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Rapid Synthesis of Boc-2′,6′-dimethyl-L-tyrosine and Derivatives and Incorporation into Opioid Peptidomimetics

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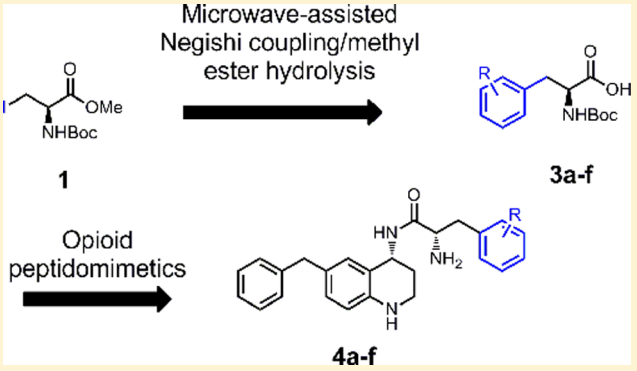
Supporting Information

ABSTRACT: The unnatural amino acid 2′,6′-dimethyl-L-tyrosine has found widespread use in the development of synthetic opioid ligands. Opioids featuring this residue at the N-terminus often display superior potency at one or more of the opioid receptor types, but the availability of the compound is hampered by its cost and difficult synthesis. We report here a short, three-step synthesis of Boc-2′,6′-dimethyl-L-tyrosine (**3a**) utilizing a microwave-assisted Negishi coupling for the key carbon−carbon bond forming step, and employ this chemistry for the expedient synthesis of other unnatural tyrosine derivatives. Three of these derivatives (**3c**, **3d**, **3f**) have not previously been examined as Tyr[1] replacements in opioid ligands. We describe the incorporation of these tyrosine derivatives in a series of opioid peptidomimetics employing our previously reported tetrahydroquinoline (THQ) scaffold, and the binding and relative efficacy of each of the analogues at the three opioid receptor subtypes: mu (MOR), delta (DOR), and kappa (KOR).



KEYWORDS: *Negishi coupling, microwave synthesis, opioid peptidomimetics, tetrahydroquinoline, 2′,6′-dimethyl-L-tyrosine*

The unnatural amino acid 2′,6′-dimethyl-L-tyrosine (Dmt)[1] has found widespread use in the synthesis of opioid peptides and small molecules.[2−5] Typically, opioid ligands containing Dmt in place of tyrosine (Tyr) at the N-terminus display increased affinity for the mu opioid receptor (MOR),[6−8] and many Dmt-containing ligands reported in the literature are potent and efficacious analgesics in preclinical pain models.[9,10] Additionally, Dmt is a component of Dmt-Tic, a delta opioid receptor (DOR) antagonist pharmacophore that is incorporated in many biologically active compounds.[11] Dmt is also an important building block for the synthesis of the mixed mu-delta opioid ligand Eluxadoline, a small molecule opioid recently approved for the treatment of irritable bowel syndrome.[12,13] Moreover, it has recently been shown that small, Dmt-conjugated peptides can be taken up by plant host cells and help mitigate oxidative stress.[14] Other studies with peptides containing this amino acid have also highlighted the antioxidant properties of Dmt.[15] The increasingly common use of this unnatural amino acid in peptide chemistry has rendered the availability of this compound extremely valuable for medicinal chemists.

Several synthetic routes to Dmt have previously been published. In one such synthesis, the key step for installing the desired L stereochemistry is the asymmetric hydrogenation of (*Z*)-2-acetamido-3-(4-acetoxy-2,6-dimethylphenyl)-2-propenoate, using the expensive chiral catalyst [Rh(1,5-COD)(*R*,*R*-DIPAMP)]BF_4.[16] Other strategies involve the alkylation of a Ni(II) complex of the chiral Schiff base derived from glycine and (*S*)-*o*-[*N*-(*N*-benzylprolyl)amino]benzophenone[17] and a stereocontrolled alkylation of a chiral 2,5-diketopiperazine synthon.[18] Although these routes are synthetically viable, we sought to develop a shorter and more direct approach for the expedient synthesis of Dmt and other novel unnatural Tyr derivatives. Additionally, we were interested in developing a synthesis in which the desired L stereochemistry is incorporated from the beginning and does not need to be installed with the use of a chiral auxiliary or catalyst.

Jackson and colleagues have disclosed that the use of $Pd_2(dba)_3$ and SPhos in a 1:2 molar ratio is a highly efficient precatalyst for the Negishi coupling of aryl halides with an organozinc reagent derived from iodoalanine intermediate **1**.[19] This strategy was shown to be effective for both aryl iodides

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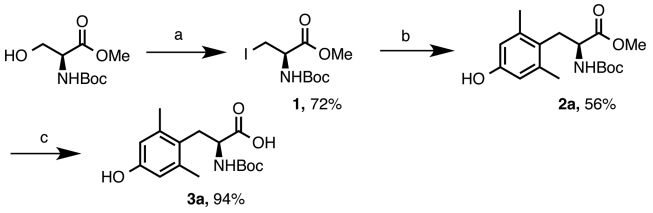
and bromides, as well as aryl halides featuring unprotected phenols and ortho substitutions. Given the synthetic utility of this approach, we reasoned that a Negishi coupling between **1** and commercially available 3,5-dimethyl-4-iodophenol was a feasible approach toward the synthesis of Dmt.

Iodoalanine intermediate **1** was synthesized under Appel conditions as previously reported starting from commercially available Boc-protected L-serine methyl ester (Boc-Ser-OMe) (Scheme 1).[20] With intermediate **1** in hand, we sought to determine the optimal conditions for the Negishi coupling with 3,5-dimethyl-4-iodophenol. Jackson and colleagues observed that the best yields for the coupling of mono-*ortho*-substituted aryl halides with **1** were obtained by using 2.5 mol % of $Pd_2(dba)_3$ and 5 mol % of SPhos with stirring at room temperature overnight. For the coupling between **1** and 3,5-dimethyl-4-iodophenol, these conditions led to the formation of desired product **2a** in 16% yield. We reasoned that the additional steric hindrance of our system contributed to the observed low yield and sought to determine a more efficient approach.

The use of microwave-assisted synthesis has been shown to be highly effective for challenging Negishi cross-coupling reactions,[21,22] and we next ran the reaction under microwave irradiation at 110 °C (Discover S-class (CEM) microwave reactor in a closed vessel with a maximum power input of 300 W) for 2 h to give **2a** in 34% yield. Increasing the mol % of $Pd_2(dba)_3$ and SPhos to 5% and 10%, respectively, under these conditions gave **2a** in 56% yield. Subsequent methyl ester hydrolysis gave Boc-2′,6′-dimethyl-L-tyrosine **3a**. We then turned our attention to using the microwave-assisted Negishi cross coupling reaction for the synthesis of other unnatural tyrosine and phenylalanine derivatives (Scheme 2).

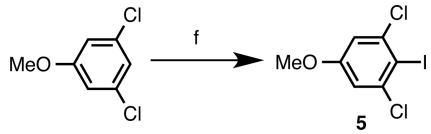
Scheme 1. Synthesis of Boc-2′,6′-dimethyl-L-tyrosine[a]



[a]Reagents and conditions: (a) I_2, PPh_3, imidazole, DCM, r.t.; (b) Zn dust, catalytic I_2, 3,5-dimethyl-4-iodophenol, $Pd_2(dba)_3$, SPhos, DMF, 110 °C, microwave irradiation; (c) LiOH, THF, H_2O, r.t.

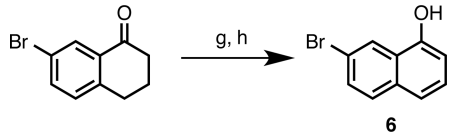
2′-Methyl-L-tyrosine (Mmt), analogue **3b**, has been previously reported in synthetic endomorphin[23] and DALDA-based[24] peptides and showed comparable binding affinity at MOR relative to the Dmt counterpart compounds. 2′,6′-Dimethyl-L-phenylalanine (Dmp), analogue **3e**, has also been incorporated into the endomorphin scaffold and has been shown to improve binding affinity at MOR and DOR compared to the naturally occurring endomorphins when substituted at the third position.[25] Additionally, phenylalanine and derivatives can sometimes serve as suitable replacements for the N-terminal tyrosine in opioid peptides, while still maintaining biological activity.[26,27] To our knowledge, compounds **3c**, **3d**, and **3f** have not been examined as Tyr replacements in opioid ligands. The synthesis of all analogues using the microwave-assisted Negishi coupling proved straightforward. Most yields were comparable to that of **2a**, although in the case of analogue **2d**, the observed lower yield of 16% was likely a result of the additional halogen substitutions on the aromatic ring in combination with the steric hindrance of the system. In the case of analogue **2d**, aryl iodide **5** was synthesized from 3,5-dichloroanisole as previously described (Scheme 3).[28] In the case of analogue **2f**, aryl bromide **6** was synthesized via halogenation and aromatization of commercially available 7-bromo-3,4-dihydronaphthalen-1(2*H*)-one (Scheme 4). After

Scheme 3. Synthesis of Aryl Iodide 5[a]



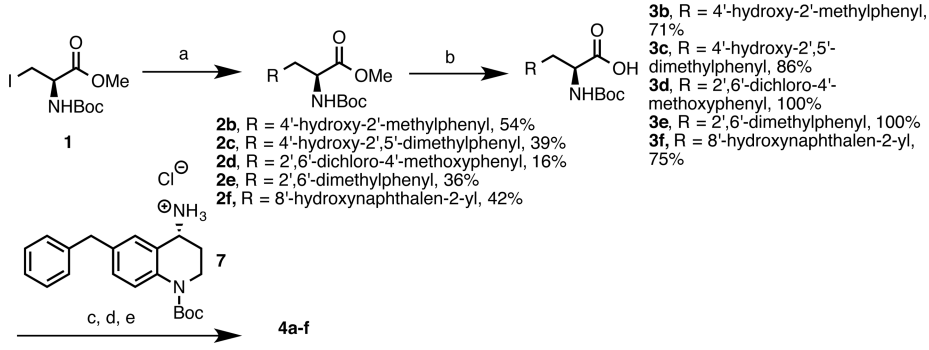
[a]Reagents and conditions: (f) I_2, Ag_2SO_4, MeCN, r.t.

Scheme 4. Synthesis of Aryl Bromide 6[a]



[a]Reagents and conditions: (g) NBS, CCl_4, reflux; (h) LiBr, Li_2CO_3, DMF, 140 °C

Scheme 2. Synthesis of Analogues 4a–f[a]



[a]Reagents and conditions: (a) Zn dust, catalytic I_2, aryl iodide or aryl bromide, $Pd_2(dba)_3$, SPhos, DMF, 110 °C, microwave irradiation; (b) LiOH, THF, H_2O, r.t.; (c) 7, PyBOP, 6-Cl-HOBt, DIPEA, DMF, r.t.; (d) TFA, DCM, r.t.; (e) BBr_3, DCM, r.t. (for intermediate **3d** only).

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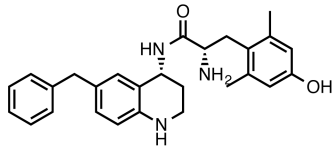
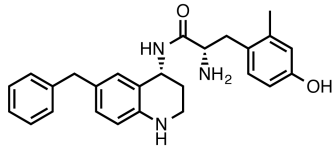
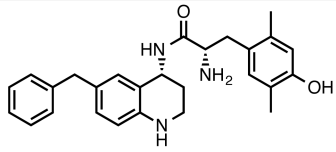
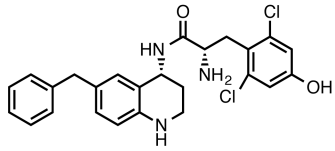
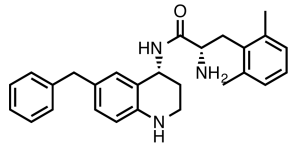
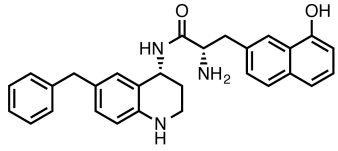
Table 1. Opioid Receptor Binding Affinities of Analogues 4a−f[a]

Compound	Structure	Binding, K_i (nM) MOR	DOR	KOR
4a		0.22±0.02[b]	9.4±0.8[b]	68±2[b]
4b		1.7±0.2	42±9	96±20
4c		6.5±2	390±120	730±80
4d		0.47±0.04	37±8	35±5
4e		18±4	660±120	130±50
4f		440±70	1600±120	2500±450

[a]Binding affinities (K_i) were obtained by competitive displacement of radiolabeled [^{3}H]diprenorphine in membrane preparations expressing MOR, DOR, or KOR. All values are expressed as the mean ± SEM of three separate assays performed in duplicate. [b]Data from ref 9.

LiOH-mediated methyl ester hydrolysis, all analogues were coupled to chiral amine salt **7**[29] under standard amide coupling conditions (Scheme 2) to give final tetrahydroquinolines **4a−f** after Boc-deprotection, and in the case of **4d**, after an additional deprotection of the aryl methoxy group with BBr_3 (Scheme 2). Chiral HPLC analysis indicated high enantiomeric ratios for derivatives **3a−f**, and no significant racemization of final compounds was observed by HPLC during the coupling of **7** to the Boc-protected amino acid derivatives. Final analogues were then purified by semipreparative RP-HPLC and lyophilized to give enough material for in vitro testing.

Binding affinities for compounds **4a−f** (K_i) were measured by the competitive displacement of radiolabeled [^{3}H]-diprenorphine (a nonselective opioid antagonist) in C6 cells stably expressing MOR or DOR, or Chinese Hamster Ovary (CHO) cells stably expressing KOR (Table 1). EC_{50} and % stimulation data were obtained by agonist-stimulated [^{35}S]-GTPγS binding in the same cell types (Table 2).[30,31]

As seen in Table 1, MOR binding affinity is reduced by approximately an order of magnitude for analogues **4b** and **4c** in which the 2′-methyl group is maintained, and the second aryl methyl is either deleted (**4b**) or moved to the 5′ position (**4c**). MOR affinity for 2′,6′-dichloro analogue **4d** is comparable to

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Table 2. Opioid Receptor Potencies and Relative Efficacies of Analogues 4a−f[a]

	EC_{50} (nM)			% stimulation		
compd	MOR	DOR	KOR	MOR	DOR	KOR
4a	1.6 ± 0.3[b]	110 ± 6[b]	540 ± 72[b]	81 ± 2[b]	16 ± 2[b]	22 ± 2[b]
4b	44 ± 20	dns	2400 ± 80	69 ± 6	dns	41 ± 8
4c	39 ± 8	5900 ± 2200	370 ± 200	89 ± 8	53 ± 8	22 ± 6
4d	33 ± 4	dns	1200 ± 290	83 ± 3	dns	32 ± 4
4e	280 ± 50	dns	4800 ± 1100	86 ± 8	dns	38 ± 5
4f	nt	nt	nt	nt	nt	nt

[a]Efficacy data were obtained using agonist induced stimulation of [^{35}S]GTPγS binding in membrane preparations expressing MOR, DOR or KOR. Efficacy is represented as EC_{50} (nM) and percent maximal stimulation relative to standard agonist DAMGO (MOR), DPDPE (DOR), or U69,593 (KOR) at 10 μM. All values are expressed as the mean ± SEM of three separate assays performed in duplicate. dns = does not stimulate; nt = not tested, due to low binding affinity (Table 1). [b]Data from ref 9.

the parent compound **4a**, which is not entirely surprising given the similar size of the methyl and chloro substituents. Analogues **4e** and **4f** display a more pronounced decrease in MOR binding, and analogues **4c**, **4e**, and **4f** lose significant binding affinity at DOR. The data in Table 2 show that analogues **4b**−**e** all maintain a high level of agonist efficacy (as measured by [^{35}S]GTPγS binding) compared to DAMGO at MOR, but with reduced potency as compared to **4a**. The 2′,5′-dimethyl analogue **4c** displays reduced potency at DOR as compared to **4a**, but with higher maximal stimulation (53% compared to the full agonist DPDPE). The naphthol analogue **4f** shows a significant decrease in binding affinity for all three receptors and thus was not evaluated in the [^{35}S]GTPγS assay.

The results observed with **4d** merit special mention. Compared with the lead compound **4a**, **4d**, which replaces the 2′, 6′ methyls of Dmt with metabolically less labile chloro substituents, maintains the high MOR affinity observed for **4a**, while providing considerable selectivity over DOR and KOR. While in this particular example, **4d** shows 20-fold lower potency in the [^{35}S]GTPγS assay compared to **4a**, the results suggest that 2′,6′-dichloro-L-tyrosine may prove useful for the development of opioids with improved metabolic stability toward benzylic oxidation.

In this report we have described a short, convenient synthesis of Boc-2′,6′-dimethyl-L-tyrosine for the more expedient production of this important unnatural amino acid. We have also demonstrated the utility of this chemistry for the synthesis of new Tyr derivatives and have shown that the incorporation of **3b** and **3e** and the previously unexplored derivatives **3c**, **3d**, and **3f** into our THQ scaffold yields novel opioids with interesting in vitro profiles at MOR, DOR, and KOR. These and future Tyr analogues should find use in the development of new Tyr-containing peptides and small molecules.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.5b00344.

Experimental procedures and NMR data for all compounds, and copies of ^{1}H NMR spectra for all final compounds (PDF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

Boc, *tert*-butyloxycarbonyl; $Pd_2(dba)_3$, tris(dibenzylideneacetone)dipalladium(0); SPhos, 2-dicyclohexylphosphino-2′,6′-dimethoxybiphenyl; DCM, dichloromethane; DMF, *N*,*N*-dimethylformamide; THF, tetrahydrofuran; PyBOP, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate; 6-Cl-HOBt, 6-chloro-1-hydroxybenzotriazole; DIPEA, diisopropylethylamine; TFA, trifluoroacetic acid; MeCN, acetonitrile; NBS, *N*-bromosuccinimide

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