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Synthesis of a metabolite of an anti-angiogenic lead candidate based on a D-glucosamine motif

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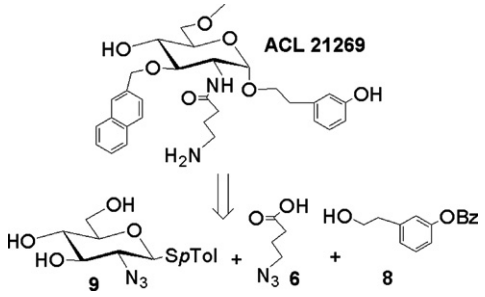
ABSTRACT

A rapid synthetic access to **ACL 21269** was established in 12 steps starting from thiogylcoside **9** utilizing synthons **8** and **6** to introduce the pharmacophores at positions 1 and 2. The functional groups decorating the glucosamine scaffold were introduced in a particular order and common protecting groups were employed to establish a robust synthetic process.

Inhibition of tissue neovascularization has presented a major therapeutic advantage in the treatment of cancer and age-related macular degeneration (AMD)[1] where several anti-angiogenic drugs are currently approved. The concept of presenting pharmacophoric groups around carbohydrate motifs to bind to pharmacologically important receptors has been exploited in the past and was first published by Hirschmann et al. and other groups.[2] *Alchemia* has developed a VAST™ (Versatile Assembly on Stable Templates) drug discovery platform,[3a,b] a technology based on carbohydrate scaffolds, which are used in the production of focused libraries. Using the VAST™ technology, lead candidates have been produced, which appear through their anti-angiogenic activity to have application in the therapeutic areas oncology, AMD and diabetic retinopathy.

During the non-clinical characterization of a VAST™ anti-cancer lead candidate, it was demonstrated that this compound was microsomally metabolized into a novel intermediate, which exhibited a long half-life and novel structural elements. Its structure is shown in Scheme 1 and the compound is further referred to as **ACL 21269**. To further elucidate the pharmacokinetic properties of this compound, a rapid synthetic access was developed utilizing readily available thioglycoside **9**[4] (Scheme 4) as starting material.

We devised a synthetic strategy (Scheme 4), which involved 12 protecting group manipulations around a D-glucosamine motif. Common protecting groups were applied to facilitate scalability of the individual steps. It was important to decorate the scaffold with the pharmacophoric groups in a certain order and to use properly adjusted protecting groups. Therefore, the 2-naphthylmethyl moiety (2NM) was introduced first, and the position 6 was O-methylated only after protecting the 4-OH-group. The acid labile *m*-hydroxyphenethyl moiety **8** was introduced via glycosylation to the protected thioglycoside **16**. The benzoyl protection in the aglycon **8** further enhanced the stability of the phenethyl glycoside **17α**, towards acidic cleavage. After reduction of the 2-azido group, the γ-aminobutyrate ('GABA') side chain was introduced under standard coupling conditions utilizing γ-azidobutyrate **6** as the synthon. The overall deprotection was achieved in two steps to produce the target molecule in high yields.



Scheme 1. ACL 21269 was synthesized using *p*-thiocresyl glycoside **9** and synthons **8** and **6** for introducing the pharmacophores at positions 1 and 2.

The synthesis of the GABA-synthon **6** was executed in five steps, starting from *N*-DTPM-protected γ-aminoalcohol **1**[5a–c] and is shown in Scheme 2. Silylation of alcohol **1** followed by removal of the amino protection and ensuing diazo transfer reaction[6] led to γ-azido-butanol **4**. Subsequent removal of the silyl ether in **4**

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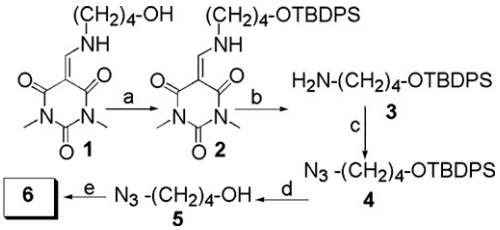
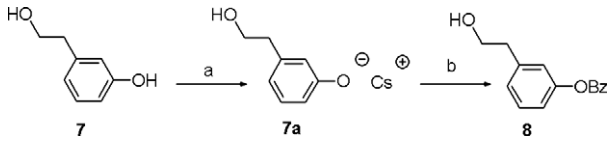


Scheme 2. Synthesis of γ-azido butyric acid **6**. Reagents and conditions: (a) TBDPSCl, imidazole, DMF, (89%); (b) 5% N_2H_4 in MeOH;[5b] (c) TfN_3, DMAP, MeCN, CH_2Cl_2,[6] (66%, 2 steps); (d) TBAF, HOAc, THF (77%); (e) NaOCl, $NaClO_2$, TEMPO, pH 7.8, H_2O, CH_3CN[7] (88%).

followed by TEMPO-mediated oxidation of the hydroxy group with $NaOCl/NaClO_2$ in a phosphate buffer[7] facilitated the carboxylic acid **6** as a brown viscous liquid.

The synthesis of the benzoyl protected *m*-hydroxyphenethyl aglycon **8** was achieved in two steps, starting from unprotected *m*-hydroxyphenethyl alcohol **7** (Scheme 3). First, alcohol **7**[8a] was transformed into the cesium phenolate **7a**. Subsequent reaction of crude **7a** with benzoyl chloride under vigorous stirring produced aglycon **8** in good yield. Finally, purification was accomplished by

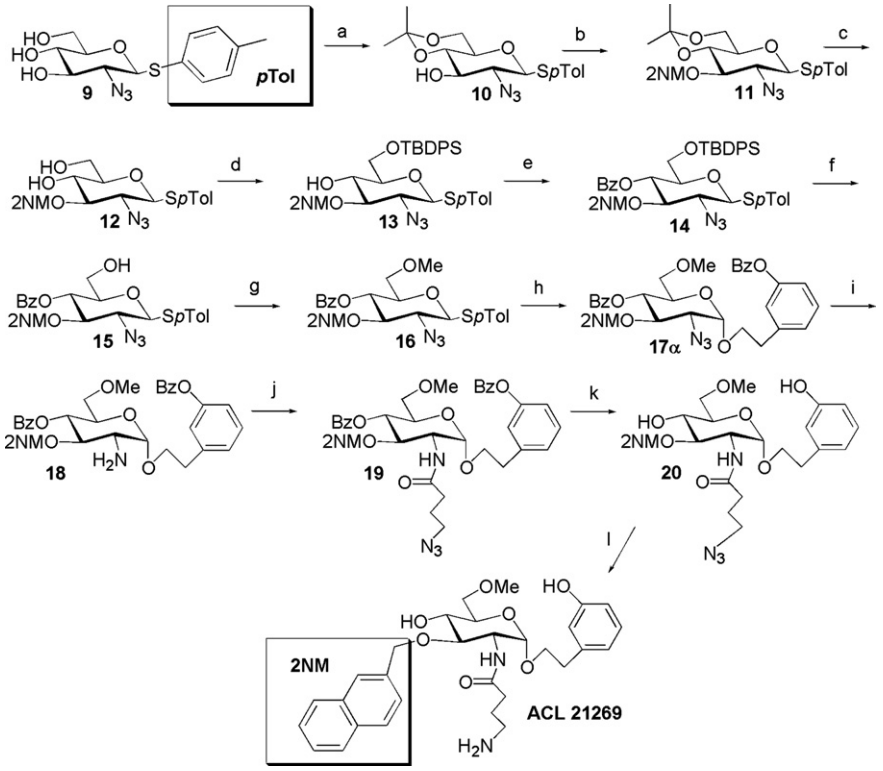


Scheme 3. Synthesis of aglycon **8**. Reagents and conditions: (a) CsOH, H_2O; (b) BzCl, CH_2Cl_2 (73%, 2 steps).

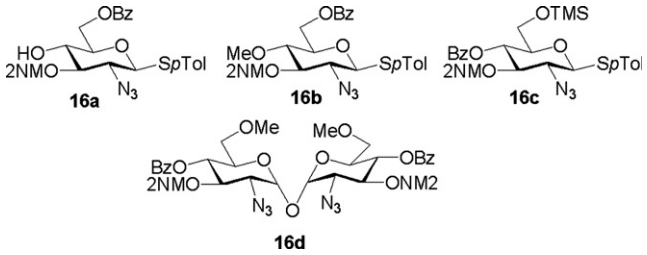
aqueous work-up and short silica gel column chromatography to furnish compound **8** as a pale yellow syrup.

Having prepared the side chain synthons **6** and **8**, the synthesis of thioglycoside **16** was next pursued (Scheme 4). The first six steps of the synthesis (steps a–f) were performed under standard conditions and did not involve any chromatography, since most of the intermediates were obtained in high purities via precipitation. It is noteworthy that due to limited steric access to the 4-OH-group in **13**, the benzoylation to compound **14** required heating (60 °C), the addition of DMAP and extended reaction time. The introduction of the methyl ether on **15** (step g) was initially attempted under basic conditions using ^{t}BuOK as base[9a] and varying the solvents and temperatures (data not shown). In all cases, we observed migration of the benzoyl group to the 6 position (**16a**, Scheme 5) concomitant with varied amounts of 4-O-methylation (**16b**, Scheme 5). The product **16** and side products **16a** and **16b** appeared at well separated retention times on the HPLC and were also isolated and the structures verified via NMR spectroscopy.[10a–c] Use of milder conditions such as MeOTf/2,6-DTBP[9b] gave sluggish reactions with multiple side products.

We next shifted our focus to perform the transformation under acidic conditions. The obvious choice for this purpose would have been the use of diazomethane.[11a,b] However, due to its hazardous nature, we replaced it with $TMSCHN_2$.[11c] Stirring of **15** with $TMSCHN_2$ over silica gel[11d] in CH_2Cl_2 also resulted in a sluggish reaction. Therefore, we next applied stronger acids to effect this conversion. Methylation via aqueous HBF_4 and $TMSCHN_2$[11e] gave ~70% of the product **16** and trehalose **16d**[10d] (Scheme 5) as the major byproduct. Alternatively, $BF_3{\cdot}OEt_2$ as Lewis acid[11f] and $TMSCHN_2$ facilitated the conversion in a clean fashion but stopped at ~50% conversion with **16c** (Scheme 5) as the only side product. We modified the conditions such that, following methylation, the



Scheme 4. Synthesis of **ACL 21269** starting from thioglycoside **9**. Reagents and conditions: (a) $(CH_3)_2C(OMe)_2$, CSA, DMF, (72%); (b) 2-NMBr, DMF, NaH, 0 °C (98%); (c) TFA, CH_2Cl_2, H_2O (95%); (d) TBDPSCl, imidazole, DMF, 60 °C, 2 h; (e) BzCl, pyridine, DMAP, 60 °C, 18 h (92%, 2 steps); (f) TBAF, HOAc, THF, 18 h (91%); (g) (i) $TMSCHN_2$, cat. $BF_3{\cdot}OEt_2$, CH_2Cl_2, 0 °C; (ii) TBAF, HOAc, THF (48%); (h) **8**, MeOTf, diethyl ether, MS 4 Å, 5 °C to rt (31% of **17α**, 19% of **17β**); (i) Zn, NH_4Cl_{aq}, DMF, MeOH, (98% crude); (j) $N_3{-}(CH_2)_3{-}CO_2H$ (**6**), DCC, DIPEA, CH_2Cl_2 (85%); (k) 1 M LiOH, H_2O_2, THF, 18 h (87%); (l) Zn, NH_4Cl_{aq}, DMF, MeOH, 2 h (91%).



Scheme 5. Observed side products during the methylation of **15**.

crude reaction mixture was treated with TBAF. Subsequent chromatography furnished product **16** in 48% yield with almost complete recovery of unreacted starting material **15**.

For the coupling of thioglycoside **16** with aglycon **8** (step h), we evaluated NIS/TMSOTf[12a] and MeOTf[12b,c] as promoters in combination with varying solvents and temperatures. These experiments showed that the β-glycoside **17β**[13a] was the preferred product (data not shown). The best α-selectivities were obtained with MeOTf in diethyl ether at ambient temperatures (0–25 °C) forming **17α**[13b] in moderate yields. Despite the non-stereoselective reaction, the two stereoisomers could be separated with ease via silica gel column chromatography. Further improvements to enhance the stereoselectivity might be possible by examining different leaving groups (e.g., sulfoxides,[12d] trichloroacetimidates[12e] or phosphates[12f]) or the use of more powerful promoters (e.g., *N*-(phenylthio)-ε-caprolactam[12g]).

The reduction of the azido group in **17α** was attempted with Zn under mild acidic conditions (step i) to produce 2-amino compound **18**. The choice of a proper protected GABA-synthon was crucial for coupling to **18**. Initially, we attempted to use commercially available, N-protected GABA synthons Fmoc-GABA,[8b] Boc-GABA[8c] and DTPM-GABA,[14] but only low coupling yields were obtained (data not shown). Therefore, γ-azido butyric acid **6** was selected as the side chain synthon, which offers the advantage of releasing the amino group under mild reaction conditions. As expected, the DCC mediated coupling (step j) proceeded in a rapid and clean fashion to afford the product **19** in high yield after normal phase purification. The deprotection of **19** to the target molecule was achieved in two steps. In the first step, the two benzoyl moieties were removed via LiOH mediated saponification (step k) under aqueous conditions to furnish compound **20** in high yield. The final target, **ACL 21269**, was obtained via reduction of the γ-azido group under mild acidic conditions. The product was obtained after filtration, extractive workup and freeze drying as a white amorphous solid. Its identity was confirmed via LCMS-analysis and NMR-spectroscopy.[15]

References and notes

1. Ferrara, N.; Kerbel, R. S. *Nature* **2005**, *438*, 967. and references cited therein.
2. (a) Hirschmann, R.; Nicolaou, K. C.; Pietranico, S.; Salvino, J.; Leahy, E. M.; Arison, B.; Cichy, M. A.; Spoors, P. G.; Shakespeare, W. C.; Sprengeler, P. S.; Hamley, P.; Smith, A. B., III; Reisine, T.; Raynor, K.; Donaldson, C.; Vale, W.; Maechler, L.; Freidinger, R. M.; Cascieri, M. A.; Strader, C. D. *J. Am. Chem. Soc.* **1993**, *115*, 12550; (b) Wunberg, T.; Kallus, C.; Opatz, T.; Henke, S.; Schmidt, W.; Kunz, H. *Angew. Chem., Int. Ed.* **1998**, *37*, 2503; (c) Sofia, M. J.; Hunter, R.; Chan, T. Y.; Vaughan, A.; Dulina, R.; Wang, H.; Gange, D. *J. Org. Chem.* **1998**, *63*, 2802; (d) Kallus, C.; Opatz, T.; Wunberg, T.; Schmidt, W.; Henke, S.; Kunz, H. *Tetrahedron Lett.* **1999**, *40*, 7783.
3. (a) Becker, B.; Condie, G. C.; Thanh Le, G.; Meutermanns, W. *Mini-Rev. Med. Chem.* **2006**, *6*, 1; (b) Thanh Le, G.; Abbenante, G.; Becker, B.; Grathwohl, M.; Halliday, J.; Tometzki, G.; Zuegg, J.; Meutermanns, W. *Drug Discovery Today* **2003**, *8*, 701.
4. Compound **1** is accessible from 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl acetate[4d] in 3 steps: (a) *p*-Cres-SH, $SnCl_4$, CH_2Cl_2[4e]; (b) $(H_2NCH_2)_2$, *n*-BuOH, 100 °C;[4g] (c) Tf–N_3,[4f] $CuSO_4$, Et_3N, CH_2Cl_2, MeOH (63%, 3 steps); (d) Lemieux, R. U.; Takeda, T.; Chung, B. Y. *A.C.S. Symp. Ser.* **1976**, *39*, pp 90–115; (e) Loenn, H. *Carbohydr. Res.* **1985**, *139*, 105; (f) Alper, P. B.; Hung, S.-C.; Wong, C.-H. *Tetrahedron Lett.* **1996**, *37*, 6029; (g) Kanie, O.; Crawley, S. C.; Palcic, M. M.; Hindsgaul, O. *Carbohydr. Res.* **1993**, *243*, 139.
5. (a) Singh, L.; Seifert, J. *Tetrahedron Lett.* **2001**, *42*, 3133; (b) Dekany, G.; Bornaghi, L.; Papageorgiou, J.; Taylor, S. *Tetrahedron Lett.* **2001**, *42*, 3129; (c) compound **1** is accessible by reaction of 4-amino-1-butanol with a solution of DTPM-NMe_2 in MeOH.[5b]
6. Vasella, A.; Witzig, Ch.; Chiara, J.-L.; Martin-Lomas, M. *Helv. Chim. Acta* **1991**, *74*, 2073.
7. Zhao, M.; Li, J.; Mano, E.; Song, Z.; Tschaen, D. M.; Grabowski, E. J. J.; Reider, P. J. *J. Org. Chem.* **1999**, *64*, 2564.
8. (a) 3-Hydroxyphenethyl alcohol was purchased from Sigma Aldrich.; (b) Fmoc-4-aminobutyric acid (Fmoc-GABA) was purchased from Neo MPS (Strasbourg, France).; (c) Boc-γ-Aminobutyric acid (Boc-GABA) was purchased from Nova Biochem.
9. (a) Greene, T. W.; Wuts, P. G. M. *Protecting Groups in Organic Synthesis*, 3rd ed.; John Wiley & Sons, 1999. pp 23–27; (b) Marshall, J. A.; Xie, S. *J. Org. Chem.* **1995**, *60*, 7230.
10. Selected NMR data of intermediates; ^{1}H NMR spectra were recorded at 400 MHz, ^{13}C NMR at 100 MHz; the solvent is mentioned in brackets after the compound number, (a) Compound **16** ($CDCl_3$); ^{1}H NMR: δ = 5.17 (dd, 1H, $J_{3,4}\sim J_{4,5}$ = 9.6 Hz, H-4), confirming a benzoyl ester at position 4.(b) Compound **16a** ($CDCl_3$); ^{1}H NMR: δ = 4.74 (dd, 1H, $J_{5,6a}$ = 3.5 Hz, J_{gem} = 11.9 Hz, H6a), 4.56 (dd, 1H, $J_{5,6b}$ = 2.2 Hz, J_{gem} = 11.9 Hz, H6b), 4.41 (d, 1H, $J_{1,2}$ = 10.1 Hz, H-1β), 3.54 (m, 1H, H-5), 3.49 (dd, 1H, not assigned), 3.43 (dd, 1H, not assigned), 3.27 (dd, 1H, not assigned).(c) Compound **16b** ($CDCl_3$); ^{1}H NMR: δ = 4.74 (dd, 1H, $J_{5,6a}$ = 2.2 Hz, J_{gem} = 11.9 Hz, H6a), 4.46 (dd, 1H, $J_{5,6b}$ = 4.4 Hz, J_{gem} = 11.9 Hz, H6b), 4.36 (d, 1H, $J_{1,2}$ = 10.1 Hz, H-1β), 3.56 (s, 3H, OCH_3), 3.55 (m, 1H, H-5), 3.51 (dd, 1H, not assigned), 3.32–3.25 (2dd, 2H, not assigned).(d) Compound **16d** ($CDCl_3$); ^{1}H NMR: δ = 5.81, 5.67 (2d, 2H, *J* = 2.6 Hz, H-1α, H-1α′), 5.47 (dd, 2H, $J_{3,4}\sim J_{4,5}$ = 9.8 Hz, H-4, H-4′), 4.96, 4.70 (2d, 4H, J_{gem} = 11.1 Hz, CH_2-2NM), 4.19 (m, 2H, H-5, H-5′), 4.17 (dd, 2H, $J_{2,3}$ = 9.8 Hz, H-3, H-3′), 3.68, 3.62 (2dd, 2H, $J_{2,3}$ = 10.1 Hz, $J_{1,2}$ = 2.6 Hz, H-2, H-2′), 3.54 (dd, 2H, *J* = 2.6 Hz, J_{gem} = 11.0 Hz, H-6a, H-6a′), 3.44 (dd, 2H, *J* = 6.0 Hz, H-6b, H-6b′), 3.31 (s, 6H, 2 × OCH_3).
11. (a) Pizey, J. S. *Synthetic Reagents*; Wiley: New York, **1974**; Vol. 2, pp 65–142.; (b) Hudlicky, M. *J. Org. Chem.* **1980**, *45*, 5377; (c) Shioiri, T.; Aoyama, T.; Mori, S. *Org. Synth.* **1990**, *68*, 1; (d) Ohno, K.; Nishiyama, N.; Nagase, H. *Tetrahedron Lett.* **1979**, *20*, 4405; (e) Aoyama, T.; Shioiri, T. *Tetrahedron Lett.* **1990**, *31*, 5507; (f) Chavis, C.; Dumont, F.; Wightman, R. H.; Ziegler, J. C.; Imbach, J. L. *J. Org. Chem.* **1982**, *47*, 202.
12. (a) Veeneman, G. H.; van Leeuwen, S. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 1331; (b) Loenn, H. *Carbohydr. Res.* **1985**, *139*, 105; (c) Loenn, H. *Carbohydr. Res.* **1985**, *139*, 115; (d) Yan, L.; Kahne, D. *J. Am. Chem. Soc.* **1996**, *118*, 9239. and references cited therein; (e) Schmidt, R. R. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 212; (f) Plante, O. J.; Andrade, R. B.; Seeberger, P. H. *Org. Lett.* **1999**, *1*, 211; (g) Duron, S. G.; Polat, T.; Wong, C.-H. *Org. Lett.* **2004**, *6*, 839.
13. Selected NMR data of intermediates; ^{1}H NMR were recorded at 400 MHz, ^{13}C NMR at 100 MHz; the solvent is mentioned in brackets after the compound number,(a) Compound **17β** ($CDCl_3$); ^{1}H NMR: δ = 5.25 (dd, 1H, $J_{3,4}\sim J_{4,5}$ = 9.3 Hz, H-4), 4.92 (d, 1H, J_{gem} = 11.6 Hz, CH_2-2NM), 4.76 (d, 1H, J_{gem} = 11.6 Hz, CH_2-2NM), 4.38 (d, 1H, $J_{1,2}$ = 7.3 Hz, H-1 **β**), 3.36 (s, 3H, O**CH**$_3$).(b) Compound **17α** ($CDCl_3$); ^{1}H NMR: δ = 5.30 (dd, 1H, $J_{3,4}\sim J_{4,5}$ = 9.6 Hz, H-4), 5.01 (d, 1H, $J_{1,2}$ = 3.4 Hz, H-1α), 4.93 (d, 1H, J_{gem} = 11.3 Hz, CH_2-2NM), 4.76 (d, 1H, J_{gem} = 11.3 Hz, CH_2-2NM), 4.13 (dd, 1H, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 3.97 (m, 1H, α-C**H**$_2$), 3.84 (m, 1H, α-C**H**$_2$), 3.58 (m, 1H, H-5), 3.48 (dd, 1H, $J_{1,2}$ = 3.4 Hz, $J_{2,3}$ = 9.8 Hz, H-2), 3.37 (dd, 1H, $J_{5,6a}$ = 2.7 Hz, $J_{6a,6b}$ = 10.8 Hz, H-6a), 3.32 (dd, 1H, $J_{5,6b}$ = 5.8 Hz, $J_{6a,6b}$ = 10.8 Hz, H-6b), 3.26 (s, 3H, O**CH**$_3$), 3.04 (m, 2H, β-C**H**$_2$). ^{13}C NMR: δ = 165.50, 165.44 (C=O), 97.82 (C-1), 36.10 (α-CH_2), 30.04 (β-CH_2).
14. DTPM-γ-aminobutyric acid (DTPM-GABA) was prepared from **1** via oxidation as described in Scheme 2, step e (71%).
15. **ACL 21269**: *m*/*z* = 539.115 $[M+1]^+$; 1077.228 $[2M+1]^+$; ^{1}H NMR (400 MHz, CD_3CN): δ = 7.88–7.78 (m, 4H, NM), 7.52–7.43 (m, 3H, NM), 7.06 (t, 1H, H-5 Ph, $J_1\sim J_2$ = 8.0 Hz), 6.73–6.70 (m, 2H, H-6 Ph, H-2 Ph), 6.62 (m, 1H, H-4 Ph), 5.97 (d, 1H, NH, *J* = 9.3 Hz), 4.92 (d, 1H, J_{gem} = 11.7 Hz, CH_2-2NM), 4.76 (d, 1H, J_{gem} = 11.7 Hz, CH_2-2NM), 4.59 (d, 1H, H-1α, $J_{1,2}$ = 3.5 Hz), 3.95 (ddd, 1H, H-2, $J_{2,3}$ = 10.2 Hz, $J_{1,2}$ = 3.5 Hz, $J_{NH,2}$ = 9.9 Hz), 3.88 (m, 1H, α-C**H**$_a$), 3.61–3.45 (m, 6H, H-5, H-4, H-6a, H-6b, H-3, α-C**H**$_b$), 3.31 (s, 3H, O**CH**$_3$), 2.79 (m, 2H, β-C**H**$_2$), 2.59 (t, 2H, J_{vic} = 6.5 Hz, α′-C**H**$_2$), 2.02 (m, 2H, γ′-C**H**$_2$), 1.53 (quin, 2H, *J* = 7.1 Hz, β′-C**H**$_2$). ^{13}C NMR (100 MHz, CD_3CN): δ = 172.45 (C=O), 157.14 (C-3 Ph), 141.41 (C-1 Ph), 129.70 (C-5 Ph), 120.61 (C-6 Ph), 117.33 (C-2 Ph), 114.73 (C-4 Ph), 98.19 (C-1α), 80.65 (C-3), 73.86 (CH_2-NM), 72.72 (C-6), 71.49 (C-4), 70.53 (C-5), 68.34 (α-C**H**$_2$), 59.65 (O**C**H_3), 51.94 (C-2), 35.62 (β-C**H**$_2$), 34.06 (α′-C**H**$_2$), 25.73 (γ′-C**H**$_2$), 25.06 (β′-C**H**$_2$).