, 3H), 3.38 (m, 1H), 3.02 (m, 1H), 2.48 (s, 3H); CIMS *m*/*z* 436 (M+1).

5.1.26.2. Step 2: 3-{4-[3-(5-Methyl-2-phenyloxazol-4-yl)propyl]phenyl}-2-phenylpropionic acid methyl ester (50). Prepared from alkyne **25** (0.89 g, 2.04 mmol) following the general method III. Compound **50** was obtained as an oil (0.86 g, 96%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.99 (m, 2H), 1.99 (m, 2H), 7.43 (m, 3H), 7.30 (m, 5H), 7.05 (m, 4H), 3.84 (m, 1H), 3.61 (s, 3H), 3.40 (m, 1H), 2.99 (m, 1H), 2.62 (m, 2H), 2.49 (m, 2H), 2.27 (s, 3H); CIMS *m*/*z*440 (M+1).

5.1.26.3. Step 3: 3-{4-[3-(5-Methyl-2-phenyloxazol-4-yl)propyl]phenyl}-2-phenylpropionic acid (58). Prepared from ester **50** (0.86 g, 1.96 mmol) following the general method II. Purification by flash chromatography on silica gel eluting with ethyl acetate in hexanes (5% to 75%) provided **58** as a white solid (0.140 g, 17%): ^{1}H NMR ($CDCl_3$, 400 MHz,) δ 8.07 (m, 2H), 7.46 (m, 3H), 7.31 (m, 5H), 7.05 (m, 4H), 3.87 (m, 1H), 3.38 (m, 1H), 3.00 (m, 1H), 2.58 (m, 4H), 2.27 (m, 3H), 2.04 (m, 2H); CIMS *m*/*z* 426 (M+1). Anal. calcd for $C_{28}H_{27}NO_3 \cdot 0.25\ H_2O$: C, 78.21; H, 6.45; N, 3.26. Found: C, 78.32; H, 6.53; N, 2.97.

5.1.27. (*S*)-3-{4-[3-(Methyl-pyridin-2-yl-amino)-propyl]-phenyl}-2-pyrrol-1-yl-propionic acid (59)

5.1.27.1. Step 1: (*S*)-3-{4-[3-(Methyl-pyridin-2-yl-amino)-propyl]-phenyl}-2-pyrrol-1-yl-propionic acid methyl ester (51). Prepared from alkene **35** (0.430 g, 1.145 mmol) following general method III. Ester **51** was obtained as a thick oil (0.378 g, 87%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.08–8.06 (m, 1H), 7.36-7.31 (m, 1H), 6.99 (d, J = 8.0 Hz, 2H), 6.85 (d, J = 8.0 Hz, 2H), 6.64 (t, J = 2.0 Hz, 2H), 6.43 (dd, J = 6.4, 5.4 Hz, 1H), 6.33 (d, J = 8.8 Hz, 2H), 6.07 (t, J = 2.0 Hz, 2H), 6.65 (dd, J = 8.8, 6.8 Hz, 1H), 3.62 (s, 3 H), 3.45 (t, J = 7.4 Hz, 2 H), 3.31 (dd, J = 13.8, 6.4 Hz, 1 H), 3.15 (dd, J = 13.8, 8.8 Hz, 1H), 2.94 (s, 3H), 2.53 (t, J = 7.6 Hz, 2 H), 1.81 (qn, J = 7.6 Hz, 2H); CIMS *m*/*z* 378 $(M+H)^+$.

5.1.27.2. Step 2: (*S*)-3-{4-[3-(Methyl-pyridin-2-yl-amino)-propyl]-phenyl}-2-pyrrol-1-yl-propionic acid (59). Prepared from ester **51** (0.378 g, 1.001 mmol) by the general method II. Purification by flash chromatography eluting with methanol in chloroform (0% to 15%) gave **59** as a white solid (0.280 g, 77%): mp 66–68 °C; $[\alpha]_D^{25} = -37.7$ (c = 0.53, THF); ^{1}H NMR (CD_3OD, 400 MHz) δ 7.90–7.85 (m, 1H), 7.82 (d, J = 6.0 Hz, 1H), 7.01 (t, J = 8.0 Hz, 3H), 6.94 (d, J = 8.0 Hz, 2H), 6.87 (t, J = 6.8 Hz, 1H), 6.66 (t, J = 2.0 Hz, 2H), 5.96 (t, J = 2.0 Hz, 2H), 4.80 (dd, J = 10.0, 5.6 Hz, 1H), 3.55 (t, J = 7.6 Hz, 2H), 3.34 (dd, J = 14.2, 5.6 Hz, 1 H), 3.18 (dd, J = 14.2, 9.8 Hz, 1H), 3.12 (s, 3 H), 2.62 (t, J = 7.6 Hz, 2H), 1.94 (qn, J = 7.2 Hz, 2H); CIMS *m*/*z* 364 $(M+H)^+$. Chiral HPLC (SFC conditions): chiralcel AS-H, 4.6 mm × 250 mm; mobile phase A: CO_2; mobile phase B: methanol; gradient: 95% A to 40% A in 15 min; flow rate: 4.0 mL/min; injection volume: 15 μL; detection: (DAD 254 nm): t_R = 2.02 min; purity: >99%. Anal. calcd for $C_{22}H_{25}N_3O_2 \cdot 0.3\ CHCl_3$: C, 67.08; H, 6.39; N, 10.52. Found: C, 67.30; H, 6.39; N, 10.21.

5.1.28. (*S*)-3-{4-[3-(Methyl-phenyl-amino)-propyl]-phenyl}-2-pyrrol-1-yl-propionic acid (60)

5.1.28.1. Step 1: (*S*)-3-{4-[3-(Methyl-phenyl-amino)-propyl]-phenyl}-2-pyrrol-1-yl-propionic acid methyl ester (52). Prepared from alkyne **27** (0.648 g, 1.739 mmol) following the general method III. Purification by flash chromatography eluting with hexanes:ethyl acetate (6:1) gave **52** as an oil (0.439 g, 67%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.20 (t, J = 8.0 Hz, 2H), 7.05 (d, J = 7.6 Hz, 2H), 6.92 (d, J = 8.0 Hz, 2H), 6.71 (t, J = 2.0 Hz, 2H), 6.65 (t, J = 7.8 Hz, 3H), 6.14 (t, J = 2.0 Hz, 2H), 4.73 (dd, J = 8.8, 6.4 Hz, 1H), 3.69 (s, 3H), 3.31 (dd, J = 13.9, 6.6 Hz, 1H), 3.15 (dd, J = 13.9, 8.8 Hz, 1H), 3.30 (t, J = 7.4 Hz, 2H), 2.90 (s, 3H), 2.59 (t, J = 7.6 Hz, 2 H), 1.86 (qn, J = 7.6 Hz, 2H); CIMS *m*/*z* 377 $(M+H)^+$.

5.1.28.2. Step 2: (*S*)-3-{4-[3-(Methyl-phenyl-amino)-propyl]-phenyl}-2-pyrrol-1-yl-propionic acid (60). Prepared from ester **52** (0.223 g, 0.592 mmol) following the general method II. Purification by chromatography on silica gel eluting with methanol in chloroform (0% to 5%) containing formic acid (0% to 0.1%) gave pure **60** as a pale brown solid (0.190 g, 88%): $[\alpha]_D^{25} = -59.6$ (c = 0.51, THF); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.33 (m, 2H), 7.12–7.06 (m, 3H), 6.95 (d, J = 8.3 Hz, 2H), 6.91 (d, J = 8.3 Hz, 2H), 6.69 (t, J = 2.0 Hz, 2H), 6.10 (t, J = 2.0 Hz, 2H), 4.73 (dd, J = 9.1, 6.3 Hz, 1 H), 3.39 (dd, J = 13.9, 6.1 Hz, 1H), 3.23 (t, J = 9.0 Hz, 2 H), 3.20 (dd, J = 13.9, 9.3 Hz, 1H), 2.52 (t, J = 7.3 Hz, 2H), 1.88-1.81 (m, 2 H); CIMS *m*/*z* 363 $(M+H)^+$. Chiral HPLC (SFC conditions): chiralpak AS-H, 4.6 mm × 250 mm; mobile phase A: CO_2; mobile phase B: methanol + 0.5% isopropylamine; gradient: 95% A to 40% A in 15 min; flow rate: 4.0 mL/min; injection volume: 15 μL; detection: DAD 254 nm: t_R = 2.16 min; purity: >99%. Anal. calcd for $C_{23}H_{26}N_2O_2 \cdot 0.1\ H_2O$: C, 75.84; H, 7.25; N, 7.69. Found: C, 75.73; H, 7.40; N, 7.50.

5.1.29. (*S*)-3-{4-[2-(5-Methyl-2-phenyl-oxazol-4-yl)-ethylamino]-phenyl}-2-pyrrol-1-yl-propionic acid (66)

5.1.29.1. Step 1: (*S*)-3-(4-Nitro-phenyl)-2-pyrrol-1-yl-propionic acid methyl ester (61). A solution of (*S*)-2-amino-3-(4-nitrophenyl)-propionic acid methyl ester (4.90 g, 22.00 mmol), 2,5-dimethoxytetrahydrofuran (4.8 mL, 37.00 mmol), and sodium acetate (3.40 g, 41.50 mmol) in acetic acid (50 mL) and water (50 mL) was heated at 100 °C for 30 min and then cooled to room temperature and treated with water. The mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic

extracts were dried over $MgSO_4$, filtered, and the solvent was removed. Ester **61** was obtained as an oil and used without further purification (3.0 g, 50%).

5.1.29.2. Step 2: (*S*)-3-(4-Amino-phenyl)-2-pyrrol-1-yl-propionic acid methyl ester (62). A solution of ester **61** (3.00 g, 10.90 mmol) in methanol (30 mL) was hydrogenated over Raneyl nickel. After the reaction was finished, the catalyst was filtered off and the filtrate concentrated to leave a brown residue. The residue was purified by flash chromatography on silica gel eluting with ethyl acetate in hexanes (0 to 20 %). Ester **62** was obtained as a thick oil (0.780 g, 29% in two steps): ^{1}H NMR (DMSO *d*-$_6$, 400 MHz) δ 6.72 (t, J = 2.2 Hz, 2H), 6.67 (d, J = 8.5 Hz, 2H), 6.33 (d, J = 8.5 Hz, 2H), 5.89 (t, J = 2.1 Hz, 2H), 4.91 (dd, J = 9.1, 6.6 Hz, 1H), 4.82 (s, 3H), 3.55 (s, 3H), 3.11 (dd, J = 13.9, 6.6 Hz, 1H), 2.99 (dd, J = 13.9, 9.3 Hz, 1H); CIMS *m*/*z* 245 (M+1).

5.1.29.3. Step 3: (5-Methyl-2-phenyl-oxazol-4-yl)-acetaldehyde (64). Dess–Martin reagent (1.060 g, 2.50 mmol) was dissolved in dichloromethane (10 mL), and 2-(5-methyl-2-phenyl-oxazol-4-yl)-ethanol (0.50 g, 2.50 mmol) was added to the solution. Saturated sodium bicarbonate solution was added to the reaction mixture after 1 h. This was followed by the addition of sodium thiosulfate until no more gas formation was observed. The reaction mixture was extracted with diethyl ether. The phases were separated and the organic extracts were dried over $MgSO_4$, filtered, and the solvent was removed. Aldehyde **64** was obtained as an oil (0.440 g, 87%): ^{1}H NMR (DMSO *d*-$_6$, 400 MHz) δ 9.61 (t, J = 1.7 Hz, 1H), 7.87–7.78 (m, 2H), 7.48–7.40 (m, 3H), 3.65 (s, 2H), 2.29 (s, 3H); CIMS *m*/*z* 202 (M+1).

5.1.29.4. Step 4: (*S*)-3-{4-[2-(5-Methyl-2-phenyl-oxazol-4-yl)-ethylamino]-phenyl}-2-pyrrol-1-yl-propionic acid methyl ester (65). A solution of **62** (0.213 g, 1.060 mmol) and aldehyde **64** (0.285 g, 1.170 mmol) in 1,2-dichloroethane (5 mL) and diethyl ether (5 mL) was cooled in an ice bath. Sodium triacetoxyborohydride (0.337 g, 1.600 mmol) and acetic acid (0.07 mL) were added and the reaction was allowed to warm to room temperature gradually and stirred for 24 h. At this time, it was diluted with diethyl ether (50 mL) and washed with saturated sodium bicarbonate (2 × 30 mL). The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by flash chromatography on silica gel eluting with ethyl acetate in hexanes (0% to 20 %). Ester **65** was obtained as an oil (0.150 g, 35%): ^{1}H NMR (DMSO *d*-$_6$, 400 MHz) δ 7.87–7.84 (m, 2 H), 7.47–7.40 (m, 3 H), 6.74 (d, J = 8.8 Hz, 2H), 6.73 (t, J = 2.2 Hz, 2 H), 6.38 (d, J = 8.5 Hz, 2H), 5.89 (t, J = 2.2 Hz, 2 H), 5.43 (t, J = 5.9 Hz, 1H), 4.91 (dd, J = 9.1, 6.6 Hz, 1H), 3.55 (s, 3H), 3.18 (q, J = 6.3 Hz, 2H), 3.13 (dd, J = 13.9, 6.6 Hz, 1H), 3.01 (dd, J = 13.9, 9.0 Hz, 1 H), 2.61 (t, J = 6.9 Hz, 2H), 2.18 (s, 3H); CIMS *m*/*z* 430 (M+1).

5.1.29.5. Step 5: (*S*)-3-{4-[2-(5-Methyl-2-phenyl-oxazol-4-yl)-ethylamino]-phenyl}-2-pyrrol-1-yl-propionic acid (66). Prepared from ester **65** (0.150 g, 0.350 mmol) following the general procedure II. Acid **66** was obtained as a beige solid (0.117 g, 78%): mp 85.1–87.4 °C; $[\alpha]_D^{25} = -31.7$ (c = 0.53, MeOH); ^{1}H NMR (DMSO *d*-$_6$, 400 MHz) δ 7.87–7.84 (m, 2 H), 7.47–7.40 (m, 3 H), 6.76 (d, J = 8.3 Hz, 2H), 6.70 (t, J = 2.2 Hz, 2 H), 6.40 (d, J = 7.8 Hz, 2H), 5.87 (t, J = 2.1 Hz, 2 H), 4.77 (dd, J = 9.1, 6.3 Hz, 1H), 3.25–3.10 (m, 3H), 2.98 (dd, J = 13.9, 9.3 Hz, 1H), 2.61 (t, J = 6.9 Hz, 2H), 2.19 (s, 3 H); CIMS *m*/*z* 416 (M+1). Chiral HPLC (SFC conditions): chiralcel OJ-H, 4.6 mm × 250 mm; mobile phase A: CO_2; mobile phase B: 50:50 methanol:ethanol; gradient: 95% A to 40% A in 15 min; flow rate: 4.0 mL/min; injection volume: 15 μL; detection: (DAD 254 nm): t_R = 2.85 min; purity: 96.60%. Anal. calcd for $C_{25}H_{25}N_3O_3 \cdot 0.3$ HCl: C, 70.42; H, 5.98; N, 9.85. Found: C, 70.36; H, 5.97; N, 9.65.

5.1.30. (*S*)-3-(4-{[(5-Methyl-2-phenyl-oxazol-4-ylmethyl)-amino]-methyl}-phenyl)-2-pyrrol-1-yl-propionic acid (74)

5.1.30.1. Step 1: (*S*)-2-Pyrrol-1-yl-3-(4-formyl-phenyl)-propionic acid methyl ester (67). A mixture of triflate **14** (3.0 g, 7.950 mmol), $Pd(OAc)_2$ (0.065 g, 0.289 mmol), dppp (0.122 g, 0.295 mmol), triethylamine (0.92 mL, 6.60 mmol), trioctyl silane (2.38 mL, 5.298 mmol) in DMF (45 mL) was stirred and heated at 70 °C under 500 psi of carbon monoxide atmosphere for 14 h. The solvent was removed and the residue was diluted with diethyl ether (50 mL) and washed with water (70 mL). The aqueous phase was extracted with diethyl ether (4 × 50 mL). The combined extracts were dried over magnesium sulfate, filtered, and the solvent was removed. The residue was purified by flash chromatography on silica gel eluting with ethyl acetate in hexanes (0 to 25 %). Ester **67** was obtained as an orange solid (1.110 g, 54%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.95 (s, 1 H), 7.74 (d, J = 8.3 Hz, 2H), 7.15 (d, J = 8.1 Hz, 2H), 6.67 (t, J = 2.2 Hz, 2H), 6.14 (t, J = 2.2 Hz, 2H), 4.76 (dd, J = 9.4, 6.0 Hz, 1H), 3.72 (s, 3H), 3.47 (dd, J = 14.0, 6.0 Hz, 1H), 3.34 (dd, J = 14.0, 9.4 Hz, 1 H); CIMS *m*/*z* 258 (M+1).

5.1.30.2. Step 2: (5-Methyl-2-phenyl-oxazol-4-ylmethyl)-carbamic acid tert-butyl ester (69). Acid **68** (3.0 g, 13.8 mmol) was dissolved in dichloromethane (70 mL) under a nitrogen atmosphere. The mixture was cooled in an ice bath and then oxalyl chloride (1.7 mL, 19.32 mmol) was added, followed by DMF (0.053 mL, 0.69 mmol). The ice bath was removed after 20 min and the reaction mixture was stirred at room temperature for 5 h. The solvent was removed. The residue was dissolved in acetone and cooled at 0 °C. A solution of sodium azide (1.19 g, 18.354 mmol) in water (4.2 mL) was added. The mixture was stirred at 0 °C for 1 h, then it was diluted with water, and extracted with toluene (4 × 35 mL). The combined extracts were dried over magnesium sulfate, filtered, and the solvent was removed. The residue was dissolved in toluene (80 mL) and *tert*-butyl alcohol (2.64 mL, 27.6 mmol) was added. The solution was heated at reflux for 18 h. The solvents were removed and the residue was purified by flash chromatography on silica gel eluting with ethyl acetate in hexanes (0 to 25 %). Carbamate **69** was obtained as a solid (2.330 g, 58%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.96 (d, J = 7.8 Hz, 2H), 7.45 (m, 3H), 5.09 (br s, 1H), 4.18

(d, J = 5.4 Hz, 2H), 2.39 (s, 3H), 1.43 (s, 9H); CIMS m/z 289 (M+1).

5.1.30.3. Step 3: (*S*)-3-(4-{[(5-Methyl-2-phenyl-oxazol-4-ylmethyl)-amino]-methyl}-phenyl)-2-pyrrol-1-yl-propionic acid methyl ester (71). Carbamate **69** (0.326 g, 1.130 mmol) was dissolved in dichloromethane (6 mL) and cooled in an ice bath. Trifluoroacetic acid (3 mL) was added and the mixture was stirred for 10 min and the ice bath was removed. The reaction mixture was stirred at room temperature for 3 h. The solvents were removed and the residue dried under high vacuum. The residue was then dissolved in 1,2-dichloroethane (15 mL) and triethylamine (0.39 mL, 2.825 mmol) was added. After 5 min, aldehyde **67** (0.215 g, 0.837 mmol) was added, followed by sodium triacetoxyborohydride (0.283 g, 1.339 mmol) after 10 min. The mixture was stirred at room temperature for 18 h. Additional sodium triacetoxyborohydride (0.300 g) was added and stirring continued for 5 h. The mixture was diluted with dichloromethane and filtered through a celite pad. Saturated $NaHCO_3$ was added and the phases were separated. The aqueous phase was extracted with dichloromethane (3×25 mL). The combined extracts were dried over magnesium sulfate, filtered, and the solvent was removed. The solvents were removed and the residue was purified by flash chromatography on silica gel eluting with methanol in chloroform (0 to 5%). Ester **71** was obtained as a thick oil (0.236 g, 66%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.98 (d, J = 8.1 Hz, 2H), 7.45–7.39 (m, 3H), 7.21 (d, J = 8.1 Hz, 2 H), 6.97 (d, J = 8.1 Hz, 2H), 6.70 (t, J = 2.2 Hz, 2 H), 6.13 (t, J = 2.2 Hz, 2H), 4.72 (dd, J = 8.8, 6.3 Hz, 1H), 3.76 (s, 2H), 3.69 (s, 3H), 3.65 (s, 2H), 3.40 (dd, J = 13.9, 6.6 Hz, 1H), 3.23 (dd, J = 13.9, 8.8 Hz, 1H), 2.29 (s, 3H); CIMS m/z 430 (M+1).

5.1.30.4. Step 4: (*S*)-3-(4-{[(5-Methyl-2-phenyl-oxazol-4-ylmethyl)-amino]-methyl}-phenyl)-2-pyrrol-1-yl-propionic acid (74). Prepared from ester **71** following the general method II. Purification by flash chromatography on silica gel eluting with methanol in chloroform (0% to 5%) gave **74** as an off-white solid (0.168 g, 74%): mp 163–165 °C; $[\alpha]_D^{25} = -34.7$ (c = 0.53, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.88 (d, J = 6.5 Hz, 2H), 7.40–7.39 (m, 3H), 7.16 (d, J = 7.8 Hz, 2H), 6.94 (d, J = 7.8 Hz, 2H), 6.35 (s, 2H), 5.86 (s, 2H), 4.32 (m, 1H), 3.87 (dt, J = 12.8, 10.2 Hz, 2H), 3.75 (dt, J = 13.9, 5.4 Hz, 2H), 3.42 (dd, J = 13.7, 5.2 Hz, 1H), 3.05 (dd, J = 13.3, 9.7 Hz, 1H), 2.23 (s, 3H); CIMS m/z 416 (M+1). Chiral HPLC (SFC conditions): chiralpak AS-H, 4.6 mm × 250 mm; mobile phase A: CO_2; mobile phase B: methanol + 0.5% isopropylamine; gradient: 95% A to 40% A in 15 min; flow rate: 4.0 mL/min; injection volume: 15 μL; detection: DAD 254 nm: t_R = 2.29 min; purity: >99%. Anal. calcd for $C_{25}H_{25}N_3O_3 \cdot 0.42\ CHCl_3$: C, 65.57; H, 5.50; N, 9.02. Found: C, 65.26; H, 5.81; N, 8.86.

5.1.31. 3-(4-{[Methyl-(5-methyl-2-phenyl-oxazol-4-ylmethyl)-amino]-methyl}-phenyl)-2-pyrrol-1-yl-propionic acid (75)

5.1.31.1. Step 1: 3-(4-{[Methyl-(5-methyl-2-phenyl-oxazol-4-ylmethyl)-amino]-methyl}-phenyl)-2-pyrrol-1-yl-propionic acid methyl ester (72). Amine **71** (0.432 g, 1.006 mmol) was dissolved in 1,2-dichloroethane (10 mL) and a 37% formaldehyde solution (0.090 mL, 1.106 mmol) was added, followed by sodium triacetoxyborohydride (0.300 g, 1.408 mmol). The mixture was stirred at room temperature for 24 h under a nitrogen atmosphere. Saturated $NaHCO_3$ (30 mL) was added and the mixture was stirred for 20 min. At this time, it was diluted with dichloromethane and the phases were separated. The aqueous phase was extracted with dichloromethane (3×30 mL). The combined organic extracts were dried over magnesium sulfate, filtered, and the solvent was removed. The solvents were removed and the residue was purified by flash chromatography on silica gel eluting with methanol in chloroform (0% to 3%). Ester **72** was obtained as a thick pale yellow oil (0.417 g, 93%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.00 (d, J = 8.0 Hz, 2H), 7.45–7.41 (m, 3H), 7.25 (s, 2H), 6.98 (d, J = 7.6 Hz, 2H), 6.96 (t, J = 2.2 Hz, 2H), 6.12 (t, J = 2.2 Hz, 2H), 4.72 (dd, J = 8.8, 6.6 Hz, 1H), 3.68 (s, 3 H), 3.50 (br s, 2H), 3.40 (dd, J = 13.8, 6.6 Hz, 1H), 3.23 (dd, J = 13.8, 8.8 Hz, 1H), 2.33 (s, 3H), 2.26 (br s, 2H), 1.58 (br s, 3H); CIMS m/z 444 (M+1).

5.1.31.2. Step 2: 3-(4-{[Methyl-(5-methyl-2-phenyl-oxazol-4-ylmethyl)-amino]-methyl}-phenyl)-2-pyrrol-1-yl-propionic acid (75). Prepared from ester **72** following the general method II. The purification was carried out by flash chromatography on silica gel eluting with methanol in chloroform (0 to 10%) to afford **75** as a solid (0.353 g, 89%): mp 133–135 °C; $[\alpha]_D^{25} = +24.6$ (c = 0.52, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.99–7.95 (m, 2H), 7.50 (d, J = 8.1 Hz, 2H), 7.47–7.44 (m, 3H), 7.16 (d, J = 8.1 Hz, 2H), 6.71 (t, J = 2.2 Hz, 2H), 6.08 (t, J = 2.2 Hz, 2H), 4.77 (t, J = 7.6 Hz, 1H), 4.25 (dt, J = 12.7, 4.9 Hz, 2H), 4.05 (s, 2H), 3.47 (dd, J = 13.8, 7.7 Hz, 1H), 3.24 (dd, J = 13.7, 7.3 Hz, 1 H), 2.65 (s, 3H), 2.31 (s, 3H); CIMS m/z 430 (M+1). Chiral HPLC (SFC conditions): chiralpak AS-H, 4.6 mm × 250 mm; mobile phase A: CO_2; mobile phase B: methanol + 0.5% isopropylamine; gradient: 95% A to 40% A in 15 min; flow rate: 4.0 mL/min; injection volume: 15 μL; detection: DAD 254 nm: t_R = 1.94 min; purity: >99%. Anal. calcd for $C_{26}H_{27}N_3O_3 \cdot 1.71\ H_2O$: C, 67.84; H, 6.66; N, 9.13. Found: C, 67.85; H, 6.39; N, 8.73.

5.1.32. (*S*)-3-(4-{[Acetyl-(5-methyl-2-phenyl-oxazol-4-ylmethyl)-amino]-methyl}-phenyl)-2-pyrrol-1-yl-propionic acid (76)

5.1.32.1. Step 1: (*S*)-3-(4-{[Acetyl-(5-methyl-2-phenyl-oxazol-4-ylmethyl)-amino]-methyl}-phenyl)-2-pyrrol-1-yl-propionic acid methyl ester (73). Amine **71** (0.245 g, 0.570 mmol) was dissolved in dichloromethane (10 mL) and cooled in an ice bath. Pyridine (0.115 mL, 1.425 mmol) was added followed by acetic anhydride (0.107 mL, 1.14 mmol). The ice bath was removed after 30 min and the mixture stirred at room temperature for 20 h. Dichloromethane (50 mL) was added and the solution was washed with 10% HCl, saturated $NaHCO_3$, and brine. The organic phase was dried over magnesium sulfate, filtered, and the solvent was removed. The solvents were removed and the residue was dried under high vacuum overnight to give **73** as a pale yellow oil (0.295 g, 100%): ^{1}H NMR ($CDCl_3$, 400 MHz) major rotamer δ 8.04–7.95 (m, 2H), 7.46–7.42 (m, 3H), 7.10 (dd,

J = 7.9, 5.4 Hz, 2H), 6.98 (d, J = 8.0 Hz, 1 H), 6.93 (d, J = 8.0 Hz, 1 H), 6.68 (t, J = 2.0 Hz, 2 H), 6.13 (t, J = 2.2 Hz, 2 H), 4.74 (s, 2 H), 4.72–4.68 (m, 1 H), 4.43 (s, 2 H), 3.70 (s, 3 H), 3.39 (dd, J = 13.9, 6.1 Hz, 1 H), 3.21 (dd, J = 13.9, 8.5 Hz, 1 H), 2.94 (s, 3 H), 2.87 (s, 3 H); CIMS m/z 472 (M+1).

5.1.32.2. Step 2: (*S*)-3-(4-{[Acetyl-(5-methyl-2-phenyl-oxazol-4-ylmethyl)-amino]-methyl}-phenyl)-2-pyrrol-1-yl-propionic acid (76). Prepared from ester **73** (0.268 g, 0.570 mmol) following the general method II. Compound **76** was obtained as a solid (0.241 g, 92%): mp 97–100 °C; $[\alpha]_D^{25} = -43.1$ (c = 0.51, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) major rotamer δ 8.01–7.94 (m, 2 H), 7.45–7.42 (m, 3 H), 7.08 (dd, J = 8.0, 2.2 Hz, 2 H), 6.99 (d, J = 8.0 Hz, 1 H), 6.93 (d, J = 8.0 Hz, 1 H), 6.69 (t, J = 2.0 Hz, 2 H), 6.13 (t, J = 2.1 Hz, 2 H), 4.73–4.66 (m, 1 H), 4.70 (s, 2 H), 4.44 (s, 2 H), 3.39 (dd, J = 14.7, 6.0 Hz, 1 H), 3.23–3.17 (m, 1 H), 2.36 (s, 3 H), 2.09 (s, 3 H); CIMS m/z 458.2 (M+1). Chiral HPLC (SFC conditions): chiralpak AS-H, 4.6 mm × 250 mm; mobile phase A: CO_2; mobile phase B: methanol + 0.5% isopropylamine; gradient: 95% A to 40% A in 15 min; flow rate: 4.0 mL/min; injection volume: 15 μL; detection: DAD 254 nm: t_R = 2.50 min; purity: > 99%. Anal. calcd for $C_{27}H_{27}N_3O_4 \cdot 0.5\ H_2O$: C, 69.51; H, 6.05; N, 9.01. Found: C, 69.32; H, 6.02; N, 8.73.

5.2. General biology

The PPARα scintillation proximity assay was carried out as reported elsewhere.[55]

5.2.1. PPAR-GAL4 chimeric transactivation assays. HepG2 cells (obtained from ATCC) were plated at 3×10^6 cells in 10 mL on 100-mm plates in Minimum Essential Medium (Eagle) supplemented with Earle's balanced salt solution, non-essential amino acids, 1 mM sodium pyruvate, 2 mM L-glutamine, 1500 mg sodium bicarbonate/L, and 10% fetal calf serum and incubated for 24 h at 37 °C with 5% CO_2. Each 100-mm plate was transiently co-transfected with 15 μg pCMV β-galactosidase control vector and 15 μg luciferase reporter plasmid pG5E1bLuc, and 15 μg of a Gal4-binding domain construct fused to either the PPARγ-LBD or the PPARα-LBD. Cells were transfected using Opti-MEM (Invitrogen) and Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. After 24 h, cells were pooled and plated in 96 well plates at a cell density of 3.4×10^5 cells/mL (150 μL/well), and dosed in duplicate with compounds in DMSO (0.5% final concentration). After 24 h of exposure, luciferase activity was measured using the Dual Light Luciferase and a β-galactosidase kit (Applied Biosystems) following the manufacturer's instructions. Detection was performed on a MicroLumat Plus LB 96 V 96 well plate reader (Berthold Technologies, Oak Ridge, TN). PPAR activation was calculated as fold activity over vehicle control by deriving the ratio of luciferase to β-gal for each data point divided by the wells containing vehicle alone. All experiments were repeated twice and the geometric mean was calculated for each EC_{50}.

5.2.2. PPAR Gal4 assays with rat PPARα and mouse PPARγ. HepG2 cells were transfected under standard conditions with 50 ng 5× Gal4RE-luciferase, 40 ng of Gal4:PPAR LBD, and 25 ng of CMV β-gal using Fugene 6. Transfected cells were treated with compounds for 18–24 h, lysed and luciferase and β-gal assays performed (Promega) using a Dynex luminometer and Molecular Devices Plate reader. Luciferase values were corrected for transfection efficiency using β-gal. Normalized luciferase values were plotted against dose and EC_{50} values were determined using GraphPad Prism.

5.2.3. PPARγ scintillation proximity assay. Scintillation proximity (SPA) assay buffer consisting of calcium and magnesium free PBS, 9% glycerol, 12 mM β-mercaptoethanol, 0.00018% Tween-20, and 34 nM ^{3}H-Darglitazone was added to 96-well clear bottom plates. 1:3 serial dilution curves of test compounds were added to the assay buffer along with total and non-specific binding controls of DMSO and 100 μM Rosiglitazone, respectively. Human 6His-PPARγ ligand-binding domain (hPPARγ LBD, amino acid sequence 207–477) peptide was then added to the mixture, followed by 0.7 mg yttrium silicate polylysine SPA beads (Amersham). The plates were sealed with press-on adhesive sealer and covered with aluminum foil. The plates were incubated at room temperature while shaking at 700 rpm on an IKA-Schuttler MST 4 titer plate shaker for one hour. After 30 min of settling, radioligand displacement was measured using a Wallac scintillation counter (Wallac Trilux 1450 Microbeta Liquid Scintillation and Luminescence counter). Ligand binding was calculated as percent displacement of total radioligand binding (DMSO control). The binding signal (cpm) in duplicates was plotted as a function of compound concentrations (M) and the plot was fitted to an equation by non-linear regression and IC_{50} derived from those plots using Spotfire. All experiments were repeated twice and the geometric mean was calculated for each IC_{50}.

5.2.4. In vivo *ob*/*ob* mouse studies. Male *ob*/*ob* (B6.V-*Lep*^{ob}/*J*) mice were obtained from Jackson Laboratories (Bar Harbor, ME). The *ob*/*ob* mouse normally presents with transient hyperglycemia, hyperinsulinemia, and hypertriglyceridemia. Mice were pair housed under a 12-h light/dark cycle with free access to water and standard Purina 5001 rodent chow (Purina Mills, Richmond, IN). Studies were initiated when 4-h fasted blood glucose obtained from tail venipuncture in non-anesthetized mice reached >300 mg/dL (HemoCue B-Glucose Monitors, Ryan Diagnostics). Mice were administered a once daily oral dose for 14 days with suspensions of vehicle alone (1.5% carboxymethyl-cellulose, 0.2% Tween 20), or vehicle plus test compound at the specified dose. A final cardiac puncture post CO_2 inhalation provided blood sample for insulin, triglycerides, and cholesterol measurements. Insulin was determined by ELISA (Alpco, Inc.) and lipids determined enzymatically (Wako, Richmond) on a Cobas Mira Analyzer (Roche).

5.2.5. In vivo ZDF rat studies. Male Zucker Diabetic Fatty (ZDF/Crl-*Lepr*^{fa}) rats were obtained from Charles River Laboratories (Wilmington, MA).[44] The ZDF

male rat normally presents with NIDDM, transient hyperinsulinemia, and hypertriglyceridemia. Rats were pair housed under a 12-h light/dark cycle with free access to water and Purina 5008 rat chow (Protein 26.8%, fat 16.7%, carbohydrates 56.5% kcal/vol; Purina Mills, Richmond, IN). Prior to the onset of diabetic hyperglycemia (approximately 6 weeks of age, fed blood glucose <200 mg/dL), rats were allocated into groups by following a post-prandial, conscious tail venipuncture. Tail venipuncture in non-anesthetized, post-prandial animals was performed weekly to determine blood glucose, insulin, triglycerides, cholesterol, and free fatty acid measurements. Insulin and lipids were analyzed as above. Rats were administered a once daily oral dose for 4 weeks with suspensions of vehicle alone (1.5% carboxymethyl-cellulose, 0.2% Tween 20), or vehicle plus test compound at the specified dose. All experiments utilizing animals were reviewed and approved by Pfizer's Institutional Animal Care and Use Committee.

5.3. Pharmacokinetic studies

Overnight fasted male Sprague–Dawley rats ($n = 3$/group) were either administered a single dose as a 5 min intravenous infusion or a single oral dose by gavage in a parallel design. Serial blood samples (for plasma) were collected from each rat over a 24 h period after dosing. The plasma samples were analyzed for drug concentrations using a LC/MS/MS method. Pharmacokinetic parameters were determined from the plasma concentration-time data using non-compartmental methods.

5.4. X-ray crystallography

A truncated construct of human PPARγ ligand-binding domain (PPARγ-LBD) containing residues Glu207 to Tyr477 was recombinantly expressed in *Escherichia coli* and purified as previously described.[42] A 12 mg/mL solution of apo human PPARγ-LBD was crystallized at 13 °C using the hanging drop vapor diffusion method over a 0.6 mL well solution consisting of 0.6–0.8 M trisodium citrate, 0.1 M imidazole, pH 8, and 1 mM TCEP. Crystals were soaked overnight in a 0.8 mM solution of **53** at room temperature prior to flash-cooling in 25% glycerol. X-ray diffraction data were collected at a wavelength of 1.00 Å at 100 K on the Industrial Macromolecular Crystallographer Association (IMCA) beamline 17-ID at the Advanced Photon Source, Argonne National Labs. Diffraction data were processed using Denzo and Scalepack in the HKL2000 program suite.[56] The space group was determined to be centered monoclinic C2 with two molecules per asymmetric unit, corresponding to a solvent content of 52.2%.[57] The structure was determined by the method of molecular replacement using a monomer of human PPARγ-LBD (1I7I) with the ligand and waters removed as a search model with the program Molrep in the CCP4i suite.[25,58–60] Structural refinement calculations and electron density maps were calculated with the program Refmac5 in the CCP4i suite using the complete data with no resolution or sigma cutoff.[60,61] Manual fitting and real space refinement of the model was performed with the program QUANTA-2000 (Accelrys Inc., San Diego, CA). The final model of PPARγ-LBD consists of two monomers of PPARγ-LBD, 1 molecule of **53**, and 360 water molecules with the refinement statistics, $R_{work} = 21.3\%$, $R_{free} = 27.1\%$ (See Supplementary material). The coordinates and structure factors may be found in the Protein Data Bank under ID code 2Q8S.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.03.043.

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