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Synthesis of fluorescent D-amino acids with 4-acetamidobiphenyl and 4-*N*,*N*-dimethylamino-1,8-naphthalimido containing side chains

Jyotirmoy Maity, Dmytro Honcharenko *, Roger Strömberg *

Department of Biosciences and Nutrition, Karolinska Institute, Novum, Huddinge SE-141 83, Sweden

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ABSTRACT

We report the synthesis and fluorescence properties of two aromatic D-amino acids. The key step for the synthesis of (*R*)-2-amino-3-(4′-acetamido-[1,1′-biphenyl]-4-yl)propanoic acid was a Suzuki cross-coupling reaction between the pinacol diester of *N*-acetylphenylboronic acid and Fmoc-D-*p*-bromo-phenylalanine. The second amino acid, (*R*)-2-amino-4-(4′-*N*,*N*-dimethylamino-1,8-naphthalimido)butanoic acid [(*R*)-2-amino-4-DMNA-butanoic acid], was synthesized in four steps from 4-bromo-1,8-naphthalic anhydride. The amino acids were prepared in a form that could be readily incorporated into a peptide sequence by solid phase peptide synthesis using the Fmoc-strategy. Evaluation of the fluorescence properties of the biphenyl amino acid showed a maximum emission at 384 nm (excitation at 295 nm) while the naphthalimido amino acid showed a maximum emission at 545 nm (excitation at 450 nm).

In recent years, fluorescence spectroscopy techniques have been applied as powerful tools for studying the structure and function of proteins. Unnatural aromatic amino acids with fluorescence properties are widely used for this purpose and have been deployed as a fluorescent labels at the N-terminus of proteins,[1] utilized for FRET analysis of protein conformation change,[2] incorporated into proteins as alternative chromophores,[3] placed in dihydrofolate reductase to study its conformational change upon inhibitor binding,[4] included in a model peptide system villin headpiece HP35 fragment to quench various fluorophores,[5] used as a substrate to establish a sensitive assay procedure for D-amino acid oxidase activity in mammalian tissues,[6] and incorporated into the molecular chaperone GroEL for its in vivo visualization.[7] Modified peptides with unnatural fluorescent amino acids have been used to screen and quantify EGF receptor-binding peptides[8] and in the study of dynamic protein interactions.[9] An unnatural fluorescent amino acid Lys(BODIPYFL) was incorporated into the mouse muscle nicotinic acetylcholine receptor (nAChR) β-subunit M2 domain 19′ position (β19′) and studied for cell-based single molecule detection.[10] Efficient methods have even been developed to genetically encode unnatural amino acids for incorporation into proteins to enhance their properties. Among these, there are quite a few unnatural aromatic amino acids, such as the *para*-substituted derivatives of phenyl alanine,[11–13] 2-amino-3-(8-hydroxyquinolin-5-yl)propanoic acid (HqAla),[12] and 3-(6-acetylnaphthalen-2-ylamino)-2-aminopropionic acid (Anap).[14] The assortment of fluorescent amino acids with diverse properties has enabled researchers to examine the biological processes of protein binding, trafficking and localization and also protein conformational studies involving them.[15]

To access libraries of unnatural aromatic amino acids with different fluorescent properties and/or biological activities, there is still a demand for the synthesis of new aromatic amino acids. Cost effective, enantioselective and efficient synthetic protocols have been developed for the synthesis of coumarin amino acids,[16–19] 5-substituted tryptophans,[6] Fmoc-L-diaminopropyl-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-OH,[20] benz-X-asparagine derivatives,[21] bis-alanine derivatives bearing (oligo)thiophene and benzoxazole units as the heteroaromatic bridge,[22,23] alanine-substituted benzoacridone derivatives,[24] and amino acid derivatives of 4-ethoxymethylene-2-[1]-naphthyl-5(4*H*)-oxazolone.[25] However, among the synthetically accessible amino acids with fluorescence properties, very few are D-amino acids. As an example, the aromatic amino acid D-tryptophan has been synthesized and evaluated for its biological significance[25] showing an enhancement of MC receptor potency when substituted in place of L-Trp.[26]

The broad range of applications of unnatural aromatic amino acids in biochemistry encouraged us to explore the synthesis of two novel aromatic D-amino acids. Herein we describe the synthesis of (*R*)-2-amino-3-(4′-acetamido-[1,1′-biphenyl]-4-yl)propanoic acid (**1**) and (*R*)-2-amino-4-(4′-*N*,*N*-dimethylamino-1,8-naphthalimido)butanoic acid [(*R*)-2-amino-4-DMNA-butanoic acid] (**2**) in their Fmoc-protected form, compounds **6** and **11**, respectively, making them suitable for incorporation into a peptide sequence

* Corresponding authors.
E-mail addresses: dmytro.honcharenko@ki.se (D. Honcharenko), roger.stromberg@ki.se (R. Strömberg).

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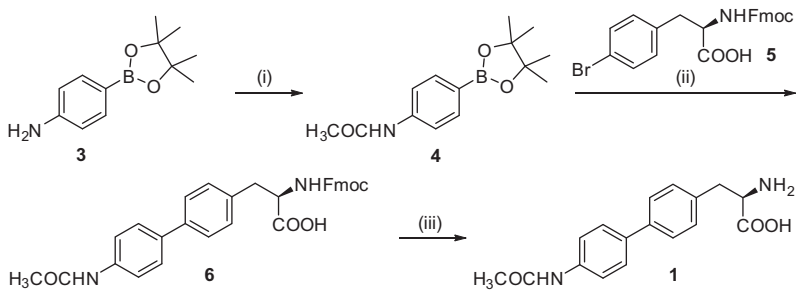


Scheme 1. Reagents and conditions: (i) Ac_2O, rt, 1 h, quant;[29] (ii) **5**, $PdCl_2$ (5 mol %), THF–ethylene glycol (10:1), Na_2CO_3, 66 °C, 3 h, 81%; (iii) 33% aq NH_3, 55 °C, 18 h, quant.

Table 1
Standardization of reaction conditions for the Suzuki coupling reaction of **4** and **5**

Entry	Solvent system	Catalyst (5 mol %)	Time (h)	Base	Temp (°C)	Yield of **6** (%)
1	THF–H_2O (10:1)	10% Pd/C	16	NaH_2PO_4/Na_2CO_3 (2:1)	22	–
2	THF–H_2O (10:1)	10% Pd/C	16	Na_3PO_4	22	–
3	THF	$Pd(PPh_3)_4$	4	Na_3PO_4	66	~40[a]
4	THF	$Pd(PPh_3)_4$	4	Na_2CO_3	66	~40[a]
5	THF	$PdCl_2$	4	K_2CO_3	66	~50 [a]
6	THF–ethylene glycol (10:1)	$PdCl_2$	3	K_2CO_3	66	72[b]
7	THF–ethylene glycol (10:1)	$PdCl_2$	3	Na_2CO_3	66	81[b]

[a] Product formation as judged by visualization by TLC under UV (254 nm).
[b] isolated yield.

using the solid phase peptide synthesis (SPPS) Fmoc-strategy.[27,28] The fluorescence properties of these unnatural aromatic amino acids in their unprotected form were also evaluated.

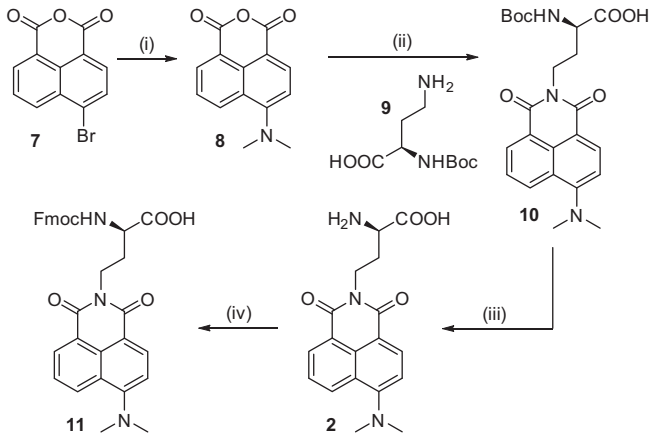
Our strategy for the synthesis of biphenyl aromatic amino acid **6** began with acetylation of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (**3**) at the *para*-amino position[29] to produce compound **4** (Scheme 1). Subsequently, the biphenyl moiety was constructed by a Suzuki cross-coupling reaction between the pinacol diester of 4-*N*-acetamidophenylboronic acid (**4**) and Fmoc-D-*p*-bromo-phenylalanine (**5**) to afford N^{α}-Fmoc-(*R*)-2-amino-3-(4′-acetamido-[1,1′-biphenyl]-4-yl)propanoic acid (**6**) (Scheme 1).

Various reaction conditions (Table 1) for the synthesis of the desired biphenyl compound were investigated using palladium on carbon (Pd/C),[30,31] $PdCl_2$[32–34] and $Pd(PPh_3)_4$[35–37] which have all been extensively used as catalysts for coupling reactions. Negligible amounts of the desired product were obtained using 10% Pd/C in THF–water, and most of the starting material was recovered (entries 1 and 2, Table 1). Different inorganic bases such as Na_3PO_4, Na_2CO_3 and K_2CO_3 as well as several solvent mixtures were examined. Decent conversions to the desired product were obtained when the reaction was performed in refluxing THF with $Pd(PPh_3)_4$ (entries 3 and 4, Table 1) and substitution of $PdCl_2$ resulted in a higher yield (entry 5, Table 1). Changing the solvent to THF–ethylene glycol (10:1) resulted in even higher yields of the cross-coupling product (81%) after isolation by chromatography (entries 6 and 7, Table 1).[38] The acetamidobiphenyl amino acid **6** was characterized by ^1H and ^{13}C NMR spectroscopy as well as by HRMS.

To carry out fluorescence spectroscopic characterization of parent amino acid the Fmoc-group was removed from compound **6** by treatment with ammonia to afford the unprotected biphenyl amino acid **1** (Scheme 1).

The second aromatic amino acid synthesized was (*R*)-4-(4′-*N*,*N*-dimethylamino-1,8-naphthalimido)-2-aminobutanoic acid (**2**) (Scheme 2). 4-Bromo-1,8-naphthalic anhydride (**7**) was converted into **8** by heating at reflux with 3-dimethylamino propionate in isoamyl alcohol.[39] Upon cooling to room temperature, compound **8** precipitated from the reaction mixture to afford sufficiently pure product that was used in the next step without purification. Anhydride **8** was reacted with N^2-(*tert*-butoxycarbonyl)-2,4-diaminobutanoic acid (Boc-D-Dab-OH, **9**) to afford (*R*)-4-(4′-*N*,*N*-dimethylamino-1,8-naphthalimido)-N^2-(*tert*-butoxycarbonyl)-2-aminobutanoic acid (**10**).[40] The Boc-protecting group was then removed using trifluoroacetic acid in dichloromethane to give the unprotected amino acid **2,** which was used for the characterization of its fluorescent properties. To obtain a suitable building block for solid phase peptide synthesis using the Fmoc-strategy, compound **2** was reprotected at the N^{α} position by treatment with Fmoc-hydroxysuccinimide ester (Fmoc-OSu)[41] affording compound **11** (Scheme 2).

Studies of the fluorescent properties of aromatic amino acids **1** and **2** were performed in a solvent mixture of 50% aqueous methanol at concentrations of 7.7×10^{-4} M and 2.9×10^{-4} M,



Scheme 2. Reagents and conditions: (i) isoamylalcohol, 3-(dimethylamino)propionitrile, 132 °C, 16 h, quant;[39] (ii) **9**, aq $NaHCO_3$, dioxane, reflux, 1 h, 66%; (iii) trifluoroacetic acid, DCM, 0 °C, 2 h; (iv) Fmoc-OSu, water–dioxane (1:1), $NaHCO_3$, 0 °C, 2 h, 71% (over two steps).

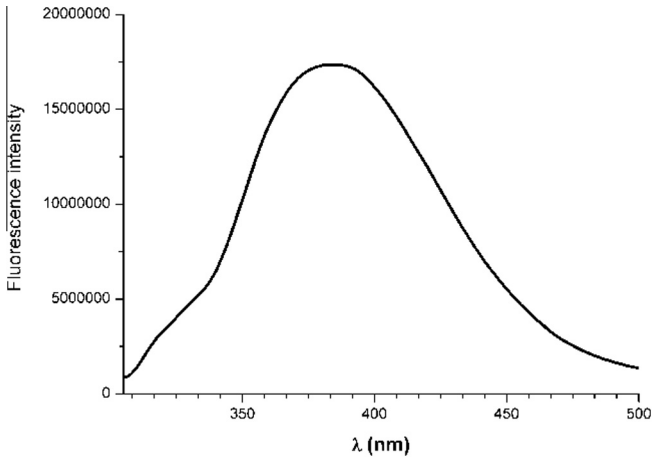


Figure 1. Fluorescence emission spectrum of (*R*)-2-amino-3-(*p*-acetamidobiphenyl)propanoic acid (**1**) in aqueous methanol (1:1) showing a maximum at 384 nm (excitation wavelength was 295 nm).

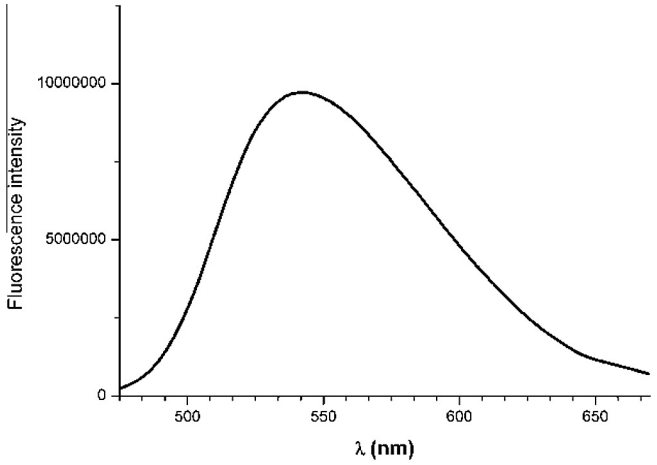


Figure 2. Fluorescence emission spectrum of (*R*)-2-amino-4-DMNA-butanoic acid (**2**) in aqueous methanol (1:1) showing a maximum at 543 nm (excitation wavelength was 450 nm).

respectively. Both compounds showed high levels of fluorescence and the relative fluorescence intensities of the aromatic amino acids were measured at various excitation wavelengths (see ESI, Figs. S12 and S13) to determine the maximum emission wavelength (λ_{em}) and maximum excitation wavelength (λ_{ex}). The acetamidobiphenyl amino acid **1** showed a maximum λ_{em} at 384 nm with a maximum λ_{ex} at 295 nm (Fig. 1). This result was comparable with the fluorescent properties of 4-aminobiphenyl.[42] The aromatic amino acid **2** displayed a maximum λ_{em} at 543 nm with a maximum λ_{ex} at 450 nm (Fig. 2). The emission for **2** was in the same range as other reported naphthalimido fluorophores.[9,43,44] A small red shift in the emission maximum was observed for **2** when fluorescence measurements were performed in pure water (maximum λ_{em} at 548 nm) compared to the emission in less polar methanol (maximum λ_{em} at 533 nm) (see ESI, Fig. S14).

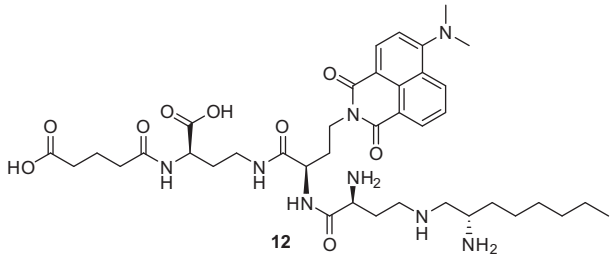


Figure 3. Chemical structure of the peptide-like compound **12** containing a fluorescent (*R*)-2-amino-4-DMNA-butanoic acid unit.

To demonstrate the incorporation of one of the fluorescent amino acids into a peptide-like structure, compound **12** (Fig. 3) was synthesized using SPPS and a recently reported triamino acid (see ESI, Scheme S1).[45]

We have synthesized two unnatural fluorescent D-amino acids with α-Fmoc protection (and α-Boc protection in one case). The synthesis of acetamidobiphenyl amino acid **6** was achieved in two steps staring from the pinacol ester of *p*-amino-phenyl boronic acid (**3**) and the fluorescent amino acid **11** was obtained from cyclic anhydride **7** in four steps. Both of these amino acids possess different fluorophores that were found to be highly fluorescent with properties that were distinctive and complementary in respect to both excitation and emission wavelengths. The acetamidobiphenyl amino acid **1** showed a maximum fluorescence intensity at a shorter wavelength (384 nm) than the naphthalimido amino acid (**2**) which had a maximum at a longer wavelength (543 nm). These aromatic amino acids with significant fluorescence properties should be valuable for incorporation into peptide sequences for studies of biological significance. Because they are D-amino acids they should be particularly valuable for incorporation into D- or D,L-peptides which are resistant to enzymatic degradation.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2015.06.059.

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38. Procedure for synthesis of (*R*)-2-amino-(9-fluorenylmethoxycarbonyl)amino-3-(4′-acetamido-[1,1′-biphenyl]-4-yl)propanoic acid (**6**): Compound **4** (64 mg, 0.25 mmol) was dissolved in a mixture of tetrahydrofuran and ethylene glycol (10:1, 15 mL) at rt. *N*-(9-Fluorenylmethoxycarbonyl)-4-bromo-D-phenylalanine (**5**, 96 mg, 0.21 mmol) was added and the mixture was kept stirring. $PdCl_2$ (10 mol %, 4 mg) was added and nitrogen was bubbled through the reaction mixture for 15 min. Na_2CO_3 (66 g, 0.62 mmol) was then added into the reaction mixture whereupon the flask was filled with nitrogen and heated at 66 °C for 3 h. When the TLC showed completion of the reaction water (20 mL) was added into the reaction mixture which was then extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with brine-water (1:1, 2 × 25 mL), dried over Na_2SO_4 and concentrated to dryness under reduced pressure. The crude product was purified by column chromatography (2–3% MeOH in DCM containing 0.1% AcOH) to afford compound **6** (87 mg, 81%). R_f = 0.25 (5% MeOH–DCM, 1% AcOH), $[\alpha]_D^{27}$ −10.0 (*c* 0.05, H_2O), ^{1}H NMR (400 MHz, CD_3OD): δ = 7.67 (d, *J* = 6.8 Hz, 2H), 7.52–7.44 (m, 6H), 7.37 (d, *J* = 7.6 Hz, 3H), 7.28–7.12 (m, 5H), 4.36 (dd, *J* = 7.6, 4.8 Hz, 1H), 4.23–4.19 (m, 1H), 4.12–4.08 (m, 1H), 4.05–4.01 (m, 1H), 3.16 (dd, *J* = 14.0, 4.8 Hz, 1H), 2.82 (dd, *J* = 14.0, 9.6 Hz, 1H), 2.04 (s, 3H) ppm. ^{13}C NMR (100.6 MHz, CD_3OD): δ = 171.7, 158.4, 145.3, 142.6, 140.3, 139.2, 137.9, 137.7, 130.9, 128.8, 128.2, 128.0, 127.7, 126.4, 126.3, 121.5, 120.9, 68.0, 56.9, 48.4, 38.4, 23.9 ppm. HRMS (ESI-TOF) (*m*/*z*): calcd for $C_{32}H_{27}N_2O_5$ $[M-H]^-$ 519.1925, found 519.1924.
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40. Procedure for the synthesis of (*R*)-2-amino-4-(4′-*N*,*N*-dimethylamino-1′,8′-naphthalimido)-N^2-(*tert*-butoxycarbonyl)butanoic acid (**10**): Compound **8** (0.266 g, 1.1 mmol) was kept under a nitrogen atmosphere in a three neck round bottom flask fitted with water condenser. Dioxane (25 mL) was added through a septum whereupon the mixture was heated at reflux and stirred vigorously. Boc-D-Dab-OH (**9**, 0.218 g, 1.0 mmol) was dissolved in an aqueous solution (5 mL) containing $NaHCO_3$ (0.42 g, 5 mmol) and added into the reaction mixture. The reaction mixture was heated at reflux for 1 h. TLC showed complete consumption of the amino acid, the mixture was concentrated under reduced pressure and water (60 mL) was added. The water phase was washed with Et_2O (2 × 60 mL) and acidified with 2 N HCl till pH 3 in an ice-bath whereupon a yellow precipitate formed. The aqueous phase was extracted with DCM (3 × 80 mL) and the combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (40–50% ethyl acetate in hexane containing 0.1% AcOH) to afford compound **10** (0.27 g, 66%). R_f = 0.2 (50% ethyl acetate–hexane, 1% AcOH), $[\alpha]_D^{22}$ −40.6 (*c* 0.032, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.49 (d, *J* = 7.2 Hz, 1H), 8.39 (d, *J* = 7.6 Hz, 1H), 8.36 (d, *J* = 8.4 Hz, 1H), 7.58 (t, *J* = 8.0 Hz, 1H), 7.02 (d, *J* = 8.4 Hz, 1H), 5.52 (d, *J* = 7.6 Hz, 1H), 4.27–4.16 (m, 3H), 3.04 (s, 6H), 2.21–2.11 (m, 2H), 1.35 (s, 9H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 174.6, 165.1, 164.6, 157.5, 156.0, 133.4, 131.9, 131.7, 130.5, 125.2, 125.0, 122.7, 114.2, 113.4, 80.4, 52.0, 44.9, 36.8, 30.8, 28.5 ppm. HRMS (ESI-TOF) (*m*/*z*): calcd for $C_{23}H_{26}N_3O_6$ $[M-H]^-$ 440.1827, found 440.1827.
41. Procedure for the synthesis of (*R*)-2-amino-4-(4′-*N*,*N*-dimethylamino-1′,8′-naphthalimido)-N^2-(9-fluorenylmethoxycarbonyl)butanoic acid (**11**): Compound **10** (0.12 g, 0.27 mmol) was dissolved in DCM (3 mL). Cold trifluoroacetic acid (3 mL) was added over 5 min and the reaction mixture was stirred for 2 h. The reaction mixture was concentrated to dryness under reduced pressure and excess TFA removed by coevaporation with chloroform (3 × 10 mL). The crude product was dried under vacuum overnight then dissolved in water (4 mL) containing $NaHCO_3$ (0.114 g, 1.35 mmol). Dioxane (10 mL) was added, followed by Fmoc-OSu (0.101 g, 0.3 mmol). The reaction mixture was stirred at 0 °C for 2 h. When the TLC showed complete consumption of the starting material the reaction mixture was diluted with water (20 mL). The aqueous layer was washed with Et_2O (2 × 20 mL) and acidified with 6 N HCl to adjust the pH to 6. The product was extracted with DCM (3 × 25 mL) and the combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude compound was purified by column chromatography (0.5–1% MeOH in DCM containing 0.1% AcOH) to afford compound **11** (0.116 g, 71%). R_f = 0.6 (2.5% MeOH–DCM, 1% AcOH), $[\alpha]_D^{22}$ −8.0 (*c* 0.05, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.51 (d, *J* = 6.8 Hz, 1H), 8.41 (d, *J* = 8.4 Hz, 1H), 8.35 (d, *J* = 8.4 Hz, 1H), 7.67 (d, *J* = 7.2 Hz, 2H), 7.59–7.52 (m, 3H), 7.31 (t, *J* = 7.2 Hz, 2H), 7.23 (t, *J* = 7.2 Hz, 2H), 7.00 (d, *J* = 8.0 Hz, 1H), 5.96 (d, *J* = 8.0 Hz, 1H), 4.35–4.31 (m, 1H), 4.29–4.19 (m, 4H), 4.13 (t, *J* = 7.2 Hz, 1H), 3.03 (s, 6H), 2.34–2.25 (m, 1H), 2.19–2.11 (m, 1H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 174.0, 165.2, 164.8, 157.7, 156.2, 144.0, 141.4, 133.6, 132.1, 131.8, 130.6, 127.8, 127.3, 125.4, 125.1, 125.0, 122.6, 120.1, 113.9, 113.3, 67.4, 52.2, 47.2, 44.9, 36.8, 31.1 ppm. HRMS (ESI-TOF) (*m*/*z*): calcd for $C_{33}H_{28}N_3O_6$ $[M-H]^-$ 562.1984, found 562.1984.
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