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Synthesis of a *C*-glucosylated cyclopropylamide and evaluation as a glycogen phosphorylase inhibitor

Philippe Bertus [a,b], Jan Szymoniak [b], Erwann Jeanneau [c], Tibor Docsa [d], Pál Gergely [d], Jean-Pierre Praly [e,f,g], Sébastien Vidal [e,f,g,*]

[a] *CNRS UMR 6011, Unité de Chimie Organique Moléculaire et Macromoléculaire (UCO2M), Université du Maine, Avenue O. Messiaen, F-72085 Le Mans Cedex 9, France*
[b] *Institut de Chimie Moléculaire de Reims, CNRS (UMR 6229), Université de Reims, 51687 Reims Cedex 2, France*
[c] *Centre de Diffractométrie Henri Longchambon, Université Claude Bernard Lyon 1, 43 boulevard du 11 Novembre 1918, F-69622 Villeurbanne Cedex, France*
[d] *Cell Biology and Signaling Research Group of the Hungarian Academy of Sciences, Department of Medical Chemistry, Research Centre for Molecular Medicine, University of Debrecen, H-4032 Debrecen, Nagyerdei krt. 98, Hungary*
[e] *CNRS, UMR5246, Institut de Chimie et Biochimie Moléculaires et Supramoléculaires, Laboratoire de Chimie Organique 2—Glycochimie, 43 Boulevard du 11 Novembre 1918, F-69622 Villeurbanne, France*
[f] *Université de Lyon, F-69622 Lyon, France*
[g] *Université Lyon 1, 43 Boulevard du 11 Novembre 1918, F-69622 Villeurbanne, France*

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ABSTRACT

The synthesis of carbohydrate-based glycogen phosphorylase inhibitors is attractive for potential applications in the treatment of type 2 diabetes. A titanium-mediated synthesis led to a benzoylated *C*-glucosylated cyclopropylamine intermediate, which underwent a benzoyl migration to afford the corresponding 2-hydroxy-*C*-glycoside. X-ray crystallographic studies revealed a unit cell composed of four molecules as pairs of dimers connected through two hydrogen bonds. The deprotection of the benzoate esters under Zemplén conditions afforded a glycogen phosphorylase inhibitor candidate displaying weak inhibition toward glycogen phosphorylase (16% at 2.5 mM).

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Glycogen phosphorylase (GP) plays an important role in the control of glycemia.[1–4] This enzyme is responsible for the depolymerization of glycogen in which a terminal glucose unit is cleaved and phosphorylated to produce glucose-1-phosphate and glycogen missing one glucose unit. GP is mostly located in the muscles for the production of glucose as a source of energy, but also in the liver where it contributes to hepatic glucose production. The inhibition of this enzyme is therefore attractive for the development of new treatments of type 2 diabetes.[5–8] GP possesses various binding sites on which several types of molecules can act as inhibitors. A large set of glucose-based molecules has been designed as ligands binding to the active site of GP, and many of them were moderate or potent competitive inhibitors of this enzyme.[5,6,9–12]

Analysis of the structures of glucose-based inhibitors of GP highlights a few preferred structural features of the aglycons. Among them, hydrogen bonds in the urea, carbonyl groups in the acylated glucosyl-ureas,[10] and hydrophobic residues as in the *C*-glucosylated 1,2,4-oxadiazoles (Fig. 1) have to be considered.

We have shown that inhibition of GP was enhanced by the hydrophobicity and the electron density of aromatic moieties in the aglycon.[11,12] Based on these observations, we designed a short synthetic route to *C*-glucosylated cyclopropylamides from a *C*-glucosyl cyanide through a titanium-mediated cyclopropanation developed recently.[13–22] This methodology was also applied to the synthesis of ester-protected ribofuranosyl cyanides.[23] The amide function would therefore act as a donor and acceptor of H-bonds, and the cyclopropyl and phenyl rings as hydrophobic residues that can be accommodated in the β-channel next to the active site of GP.

The readily available glucosyl cyanide **1**[24] was first reacted under standard[13] titanium-mediated cyclopropanation conditions (Scheme 1). However, the addition of EtMgBr to a mixture of **1** and Ti(O*i*-Pr)$_4$ in Et$_2$O, followed by BF$_3$·Et$_2$O, did not give the expected primary cyclopropylamine **2**, but the benzamide **3** in 62% yield. This amide resulted presumably from a regioselective migration of the benzoyl group from the 2-position to the amine. Interestingly, when the reaction was performed without a Lewis acid (BF$_3$·Et$_2$O), the same amide **3** was obtained in a slightly better yield (66%).[25] Changing the solvent (THF or Et$_2$O) or performing the

* Corresponding author. Tel./fax: +33 0472 44 83 49.
E-mail address: sebastien.vidal@univ-lyon1.fr (S. Vidal).

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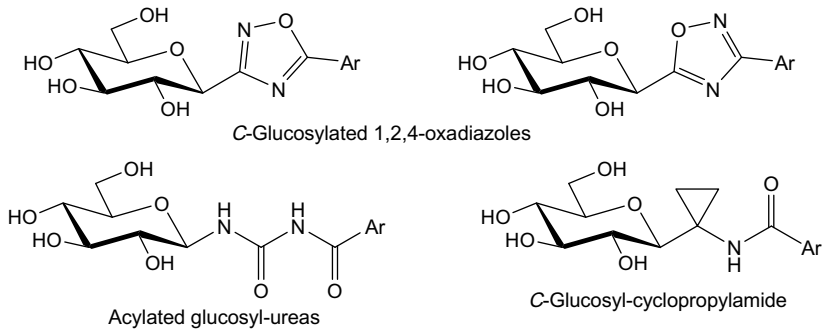
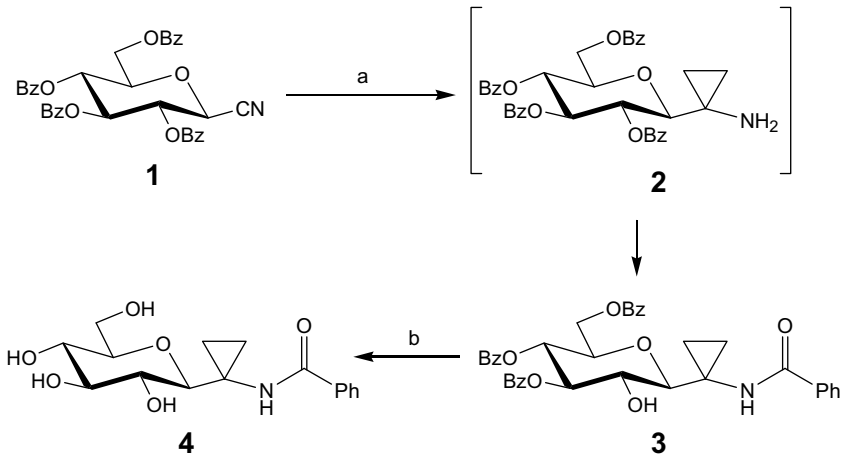


Figure 1. Structures of some glucose-based inhibitors of glycogen phosphorylase and of the cyclopropylamide inhibitor candidate.



Scheme 1. Reagents and conditions: (a) EtMgBr (2.2 equiv), Ti(O*i*-Pr)$_4$ (1.1 equiv), Et$_2$O–THF, rt, 1 h (66%); (b) NaOMe, MeOH (93%).

addition of the Grignard reagent at lower temperature did not influence the outcome of the reaction, providing invariably the amide **3** as the main product. The benzoate esters were finally removed under Zemplén conditions to obtain the expected *C*-glucosylated cyclopropyl-benzamide **4**[26] (Scheme 1).

Such a benzoyl migration in the glucopyranose series is noteworthy, since a similar amide formation was not observed for a related 2-benzoylated ribofuranose derivative (Fig. 2).[23] 2C-selective acetyl groups migration was similarly observed while preparing substituted *C*-glycopyranosyl-methylamines.[27,28]

The benzoyl group migration occurred either during the cyclopropanation process through a spiro-cyclopropylated six-membered ring system and hydrolysis (Fig. 3, path A), or after formation of the cyclopropylamine, via intramolecular aminolysis of the most accessible benzoate group (Fig. 3, path B).

Cyclopropyl-benzamide **3** afforded single crystals suitable for X-ray crystallography by slow evaporation of a solution in dichloromethane followed by washing of the crystals with diethyl ether.[29] A colorless crystal with dimensions $0.07 \times 0.07 \times 0.11$ mm^3 was selected for X-ray structure analysis (Fig. 4). The compound crystallized in the non-centrosymmetric space group $P2_1$ with an asymmetric unit consisting of four independent molecules ($Z' = 4$), which is due to the different orientations of phenyl groups from one molecule to another (Fig. 5). Two types of hydrogen bonds were observed (Table 1): an intramolecular O–H···O bond between the carbonyl of the amide and the hydroxyl group at the 2-position and an intermolecular N–H···O bond between NH of the amide and the carbonyl of the O-6 benzoate, leading to the formation of dimeric entities (Fig. 6). The three-dimensional packing was achieved through C–H···O interactions. All bond distances and angles were in agreement with the expected values.[30] The crystal packing contained solvent accessible voids of 208.7 Å^3 per unit cell (3.1% of the total volume).

The inhibition of the hydroxylated cyclopropyl-benzamide **4** was evaluated against rabbit muscle glycogen phosphorylase *b* (RMGP*b*).[31] No inhibition was observed at a concentration of 625 μM and 16% inhibition at 2.5 mM. The poor biological activity observed could be attributed to unfavorable structural and conformational features of the cyclopropyl group or to its inability to establish binding interactions with the active site of GP. Neverthe-

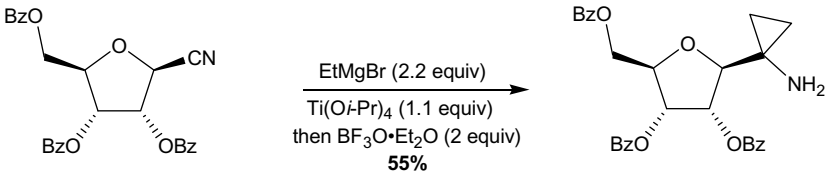


Figure 2. Titanium-mediated cyclopropanation afforded the expected cyclopropylamine in good yield in the presence of benzoyl protecting groups in the ribofuranose series.

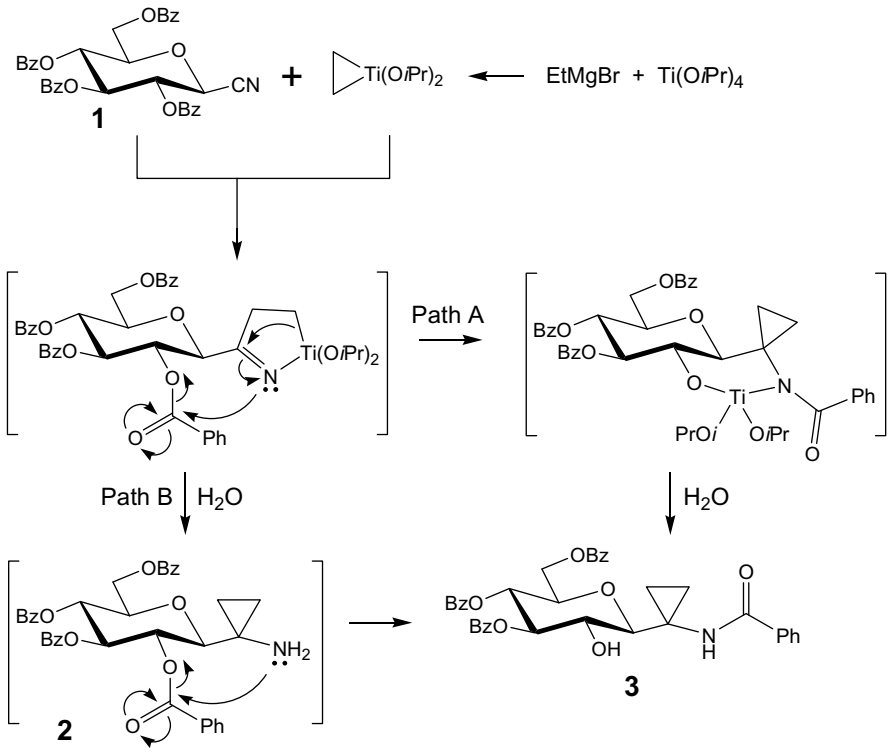


Figure 3. Proposed pathways for the formation of the cyclopropyl-benzamide **3**.

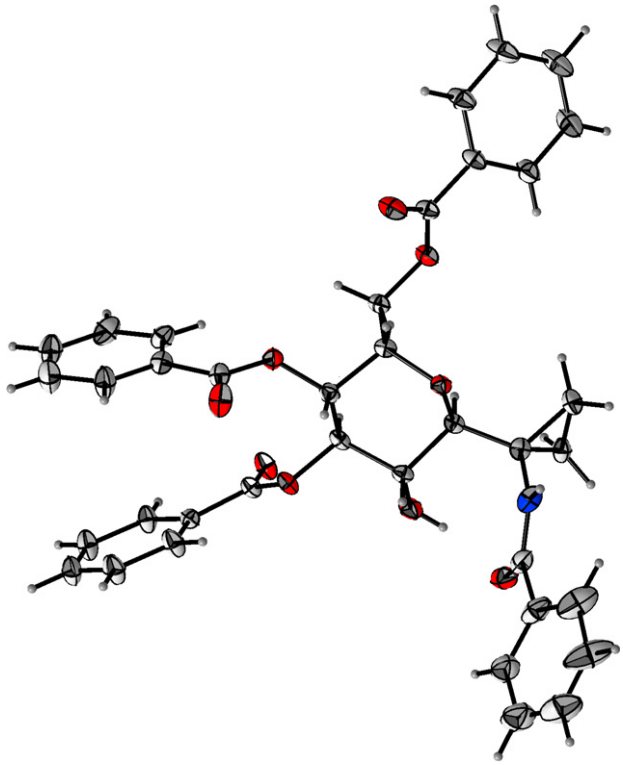


Figure 4. Representation of the X-ray crystal structure of cyclopropyl-benzamide **3**.

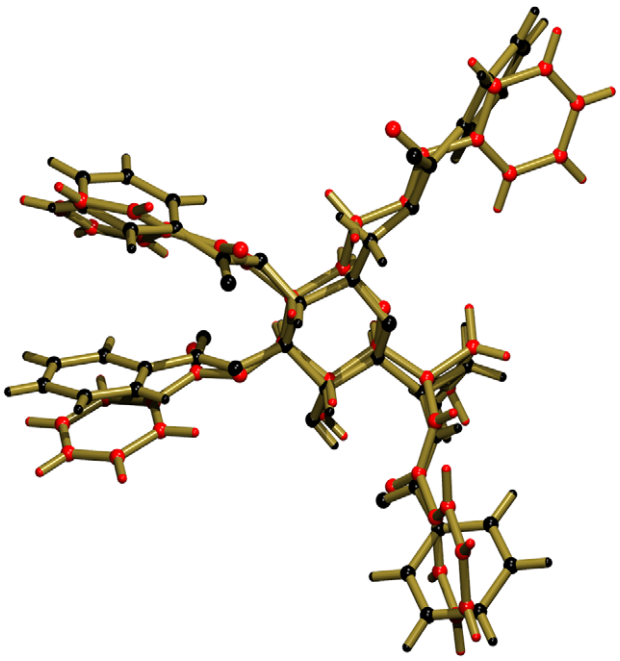


Figure 5. Overlay of two molecules of cyclopropyl-benzamide **3** in the same unit cell displaying different orientations of the phenyl rings.

less, binding at the inhibitor site cannot be ruled out due to the presence of a phenyl ring in the inhibitor's aglycon structure which could interact with the protein. The use of computer-aided strategies (e.g., fragment-based drug design) will be considered for the design of other *C*-glucosylated cyclopropyl-amides with potentially better ability to bind the active site of GP.

In conclusion, titanium-mediated cyclopropanation of the tetrabenzoylated β-D-glucopyranosyl cyanide afforded a 1-benzamide derivative. It originated from the selective migration of the 2-benzoyl group to the newly created amine. The selectivity of the benzoyl group transfer from the 2-position to the amine moiety can be advantageous for the selective synthesis of 2-deoxy-2-amino glycosides. The crystal packing of the benzamide displayed four molecules in the unit cell with two dimers held together by two

Table 1
Distances and angles characterizing hydrogen bonds observed in each molecule present in the unit cell of cyclopropyl-benzamide **3**

	D–H (Å)	H···*A* (Å)	*D*···*A* (Å)	*D*–H···*A* (°)
N25–H251···O210	0.85	2.10	2.930 (5)	165
O83–H831···O140	0.81	1.93	2.702 (5)	159
N138–H1381···O265ii	0.87	2.07	2.893 (5)	158
O181–H1811···O187	0.82	1.87	2.667 (5)	165
N185–H1851···O50	0.87	2.12	2.961 (5)	162
O243–H2431···O300	0.81	1.98	2.738 (5)	155
N298–H2981···O105vi	0.86	2.06	2.887 (5)	163
O22–H221···O27	0.84	1.98	2.778 (5)	159

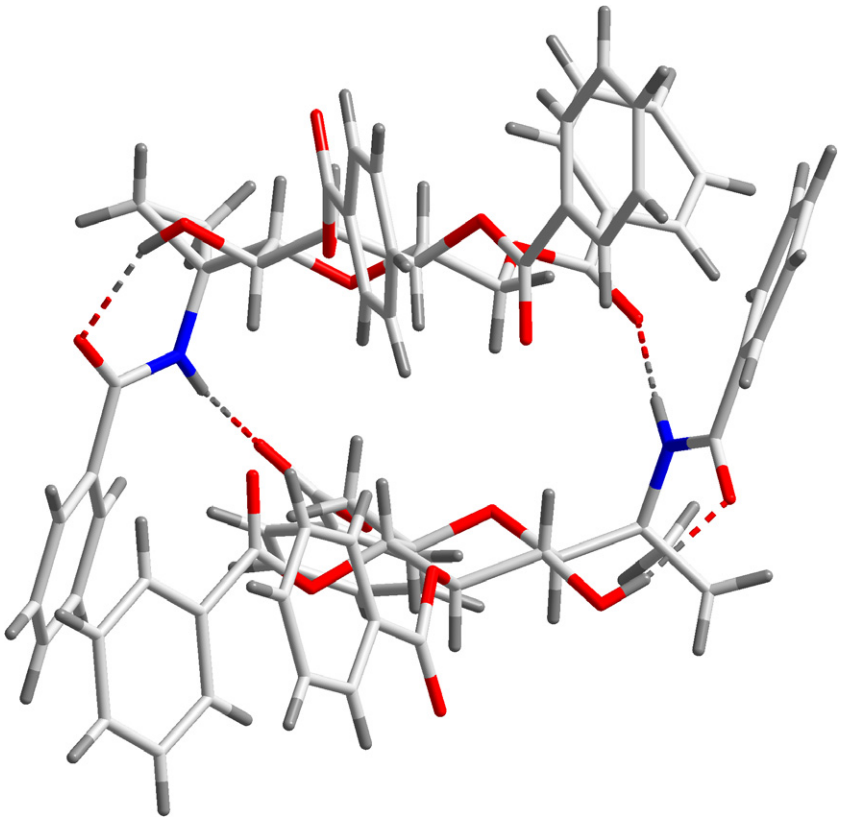


Figure 6. Dimers formed through intermolecular N–H···O hydrogen bonds (dotted lines) in the same unit cell.

N–H···O hydrogen bonds. The hydroxylated cyclopropyl-benzamide was found a weak inhibitor of GP (16% inhibition at 2.5 mM).

Acknowledgments

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25. *N-[1-(C-3,4,6-tri-O-benzoyl-β-D-glucopyranosyl)cyclopropyl]benzamide* **(3)**. EtMgBr (0.80 mL, 1.1 mmol, 1.4 M in Et_2O) was added under argon at rt to a solution of the nitrile **1** (0.30 g, 0.5 mmol) and $Ti(Oi\text{-}Pr)_4$ (0.17 mL, 0.55 mmol) in Et_2O/THF (1:1, 10 mL). The solution was stirred at rt for 1 h. Water was added (1 mL), followed by 10% aq HCl (10 mL) and EtOAc (20 mL). A 10% aq NaOH solution was added to the

transparent mixture until pH 12. The product was extracted with EtOAc (2× 20 mL). The combined organic extracts were dried ($MgSO_4$). After evaporation of the solvent, the product was purified by flash chromatography on silica gel (PE/EtOAc 1:1) to give **3** as a white solid (0.21 g, 66%). Mp = 222 °C. R_f = 0.66 (PE/EtOAc 1:1). $[\alpha]_D^{20}$ −53.0 (c = 1.05/CH_2Cl_2). IR (KBr): ν (cm^{-1}) 3351, 1724, 1702, 1645, 1602, 1582, 1525, 1452, 1287. *^{1}H NMR (250 MHz, $CDCl_3$).* δ (ppm) 0.86–0.97 (m, 2H, CH_2CH_2), 1.25–1.48 (m, 2H, CH_2CH_2), 2.85 (d, 1H, J = 9.4 Hz, H-1), 3.81 (td, 1H, J = 9.7, 3.5 Hz, H-2), 4.00 (ddd, 1H, J = 9.7, 5.3, 3.0 Hz, H-5), 4.42 (dd, 1H, J = 12.1, 5.3 Hz, H-6), 4.57 (dd, 1H, J = 12.1, 3.0 Hz, H-6′), 5.35 (d, 1H, J = 3.5 Hz, OH), 5.51 (t, 1H, J = 9.7 Hz, H-4), 5.68 (t, 1H, J = 9.7 Hz, H-3), 6.88 (s, 1H, NH), 7.28–7.58 (m, 12H, H-ar), 7.75 (d, 2H, J = 7.1 Hz, H-ar), 7.90 (d, 2H, J = 7.1 Hz, H-ar), 7.97 (d, 2H, J = 7.1 Hz, H-ar), 8.02 (d, 2H, J = 7.1 Hz, H-ar). *^{13}C NMR ($CDCl_3$, 63 MHz).* δ (ppm) 12.1, 14.3 (2s, 2C, CH_2CH_2), 32.8 (C–N), 63.6 (C-6), 69.7 (C-4), 70.3 (C-2), 74.8 (C-3), 76.2 (C-5), 86.3 (C-1), 127.1 (s, 2C, CH-ar), 128.1 (s, 2C, CH-ar), 128.3 (s, 4C, CH-ar), 128.6 (s, 2C, CH-ar), 128.8 (s, 2C, C-ar), 129.5 (s, 2C, CH-ar), 129.6 (s, 2C, C-ar), 129.7 (s, 4C, CH-ar), 132.2 (CH-ar), 132.8 (CH-ar), 133.0 (CH-ar), 133.2 (CH-ar), 165.4, 166.1, 166.2 (3s, C=O), 169.9 (PhCONH). *HR-ESIMS (positive mode).* m/z = $[M+H]^+$ $C_{37}H_{34}NO_9$ calcd 636.2234, found 636.2238. *Crystallographic data for (**3**).* $C_{37}H_{33}NO_9$, Mr = 635.64, monoclinic, space group $P2_1$ (No. 4); a = 15.651(1), b = 22.761(2), c = 19.293(1) Å, β = 102.70(1)°; V = 6704.6(8) Å^3; Z = 8; $F(000)$ = 2672; D_x = 1.259 g m^{-3}; S = 1.118 R/wR = 0.050/0.057 for 1693 parameters and 14319 reflections with $I > 2\sigma(I)$, R/wR = 0.0571/0.0662 for all 16391 independent reflections measured in the range 1.082°–27.893°. CCDC 686021 contains the supplementary crystallographic data for this molecule. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

26. *N-[1-(C-β-D-glucopyranosyl)cyclopropyl]benzamide* (**4**). A solution of cyclopropyl-benzamide **3** (337 mg) and NaOMe (5 mg) in CH_2Cl_2/MeOH (10 mL, 1:1) was stirred at room temperature for 3 h. The white precipitate was filtered and washed with petroleum ether (2× 5 mL) to afford the hydroxylated benzamide **4** (160 mg, 93%) as a white solid. Mp = 260–262 °C. R_f = 0.47 (EtOAc/MeOH 3:1). $[\alpha]_D^{20}$ −47.6 (c = 1.03/DMSO). *^{1}H NMR (300 MHz, CD_3SOCD_3).* δ (ppm) 0.70–0.96 (m, 3H, CH_2CH_2), 1.02–1.14 (m, 1H, CH_2CH_2), 2.52 (m, 1H, H-1), 2.95–3.11 (m, 3H, H-2 H-4 H-5), 3.18 (t, 1H, J = 9.0 Hz, H-3), 3.43 (dd, 1H, J = 11.6, 5.4 Hz, H-6), 3.67 (dd, 1H, J = 11.6, 1.5 Hz, H-6′), 7.44 (t, 2H, J = 7.3 Hz, H-ar), 7.54 (t, 1H, J = 7.3 Hz, H-ar), 7.86 (d, 2H, J = 7.3 Hz, H-ar). *^{13}C NMR (75 MHz, CD_3SOCD_3).* δ (ppm) 11.4,14.0 (2s, 2C, CH_2CH_2), 33.0 (C–N), 61.3 (C-6), 70.3 (C-2), 71.9 (C-4), 76.4 (C-3), 80.9 (C-5), 84.7 (C-1), 127.7 (s, 2C, CH-ar), 128.5 (s, 2C, CH-ar), 132.0 (CH-ar), 133.5 (C-ar), 169.3 (C=O). *HR-ESIMS (positive mode).* m/z = 346.2 $[M+Na]^+$, 668.8 $[2M+Na]^+$. *HR-ESIMS (negative mode).* m/z = 321.9 $[M-H]^-$, 358.0 $[M+Cl]^-$. *HR-ESIMS (positive mode).* m/z = $[M+Na]^+$ $C_{16}H_{21}NNaO_6$ calcd 346.1267, found 346.1270.
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