Synthesis of a Deoxyxylopuromycin Analogue

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Abstract: N^6-Bis-demethylated deoxyxylopuromycin was synthesized over six steps in 56% overall yield. The key steps are Mitsunobu reaction with DPPA and a Staudinger–Vilarrasa coupling.

Key words: Antibiotic, ribosome, azide, Mitsunobu, Staudinger–Vilarrasa

Synthesis of puromycin analogues has become a prominent research area ever since the antibiotic puromycin (**1**, Figure 1) was isolated from a culture broth of *Streptomyces alboniger* by Porter et al.[1] It has been found to inhibit the ribosomal protein biosynthesis by acting as a 3′-end mimic of aminoacyl tRNA.[2] Various structural analogues of puromycin have been synthesized for clarification of the relationship between the structure and biological activity.[3] Besides, studies have been carried out to lower its toxicity and to enhance its biological activity. For example, Vince and co-workers have synthesized 5′-chloro-5′-deoxypuromycin and 5′-deoxypuromycin analogues with comparable activity as puromycin but reduced toxicity on kidneys.[4] Even though the toxicity related to puromycin resulted in a very limited use as an antibiotic, its inhibitory action in the ribosome makes it a very useful tool for mechanistic studies on the mechanism of protein biosynthesis.[5] In spite of these studies, the exact nature of the catalysis of peptide bond formation, that is, the molecular reason for measured reaction rate accelerations of 10^7–10^{10}-fold with respect to an uncatalyzed ester aminolysis, still remains to be unravelled.[6]

The importance of a 2′-OH of puromycin and the 3′-terminal A76 of the aminoacyl tRNA constitutes a fundamental and long-debated research area in biochemistry[7] and its role still remains controversial.[8] The P-site A76 2′-OH of peptidyl-tRNA is located close to the nucleophilic α-amine and O-3′ leaving group, making it one of the few functional groups in a position to be directly involved in ribosomal catalysis.[9] A millionfold decrease in rate for peptidyl transfer (PT) was observed when the 2′-OH of the P-site A76 moiety was replaced by H or F.[10] A ‘proton shuttle’ mechanism for the PT was proposed based on the catalytic importance and physical proximity of this 2′-OH group.[11] Like the catalytic importance of the 2′-OH of the A76 moiety of the peptidyl-tRNA in the P-site, the catalytic importance of the 2′OH of the A76 moiety of the aminoacyl tRNA in the A-site also must be carefully evaluated. Crystallographic studies suggest that the 2′-OH group of puromycin and thus, equally, of the 3′-terminal A76 residue of any natural 3′-aminoacyl tRNA may be important for good binding to the ribosomal A site[12a] if pseudo axially oriented.[12b] A deoxypuromycin analogue **2** was synthesized for the first time by Koizumi et al. where they confirmed that a 2′-OH is necessary for puromycin's antimalarial activity.[13] The absence of the antimalarial activity of **2** can be explained by the deoxyribofuranose's preference for the 2′-*endo* (south) conformation while puromycin with its 2′-OH favors a 3′-*endo* (north) conformation.[13,14]

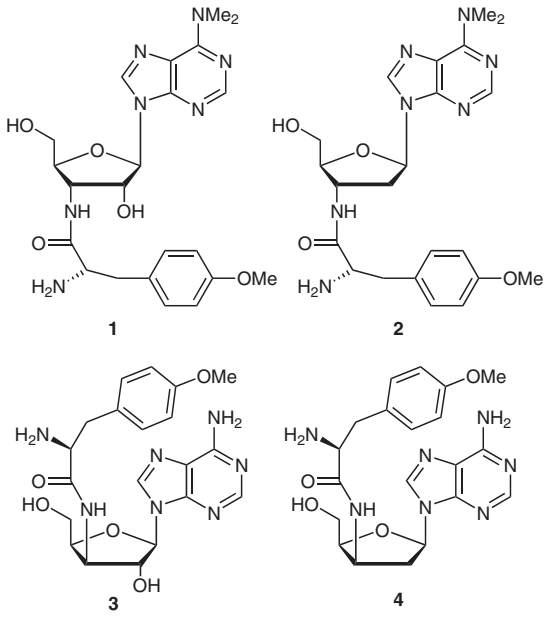

Figure 1

The configuration of the 3′-position carrying the amino acid chain also could be pivotal for the puromycin activity. We have already synthesized a xylopuromycin analogue **3** which could shed light on the importance of the stereochemistry of 3′-position where it had its 2′-OH intact.[15] Koizumi et al. synthesized the 2′-deoxypuromycin analogue in a ribo configuration. However, an inversion of the 3′-configuration along with the absence of 2′-OH has never been envisaged. This stimulated us to synthesize a 2′-deoxy analogue of puromycin in a xylo configuration. Hence, here we describe the first synthesis of deoxyxylopuromycin analogue **4** (Scheme 1).

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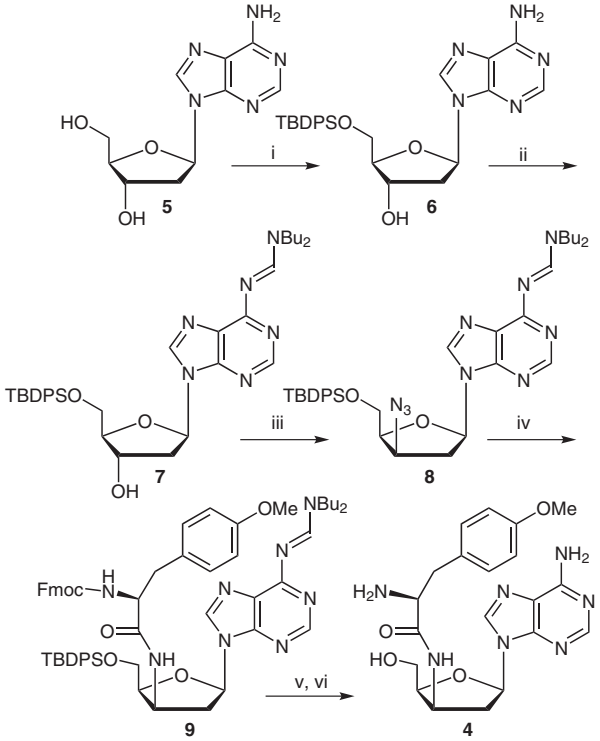

Scheme 1 *Reagents and conditions*: i) TBDPSCl, pyridine, r.t., 18 h, 88%; ii) $(CH_3O)_2CHN(nBu)_2$, MeOH, r.t, 2 h, 98%; iii) DIAD, DPPA, Ph_3P, THF, 0 °C to r.t., 7 h, 85%; iv) N-Fmoc-O-Me-L-Tyr, HOBt, DIC, Me_3P, THF, 0 °C to r.t, 4 h, 85%; v) 33% $MeNH_2$–EtOH, r.t., overnight; vi) NH_4F, MeOH, 55 °C, 90%.

We began the synthesis with the silyl protection of the 5′-OH of 2′-deoxyadenosine, using TBDPSCl by reported protocols. The N^6-amidine function was protected as *N*,*N*-dibutylformamidine (dbf) using *N*,*N*-di-*n*-butylformamide dimethylacetal, which was prepared beforehand.[3m] The key 3′-xylo-azide **8** was obtained using a Mitsunobu reaction with diphenyl phosphoryl azide (DPPA).[16] Application of Mitsunobu azidation appeared to be more convenient than the azide substitution of the 3′-*O*-triflate.[16d] Compound **7** was treated with diisopropyl azodicarboxylate (DIAD) and DPPA in the presence of triphenylphosphine to yield the xylo-azide. The major problem encountered was the purification of the desired compound from the triphenylphosphine oxide, generated as a byproduct. To solve this problem we tested trimethylphosphine instead. We anticipated that the resulting byproduct trimethylphosphine oxide would be soluble in water and could be removed by an aqueous workup. But in our hands the Mitsunobu reaction with trimethylphosphine did not work and we found the starting material unreacted. Therefore we used triphenylphosphine for the Mitsunobu azidation. Column chromatography of the crude product mixture followed by precipitation of triphenylphosphine oxide from cold diethyl ether furnished the azide intermediate **8** in acceptable purity for proceeding to the next step.[17]

The azide intermediate **8** was coupled with N-protected L-amino acid oxybenzotriazolyl ester via the Staudinger–Vilarrasa reaction which was developed to replace the usual reduction of the organo azide in to an amine, which would be the usual substrate for a coupling reaction.[3k] The use of trimethylphosphine (1 M in THF) instead of tributylphosphine in this reaction alleviated the co-migration problems of the tributylphosphine oxide byproduct, as trimethylphosphine oxide is soluble in water and was removed during the workup.[15] The coupled compound was completely deprotected with methylamine followed by NH_4F in warm methanol to get the desired deoxyxylopuromycin analogue **4**.

In conclusion N^6-bis-demethylated deoxyxylopuromycin **4** was synthesized for the first time over six steps in 56% overall yield.[18] The synthetic pathway included a Mitsunobu reaction with DPPA and a Staudinger–Vilarrasa coupling as key steps. Together with natural puromycin, other puromycin analogues, and the xylopuromycin analogue, the deoxyxylopuromycin analogue can shed light on the catalytic mechanistic aspects of peptidyl transfer reaction occurring in the ribosome. If this analogue gives interesting results in antimalarial activity, different structural analogues of **4** can be made using the same synthetic pathway.

Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett.

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(18) **5′-*O*-(*tert*-Butyldiphenylsilyl)-2′-deoxyadenosine (6)**
TBDPSCl (1.6 g, 5.7 mmol) was added to **5** (1.2 g, 4.8 mmol) in dry pyridine (24 mL), and the solution was stirred for 18 h at r.t. under N_2. H_2O (2 mL) was added, stirring was continued for 30 min, and the volatiles were evaporated. The residue was partitioned (H_2O–EtOAc), and the organic phase was washed with H_2O (2 × 50 mL) and brine, then dried (Na_2SO_4). The volatiles were evaporated, and the residue was column chromatographed (step gradient from EtOAc–cyclohexane = 1:1 to 1:0 and then to EtOAc–MeOH = 95:5) to yield **6** (2.0 g, 88%). R_f = 0.33 (EtOAc–MeOH = 9:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.06 [s, 9 H, $SiC(CH_3)_3$], 2.53 (ddd, 1 H, *J* = 13.4, 6.2, 4.0 Hz, H_A2'), 2.75 (td, 1 H, *J* = 13.1, 6.4 Hz, H_B2'), 3.79–3.98 (m, 2 H, H5′5″), 4.71–4.75 (m, 1 H, H3′), 5.84 (s, 2 H, NH_2), 6.46 (t, 1 H, *J* = 6.5 Hz, H1′), 7.31–7.45 (m, 6 H, ar), 7.62–7.65 (m, 4 H, ar), 8.02 (s, 1 H, H2), 8.29 (s, 1 H, H8). ^{13}C NMR (70 MHz, $CDCl_3$ + CD_3OD): δ = 18.7 (SiC), 26.3 [$(CH_3)_3$], 40.6 (C2′), 63.4 (C5′5″), 70.6 (C3′), 84.0 (C1′), 87.1 (C4′), 118.8 (C5), 127.3 (*p*-ar), 129.4, 129.4 (*o*-ar), 132.1, 132.4 (*i*-ar), 134.9, 135.1 (*m*-ar), 138.2 (C2), 148.4 (C4), 152.1 (C6), 155.1 (C8). MS (ESI$^+$): *m/z* = 490.1 $[MH]^+$

6-*N*-[(di-*n*-Butylamino)methylene]-5′-*O*-(*tert*-butyldiphenylsilyl)-2′-deoxyadenosine (7)
Compound **6** (600 mg, 1.3 mmol) was dissolved in MeOH (5 mL). *N*,*N*-Di-*n*-butylformamide dimethylacetal (600 mg, 2.6 mmol) was added, and the solution was stirred for 2 h at r.t. The volatiles were removed under reduced pressure, and the residue was purified using silica gel column chromatography (EtOAc–MeOH = 100:0 to 95:5 step gradient) to yield **7** (750 mg, 98%). R_f = 0.45 (CH_2Cl_2–MeOH = 9.5:0.5). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.82–0.88 (m, 6 H, 2 × $NCH_2CH_2CH_2CH_3$), 0.96 [(s, 9 H, $SiC(CH_3)_3$], 1.23–133 (m, 4 H, 2 × $NCH_2CH_2CH_2CH_3$), 1.49–1.62 (m, 4 H, 2 × $NCH_2CH_2CH_2CH_3$), 2.46 (ddd, *J* = 13.2, 6.0, 3.5 Hz, 1 H, H_A2'), 2.57–2.66 (m, 1 H, H_B2'), 3.28–3.34 (m, 2 H, $NCH_2CH_2CH_2CH_3$), 3.59–3.65 (m, 2 H, $NCH_2CH_2CH_2CH_3$), 3.72–3.85 (m, 2 H, H5′5″), 4.05–4.09 (m, 1 H, H4′), 4.64–4.68 (m, 1 H, H3′), 6.44 (t, *J* = 6.6 Hz, 1 H, H1′), 7.25–7.32 (m, 6 H, ar), 7.53–7.58 (m, 4 H, ar), 8.02 (s, 1 H, H2), 8.41 (s, 1 H, H8), 8.91 (s, 1 H, CH=N). ^{13}C NMR (70 MHz, $CDCl_3$): δ = 13.6 ($NCH_2CH_2CH_2CH_3$), 13.8 ($NCH_2CH_2CH_2CH_3$), 19.1 (SiC), 19.6 ($NCH_2CH_2CH_2CH_3$), 20.1 ($NCH_2CH_2CH_2CH_3$), 26.8 [$(CH_3)_3$], 29.1 ($NCH_2CH_2CH_2CH_3$), 30.9 ($NCH_2CH_2CH_2CH_3$), 40.6 (C2′), 45.1 ($NCH_2CH_2CH_2CH_3$), 51.7 ($NCH_2CH_2CH_2CH_3$), 64.0 (C5′5″), 71.7 (C3′), 84.01 (C1′), 87.0 (C4′), 126.1 (C5), 127.7, 129.7, 129.8, 132.7, 132.8, 135.4, 135.4 (Ar), 139.7 (C2), 151.0 (C4), 152.3 (C6), 158.3 (C8), 159.9 (CH=N). HRMS (ESI$^+$): *m/z* calcd: 629.3635 $[MH]^+$; found: 629.3636.

9-[3′-Azido-5′-*O*-(*tert*-butyldiphenylsilyl)-2′,3′-dideoxy-β-D-xylofuranosyl)-6-*N*-[(di-*n*-butylamino)methylene]-adenine (8)
To a THF (1.9 mL) solution of **7** (69 mg, 0.11 mmol) and Ph_3P (90 mg, 0.33 mmol), a mixture of DIAD (71 μL, 0.33 mmol) and DPPA (65 μL, 0.33 mmol) in THF (500 μL) was added dropwise at 0 °C under Ar. After 7 h of stirring at r.t., EtOH (1 mL) was added, and the solution was stirred for 30 min. After evaporation of all the volatiles, the oily residue was purified by silica gel column chromatography (EtOAc–cyclohexane = 3:7 to 8:2 step gradient). Ph_3PO contained in the chromatographed product was removed by precipitation from cold Et_2O (3 h, 4 °C). After the second precipitation the filtrate was evaporated to yield **8** (61 mg, 85%). R_f = 0.4 (CH_2Cl_2–MeOH = 9.5:0.5). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.94 (2 t, *J* = 7.3, 7.3 Hz, 6 H, 2 × $NCH_2CH_2CH_2CH_3$), 1.09 [s, 9 H, $SiC(CH_3)_3$], 1.21–1.45 (m, 4 H, 2 × $NCH_2CH_2CH_2CH_3$), 1.58–1.71 (m, 4 H, 2 × $NCH_2CH_2CH_2CH_3$), 2.54 (dd, *J* = 14.8, 0.9 Hz, 1 H, H_A2'), 2.83 (ddd, *J* = 14.0, 7.9, 6.0 Hz, 1 H, H_B2''), 3.38 (t, *J* = 7.3 Hz, 2 H, $NCH_2CH_2CH_2CH_3$), 3.67–3.75 (m, 2 H, $NCH_2CH_2CH_2CH_3$), 3.96–4.07 (m, 2 H, H5′5″), 4.19–4.24 (m, 1 H, H4′), 4.41–4.44 (m, 1 H, H3′), 6.41 (dd, *J* = 7.9, 2.1 Hz, 1 H, H1′), 7.42–7.70 (m, 10 H, ar), 8.19 (s, 1 H, H2), 8.52 (s, 1 H, H8), 9.02 (s, 1 H, CH=N). ^{13}C NMR (70 MHz, $CDCl_3$): δ = 13.6 ($NCH_2CH_2CH_2CH_3$), 13.9 ($NCH_2CH_2CH_2CH_3$), 19.1 (SiC), 19.7 ($NCH_2CH_2CH_2CH_3$), 20.1 ($NCH_2CH_2CH_2CH_3$), 26.8 [$(CH_3)_3$], 29.2 ($NCH_2CH_2CH_2CH_3$), 30.9 ($NCH_2CH_2CH_2CH_3$), 39.0 (C2′), 45.1 ($NCH_2CH_2CH_2CH_3$), 51.8 ($NCH_2CH_2CH_2CH_3$), 61.5 (C3′), 61.9 (C5′5″), 83.1 (C1′), 83.1 (C4′), 125.8 (C5),

125.8, 127.8, 128.3, 131.8, 131.9, 132.0, 135.4 (ar), 139.8 (C2), 151.0 (C4), 156.6 (C6), 157.3 (C8), 158.4 (CH=N). HRMS (ESI$^+$): *m/z* calcd: 654.3700 [MH]$^+$; found: 654.3700.

9-[*5′-O*-(*tert*-Butyldiphenylsilyl)-3′-[*N*-(9-fluorenyl)-methoxycarbonyl-*O*-methyl-L-tyrosyl]amido-2′,3′-dideoxy-β-D-xylofuranosyl)-6-*N*-[(di-*n*-butylamino)-methylene]adenine (9)

N-Fmoc-*O*-Me-L-Tyr (45 mg, 0.11 mmol) and HOBt (18 mg, 0.11 mmol) were co-evaporated with anhyd THF (3 × 1 mL). The mixture was dissolved in anhyd THF (1 mL) and cooled down to 0 °C under N_2 for 10 min. Diisopropylcarbodiimide (16 μL, 0.10 mmol) was added, and the reaction mixture was stirred at the same temperature for 15 min and then 10 min at r.t. Me_3P (1 M in THF, 160 μL, 0.16 mmol) was added to **8** (52.6 mg, 0.08 mmol) in THF (1 mL) and stirred at r.t. for 5 min. The amino acid solution was added to the iminophosphorane solution and stirred for 4 h at r.t. The reaction mixture was concentrated under reduced pressure and dissolved in EtOAc (20 mL) and then washed with sat. aq $NaHCO_3$ solution (15 mL) and H_2O (2 × 20 mL). The organic layer was dried over Na_2SO_4, evaporated, and the residue was purified by silica gel column chromatography (CH_2Cl_2–MeOH = 99.5: 0.5 to 98:2 step gradient) to yield 9 (70 mg, 85%). R_f = 0.45 (CH_2Cl_2–MeOH = 9.5:0.5). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.81–0.91 (m, 6 H, 2 × $NCH_2CH_2CH_2CH_3$), 1.06, 1.07 [2 s, 9 H, $SiC(CH_3)_3$], 1.22–1.39 (m, 4 H, 2 × $NCH_2CH_2CH_2CH_3$), 1.52–1.72 (m, 4 H, 2 × $NCH_2CH_2CH_2CH_3$), 1.93 (dd, *J* = 15.0, 2.9 Hz, 1 H, H_A2′), 2.72–2.74 (m, 1 H, H_B2′), 1.88 (d, *J* = 6.1 Hz, 1 H, Hβ), 3.36 (t, *J* = 7.3 Hz, 2 H, $NCH_2CH_2CH_2CH_3$), 3.56–3.59 (m, 2 H, $NCH_2CH_2CH_2CH_3$), 3.66–3.71 (m, 2 H, H5″), 3.89 (dd, *J* = 11.1, 3.9 Hz, 1 H, H5′), 4.01–4.02 (m, 1 H, H4′), 4.11–4.36 (m, 4 H, CH-Fmoc, Hα and CH_2-Fmoc), 4.73 (d, *J* = 7.4 Hz, 1 H, H3′), 5.37 (d, *J* = 7.3 Hz, 1 H, NH-Fmoc), 5.88 (dd, *J* = 8.9, 3.5 Hz, 1 H, H1′), 6.49, 6.91 [2 d, *J* = 8.5 Hz, 4 H, H-*o*-Ph(OMe)], 7.10–7.80 [m, 18 H, ar (Fmoc and TBDPS)], 8.39 (s, 1 H, H2), 8.94 (s, 1 H, H8), 9.56 (s, 1 H, CH=N). ^{13}C NMR (70 MHz, $CDCl_3$): δ = 13.6 ($NCH_2CH_2CH_2CH_3$), 13.8 ($NCH_2CH_2CH_2CH_3$), 19.1 (SiC), 19.7 ($NCH_2CH_2CH_2CH_3$), 20.1 ($NCH_2CH_2CH_2CH_3$), 23.4, 26.7 [$(CH_3)_3$], 29.2 ($NCH_2CH_2CH_2CH_3$), 30.9 ($NCH_2CH_2CH_2CH_3$), 38.1 (C2′), 38.7 (Cβ), 45.2 ($NCH_2CH_2CH_2CH_3$), 47.1 (CH-Fmoc), 48.8 (C3′), 51.8 ($NCH_2CH_2CH_2CH_3$), 57.1 (C5′5″), 62.6 (OCH_2 Fmoc), 66.8 (Cα), 82.7 (C4′), 84.5 (C1′), 113.6 [*o*-Ph(OMe)], 119.8, 125.0, 127.4, 127.5, 127.6, 128.3, 128.5, 130.2, 131.7, 131.8, 131.9, 131.9, 132.1, 133.1, 135.4, 135.5, 135.5, 141.2, 141.5, 143.7, 143.8 (Ar), 149.4 (C4), 151.8 (C2), 157.2 (C6), 157.2 (C=O), 158.3 (C8), 169.97 (CH=N). HRMS (ESI$^+$): *m/z* calcd: 1049.5085 [M + Na]$^+$; found: 1049.5084.

9-(3′-*O*-Methyl-L-tyrosyl)amido-2′,3′-dideoxy-β-D-xylofuranosyl)adenine (4)

Compound **9** (65 mg, 0.08 mmol) was dissolved in 33% $MeNH_2$–EtOH (8 mL), and the mixture was stirred overnight at r.t. in a closed vessel. The solution was concentrated under reduced pressure, and the residue was dissolved in MeOH (2 mL) and then NH_4F (16 mg, 0.43 mmol) was added. The reaction mixture was warmed to 50–55 °C for 4 h. The volatiles were evaporated, and the residue was washed with EtOAc (3×) and then purified by a preparative thin-layer column chromatography using *i*-PrOH–NH_3–H_2O (8:0.5:0.5) as the eluent to obtain **4** (24 mg, 90%). ^{1}H NMR (300 MHz, CD_3OD): δ = 1.95–2.01 (m, 1 H, H_A2′), 2.67–2.77 (m, 2 H, H_B2′, Hβ), 3.44 (t, *J* = 7.2 Hz, 1 H, Hα), 3.49 (s, 3 H, OMe), 3.61–3.65 (m, 2 H, H5′5″), 3.99 (dd, *J* = 9.7, 5.4 Hz, 1 H, H4′), 4.55–4.59 (m, 1 H, H3′), 6.03 (dd, *J* = 8.4, 5.1 Hz, 1 H, H1′), 6.58 [d, *J* = 8.6 Hz, 2 H, *o*-Ph(OMe)], 6.98 [d, *J* = 8.6 Hz, 2 H, *m*-Ph(OMe)], 8.05 (s, 1 H, H2), 8.11 (s, 1 H, H8). ^{13}C NMR (125 MHz, CD_3OD): δ = 39.4 (C2′), 39.9 (Cβ), 50.8 (C3′), 55.6 (OMe), 57.1 (Cα), 61.8 (C5′5″), 83.3 (C4′), 86.0 (C1′), 115.1 [2 × C-*o*-Ph(OMe)], 122.4 (C5), 127.9 [C-*p*-Ph(OMe)], 131.7 [2 × C-*m*-Ph(OMe)], 142.6 (C2), 148.9 (C4), 153.5 (C8), 157.7 (C6), 160.5 [C-*i*-Ph(OMe)], 169.9 (C=O). HRMS (ESI$^+$): *m/z* calcd: 428.2046 [MH]$^+$; found: 428.2047.