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Synthesis and conformational studies of 3,4-di-*O*-acylated furanoid sugar amino acid-containing analogs of the receptor binding inhibitor of vasoactive intestinal peptide☆

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Abstract—Structural analysis of the di-*O*-caprylated Gaa-containing analog of the receptor binding inhibitors of vasoactive intestinal peptide by various NMR techniques and constrained molecular dynamics (MD) simulation studies established a well-defined β-turn structure in DMSO-d_6 with an intramolecular hydrogen bond between TyrNH → MetCO.

Receptors for vasoactive intestinal peptide (VIP),[1] a widely distributed naturally occurring neuropeptide, are over-expressed in a variety of malignant tumor cells that are also associated with the synthesis and secretion of detectable levels of VIP by the malignant cells themselves.[2] VIP acts as a growth factor and plays a dominant role in the sustained or indefinite proliferation of cancer cells. Therefore, VIP receptor binding inhibitors have the potential to arrest the growth of malignant cells.[3] The peptide sequence Leu[1]-Met[2]-Tyr[3]-Pro[4]-Thr[5]-Tyr[6]-Leu[7]-Lys[8] **1** is known to be one such VIP receptor binding inhibitor.[4] The role of this octapeptide as a VIP receptor binding inhibitor[5] and its anti cancer activities in combination with other neuropeptide analogs[6] have been well established. Several novel analogs of this peptide containing α,α-dialkylated amino acids and its lipoconjugates have been synthesized and tested for their anti cancer activities.[7] We have earlier developed several analogs of octapeptide **1** by replacing some of its amino acids by dideoxy furanoid sugar amino acids.[8] Many of these analogs, such as the tetrapeptide **2**, showed either retention or enhancement of biological activities.[9]

However, for the development of peptides as novel therapeutic agents, it is essential to have them delivered efficiently to their specific sites of action.[10] To achieve this, many peptides have been modified covalently by attaching fatty acid moieties to their *C*- or *N*-termini.[11] As part of our ongoing project on sugar amino acid based molecular designs,[12] we were interested in developing 3,4-di-*O*-acylated derivatives of the peptide **2** for improved therapeutic applications. It was also essential for us to ensure that the acylated analogs do not deviate from the bioactive conformation of the native peptide. In this connection, we have studied earlier the acylated derivatives of the Gaa-containing analogs of Leu-enkephalin.[13] Herein, we describe the synthesis of acylated derivatives **3**–**5** of the VIP receptor binding inhibitor **2** and the detailed conformational analysis of the di-*O*-caprylated analog **4** by various NMR techniques and constrained molecular dynamics (MD) simulation studies that established a well-defined β-turn structure in DMSO-d_6, similar to that seen earlier in **2**,[8] with an intramolecular hydrogen bond between TyrNH → MetCO.

Scheme 1 outlines the synthesis of the 3,4-di-*O*-acylated peptides **3**–**5**. The acylated sugar amino acids[14] were synthesized following a new route which gave improved yields of the target molecules compared with our earlier methods.[13,15]

The primary hydroxyl group of the common starting material **6**, synthesized from D-mannitol following the

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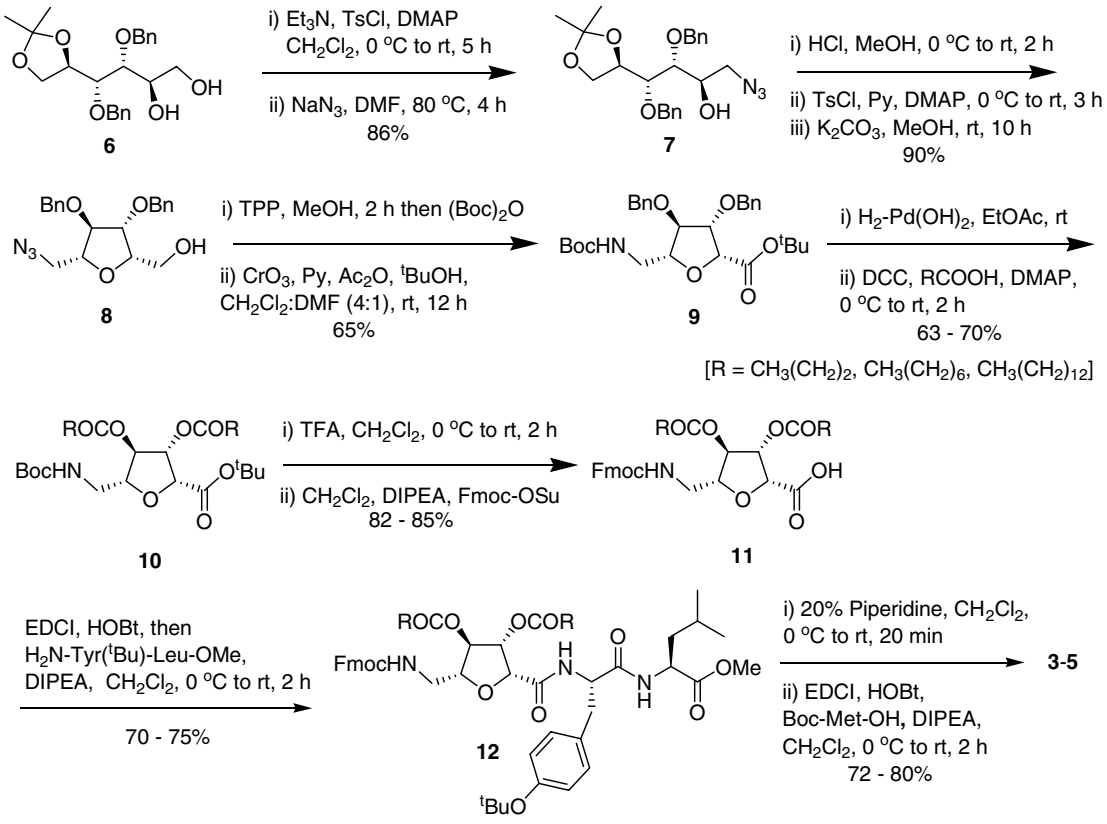
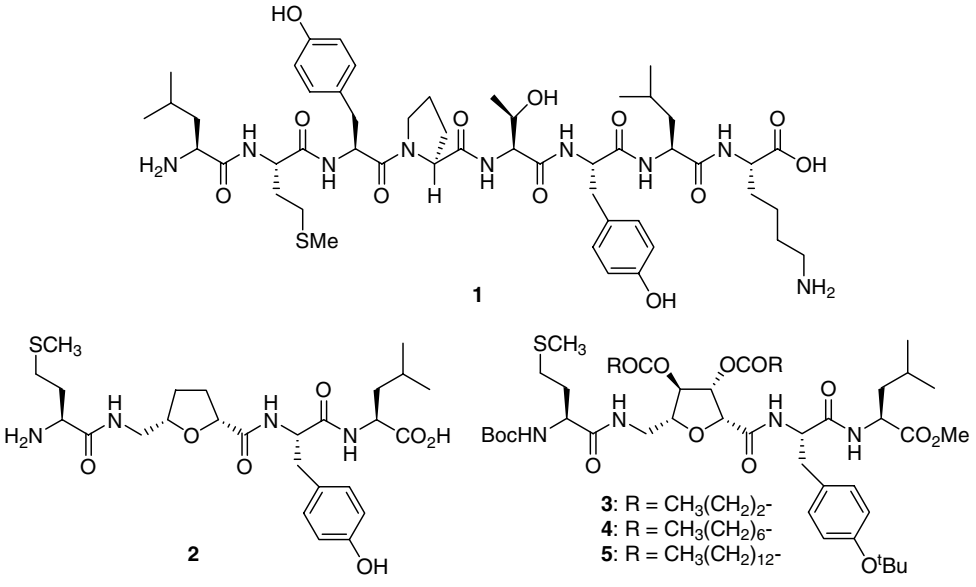


Scheme 1. Synthesis of peptides **3**–**5**.



reported procedure,[16] was converted to an azide in two steps, tosylation followed by reaction with sodium azide, to provide **7** in 86% overall yield. Next, deprotection of the acetonide was accomplished using concd HCl in MeOH followed by selective tosylation using TsCl in pyridine and treatment of the resulting tosyl intermediate with K_2CO_3 generated the tetrahydrofuran **8** in 90% overall yield. Reduction of the azide group using Ph_3P, in situ protection of the resulting amine using $(Boc)_2O$ and oxidation of the free hydroxyl with CrO_3–Py in the presence of acetic anhydride and *t*-butanol provided the *t*-butyl ester of the D-gluconic acid **9** in 65% yield.[17] Removal of the Bn-protective groups was achieved by hydrogenation using $Pd(OH)_2$–C as catalyst to afford a diol intermediate. The diol was acylated by reacting with *n*-alkanoic acid in the presence of 1,3-dicyclohexylcarbodiimide (DCC) and a catalytic amount of 4-dimethylaminopyridine (DMAP) to give the diacy-

Table 1. ^{1}H Chemical shifts (δ in ppm), coupling constants (J in Hz) and temperature coefficients ($\Delta\delta/\Delta T$ in ppb/K) of **4** in DMSO-d_6 at 500 MHz

Amino acid	NH	CαH	CβH	CγH	CδH	Others	$\Delta\delta/\Delta T$
Tyr	7.84 (d, 1H) (J = 8.8)	4.55 (ddd, 1H) (J = 9.2, 8.8, 4.6)	2.98[a] (dd, 1H, J = 13.77, 4.6) 2.89[b] (dd, 1H, J = 13.77, 9.2)			7.14, 6.85 (4H, Ph), 1.25 (s, 9H, ^{t}Bu)	−4.88
Met	6.89 (d, 1H) (J = 8.24)	4.01 (ddd, 1H) (J = 8.24, 8.14, 4.24)	1.89[a] (ddd, 1H, J = 12.7, 8.14, 4.24) 1.78[b] (ddd, 1H, J = 12.7, 3.90, 8.14)	2.43 (ddd, 2H) (J = 12.00, 4.24, 3.90)		1.51 (s, 3H, –S–Me) 1.25 (s, 9H, Boc)	−10.22
Leu	8.39 (d, 1H) (J = 7.45)	4.31 (q, 1H) (J = 7.45)	1.53 (dt, 1H, J = 13.44, 7.45)	1.63 (m, 1H)	0.89 (d, 3H, J = 6.75) 0.85 (d, 3H, J = 6.75)	3.62 (s, 3H, CO_2CH_3)	−6.88

Gaa: NH (8.17, t, 1H, J = 6.0 Hz), H-6 (pro-*S*) (3.51, ddd, 1H, $J_{NH\text{-}C6}$ = 6.0 Hz, $J_{C6\text{-}C6'}$ = 13.44 Hz, $J_{C6\text{-}C5}$ = 10.6 Hz), H-6′ (pro-*R*) (3.25, ddd, 1H, $J_{NH\text{-}C6'}$ = 6.0 Hz, $J_{C6'\text{-}C6}$ = 13.44 Hz, $J_{C6'\text{-}C5}$ = 3.6 Hz), H-5 (3.95, td, 1H, $J_{4,5\text{ and }5,6}$ = 6.7 Hz, $J_{5,6'}$ = 2.1 Hz), H-4 (4.96, m, 1H), H-3 (5.31, dd, 1H, $J_{2,3}$ = 4.6 Hz, $J_{3,4}$ = 1.4 Hz), H-2 (4.45, d, 1H, $J_{2,3}$ = 4.6 Hz), –OCOCH$_2$ (2.19–2.32, m, 4H), –OCOCH$_2$CH_2 (1.42–1.50, m, 4H), other CH$_2$s (1.23–1.26, m, 16H), 0.85 (m, 6H, methyls). $\Delta\delta/\Delta T$ = −6.22.

[a] (pro-*S*).

[b] (pro-*R*).

lated product **10** in 63–70% yields. Treatment of **10** with trifluoroacetic acid (TFA) deprotected both the *C*- and *N*-termini, and the *N*-terminus was protected using Fmoc-OSu to furnish Fmoc-Gaa(OCOR)$_2$-OH **11** in 82–85% yields.

Fmoc-Gaa(OCOR)$_2$-OH **11** was used in the synthesis of VIP receptor inhibitor analogs **3**–**5** via standard solution phase peptide synthesis methods[18] using 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole (HOBt) as coupling agents and dry CH_2Cl_2 as solvent. The tripeptide Fmoc-Gaa(OCOR)$_2$-Tyr(^{t}Bu)-Leu-OMe **12** was prepared by the condensation of Fmoc protected free acid **11** with the dipeptide H-Tyr(^{t}Bu)-Leu-OMe following the above protocol in 70–75% yield. Deprotection of the *N*-terminus of tripeptide **12** with 20% piperidine in dry CH_2Cl_2 and coupling of the resulting free amine with Boc-Met-OH gave the required peptides **3**–**5** in 72–80% yields.[19] The final products were purified by silica gel column chromatography and fully characterized by spectroscopic methods before using them in the conformational studies.

Peptide **4** was completely characterized by 1D and homonuclear ^{1}H–^{1}H 2D experiments, gDQCOSY, TOCSY,[20] and ROESY.[21] The corresponding resonance assignments of **4** are shown in Table 1. Variable temperature studies were carried out to measure the temperature coefficients of the amide proton chemical shifts ($\Delta\delta/\Delta T$), which provided information about their involvement in intramolecular hydrogen bonding[22] and the relative strength of the hydrogen bonds. The intensities of the cross-peaks in the ROESY spectrum, shown in Figure 1, were used to obtain the restraints in the simulated molecular dynamics (MD) calculations. A portion of the ROESY spectrum is shown in Figure 2.

The $^3J_{NH\text{–}C\alpha H}$ values were large (>8 Hz) for Leu and Gaa indicating that the values of ϕ for these residues were in the vicinity of −137°. The populations of the side-chain conformations about Cα–Cβ (χ1) for all the

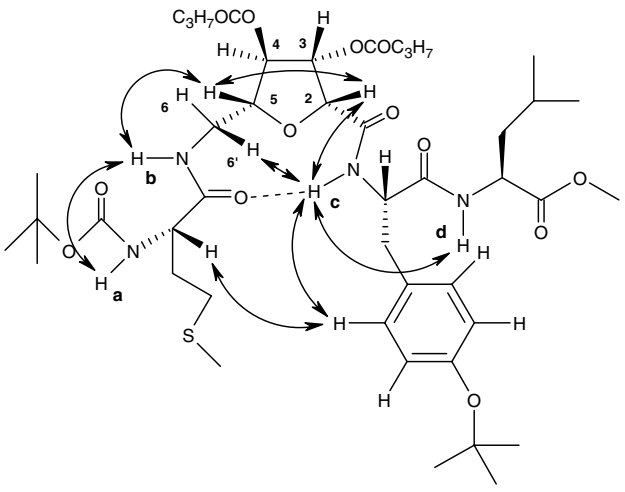


Figure 1. Schematic representation of the ROE cross peaks in the peptide **4**.

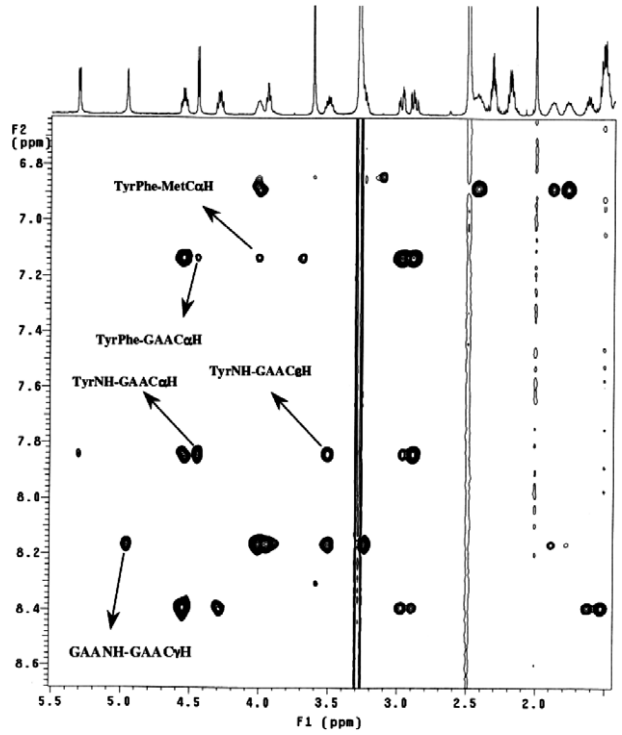


Figure 2. Expansion of some of the important cross peaks in the ROESY spectrum of **4**.

Table 2. The list of distance restraints (more than three bonds away) used in the MD simulation studies

ROEs	Distance (Å)
1. LeuNH ↔ TyrNH	2.3–3.0
2. LeuNH ↔ TyrCγH	3.2–3.9
3. LeuNH ↔ TyrCαH	2.0–2.4
4. LeuNH ↔ LeuCγH	2.6–3.2
5. TyrNH ↔ MetCαH	3.0–3.9
6. TyrNH ↔ GaaCεH	3.0–3.6
7. TyrNH ↔ GaaCαH	2.2–3.0
8. GaaNH ↔ MetNH	2.3–2.8
9. GaaNH ↔ MetCαH	3.0–3.8
10. GaaC2H ↔ GaaC5H	2.5–3.2
11. GaaC3H ↔ GaaC5H	3.1–3.9

amide protons could be estimated from the $J_{\alpha-\beta}$ values shown in Table 1. $J_{\alpha-\beta(\text{pro-}R)}$ and $J_{\alpha-\beta(\text{pro-}S)}$ are 9.2 and 4.6 Hz, respectively, for Tyr, whereas these values are 8.14 and 4.24 Hz, respectively, for Met. The ^3J values for both these residues suggest the g^- rotamer populations about $\chi 1$ to be more than 70%. The predominance of the g^- rotamers about Cα–Cβ is further supported by strong ROESY cross-peaks between NH ↔ $C\beta H_{(\text{pro-}R)}$ and NH ↔ $C\beta H_{(\text{pro-}S)}$ in these residues. The values of $J_{2,3} = 4.6$, $J_{3,4} = 1.4$ and $J_{4,5} = 6.7$ Hz in Gaa and the ROESY cross peak between $GaaC_2H \leftrightarrow C_5H$ support an envelope (C_2-*exo* or C_3-*endo*) conformer for the sugar ring.

The NOE between TyrNH ↔ $GaaC_6H_{(\text{pro-}R)}$ and the magnitude of the temperature coefficient $(\Delta\delta/\Delta T) = -4.88$ ppb/K measured over a range of 25–70 °C, for Tyr-NH, strongly support the presence of a β-turn-like ring structure bridged by a 10-membered intramolecular hydrogen bond between TyrNH → MetCO, similar to that observed earlier by us and others.[13] The molecular structure was further supported by the observed ROE cross-peaks between,TyrNH ↔ $GaaC_2H$, GaaNH ↔ $GaaC_5H$, GaaNH ↔ $GaaC_4H$ and TyrPhe ↔ MetCαH.

A number of interatomic distances derived from intensities of the ROE cross-peaks were used for obtaining the restraints in the MD calculations,[23] by following a simulated annealing protocol.[24] The long-range distance restraints (more than three bonds away) were derived based on a two-spin approximation by taking the distance between the Tyr β-protons (1.8 Å) as an internal standard. The long-range distance restraints shown in Table 2, and H-bonding restraint (1.3–2.5 Å, force constant 30 kcal/Å) were used in the MD calculations. Twenty structures were sampled during the constrained MD simulations carried out for a duration of 120 ps using 20 cycles, each of 6 ps periods, of the simulated annealing protocol. The sample structures were subsequently energy minimized and the superimposition of eleven structures from those sampled is shown in Figure 3. The alignment of the hydrogen bonded parts clearly revealed that the possible conformational arrangement

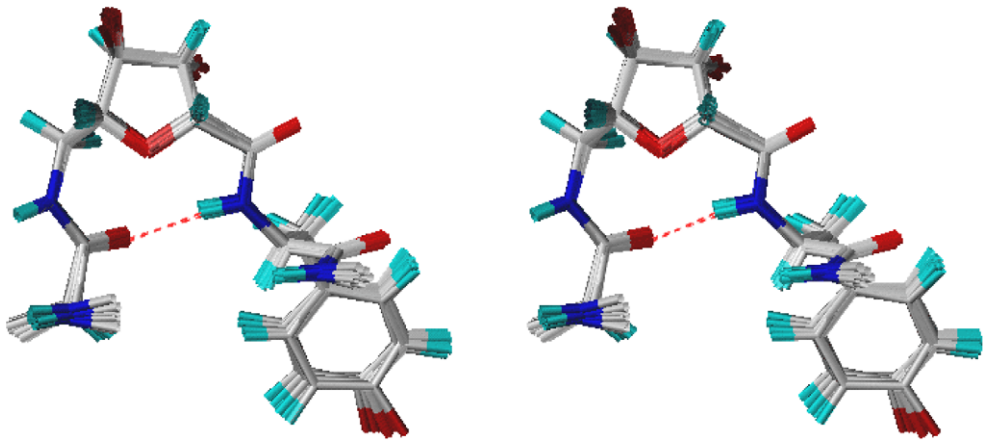


Figure 3. Stereoview of the eleven backbone-superimposed energy-minimized structures sampled during 20 cycles of the 120 ps constrained MD simulations following the simulated annealing protocol. For the sake of clarity in viewing, only the backbones are shown here omitting the amino acid side chains and fatty acid chains.

of this compound involved an intramolecular hydrogen bond between TyrNH → MetCO.

A type II β-turn is generally stabilized by an intramolecular hydrogen bond between the carbonyl group of residue *i* and the amino group of residue $(i+3)$ to form a 10-membered β-turn and is believed to be of great importance for the recognition and activity of peptides and proteins.[25] Our studies show that the acylation of the ring hydroxyls in the sugar ring did not alter the overall structure of the sugar amino acid-containing tetrapeptide **2** and the side-chain acylated compounds, prepared for improved therapeutic applications, retained the bioactive conformation of the molecule characterized by a β-turn involving an intramolecular hydrogen bond between TyrNH → MetCO. Further work is in progress.

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19. *Selected physical data of* **3**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.70 (d, $J=8.8$ Hz, 1H), 7.07 (d, $J=8.2$ Hz, 2H), 6.95 (t, $J=6.6$ Hz, 1H), 6.84 (d, $J=8.2$ Hz, 2H), 6.42 (d, $J=7.9$ Hz, 1H), 5.47 (d, $J=4.6$ Hz, 1H), 5.22 (d, $J=8.7$ Hz, 1H), 4.80 (br s, 1H), 4.64 (q, $J=8.1$ Hz, 1H), 4.57 (d, $J=4.6$ Hz, 1H), 4.46 (dq, $J=8.3$, 5.3 Hz, 1H), 4.21 (q, $J=7.5$ Hz, 1H), 4.02 (d, $J=4.6$ Hz, 1H), 3.62 (m, 4H), 3.01 (dt, $J=7.5$, 13.8 Hz, 2H), 2.49 (t, $J=7.5$ Hz, 2H), 2.25 (dt, $J=2.2$, 7.8 Hz, 2H), 2.19 (dt, $J=3.5$, 7.8 Hz, 2H), 2.03 (m, 4H), 1.87 (d, $J=7.5$ Hz, 1H), 1.77 (br s, 1H), 1.58–1.51 (m, 6H), 1.38 (s, 9H), 1.25 (s, 9H), 0.89 (t, $J=7.5$ Hz, 3H), 0.83 (m, 9H); MS (ESIMS) *m*/*z* (%) 895 (30) $[M+1]^+$, 917 (99) $[M+Na]^+$. *Selected physical data of* **4**: ^{1}H NMR (DMSO-d_6,

500 MHz) listed in Table 1; MS (ESI) *m*/*z* (%) 1007 (48) $[M+1]^+$, 1029 (100) $[M+Na]^+$. *Selected physical data of* **5**: ^1H NMR ($CDCl_3$, 200 MHz); δ 7.75 (d, $J = 8.5$ Hz, 1H), 7.61 (d, $J = 7.4$ Hz, 1H), 7.13 (d, $J = 8.2$ Hz, 3H), 6.87 (d, $J = 8.2$ Hz, 2H), 6.49 (d, $J = 8.9$ Hz, 1H), 5.49 (d, $J = 5.3$ Hz, 1H), 5.31 (d, $J = 8.1$ Hz, 1H), 4.82 (s, 1H), 4.68 (m, 1H), 4.61 (d, $J = 5.3$ Hz, 1H), 4.48 (m, 1H), 4.22 (m, 1H), 4.03 (m, 1H), 3.68 (s, 3H), 3.51 (m, 2H), 3.07 (d, $J = 7.0$ Hz, 1H), 2.52 (m, 1H), 2.34–2.22 (m, 4H), 1.98–1.89 (m, 2H), 1.58 (m, 12H), 1.31–1.25 (m, 56H), 0.88 (m, 12H); MS (ESIMS) *m*/*z* (%) 1175 (30) $[M+1]^+$, 1197 (100) $[M+Na]^+$.

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