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Synthesis of N^1-Substituted Analogues of (2*R*,4*R*)-4-Aminopyrrolidine-2,4-dicarboxylic Acid as Agonists, Partial Agonists, and Antagonists of Group II Metabotropic Glutamate Receptors

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Abstract—The chemical synthesis of a series of N^1-substituted derivatives of (2*R*,4*R*)-4-aminopyrrolidine-2,4-dicarboxylic acid [(2*R*,4*R*)-APDC] as constrained analogues of γ-substituted glutamic acids is described. Appropriate substitution of the N^1-position results in agonist, partial agonist, or antagonist activity at mGluR2, mGluR3, and/or mGluR6.

The amino acid glutamate plays a pivotal role in biological processes ranging from memory and learning to neuronal degeneration. This major excitatory amino acid (EAA) acts through disparate glutamate receptors, which can be categorized into two distinct types, the so-called ionotropic receptors (iGluRs) and the metabotropic receptors (mGluRs).[1] iGluRs are associated with integral cation-specific ion channels and include the *N*-methyl-D-aspartate (NMDA), 2-amino-3-(5-methyl-3-hydroxyisoxazol-4-yl)propanoic acid (AMPA), and kainate subtypes. On the other hand, mGluRs are coupled to cellular effectors through GTP-binding proteins. Pharmacologically, mGluRs have been distinguished from the iGluRs by the use of the mGluR-selective agonist, (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid [(1*S*,3*R*)-ACPD], generally through measurements involving phosphoinositide (PI) hydrolysis or Ca^{2+} mobilization. To date the use of expression cloning techniques has led to the identification of eight mGluR subtypes, which have been placed into three major categories based on their molecular structure, signal transduction mechanisms, and pharmacological properties. Group I mGluRs (mGluR1 and 5) are coupled to PI hydrolysis, whereas group II (mGluR2 and 3) and group III (mGluR4, 6, 7, and 8) are negatively linked to adenylyl cyclase activity. The group I receptors are more sensitive to quisqualic acid than they are to ACPD, the group II receptors are more sensitive to ACPD than to quisqualic acid, and the group III receptors are most sensitive to 2-amino-4-phosphonobutyric acid (L-AP4).[2]

In order to better characterize the roles of GluRs in physiological processes,[3–5] there is an important need to identify novel, high affinity ligands that are family and subtype specific.[6] While a number of mGluR selective compounds (Scheme 1) have been described to date, such as LY354740 and its heteroatom analogues,[7] APDC,[8] ABHxD-I,[9] 1-benzyl-APDC,[10] and 1-amino-APDC,[11] the goal of having agonists, partial agonists, and antagonists of exquisite selectivity for each of the known subtypes has not been fully achieved. Herein we report on additional N^1-substituted analogues of (2*R*,4*R*)-4-aminopyrrolidine-2,4-dicarboxylic acid (APDC). Of interest is our finding that depending upon the nature of the ring nitrogen substitutent, one is able to identify ligands capable of acting as agonists, partial agonists, or antagonists at mGluR2 while maintaining the same rigidified glutamate template, namely APDC. As APDC itself is a full agonist of the group II receptors,[8] the

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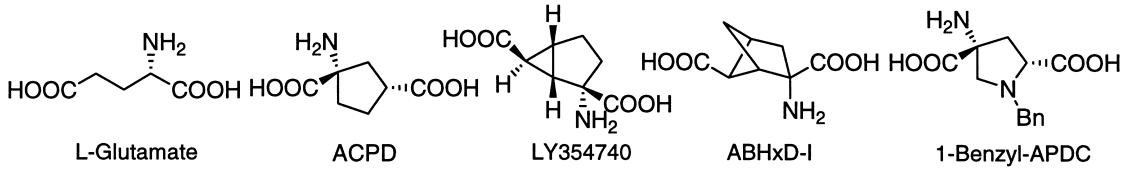
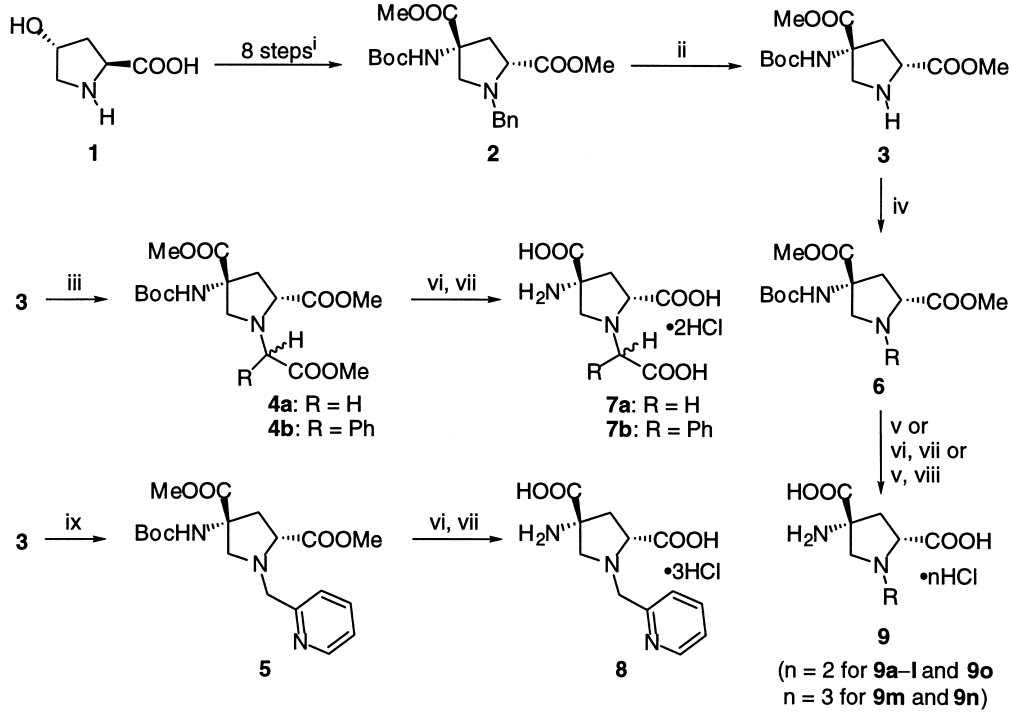
present finding opens up another venue for mGluR compound design.

We have previously reported on synthetic routes to 1-amino-APDC and to 1-benzyl-APDC, and much of the same chemistry was employed in the preparation of the compounds detailed herein. The synthesis of compounds **7a**–**b**, **8**, and **9a**–**o** is depicted in Scheme 2. The intermediate **2** was prepared starting from *cis*-4-hydroxy-D-proline (**1**) following the previously reported procedure.[11,12] Compound **2** was debenzylated by hydrogenolysis over $Pd(OH)_2/C$ to give **3** which is the key intermediate for the preparation of the various N^1-substituted APDC derivatives.

Alkylation at the pyrrolidine ring nitrogen atom (N^1) with a variety of alkylating agents (Scheme 2) was carried out under standard conditions and afforded intermediates **4a**–**b**, **5**, and **6a**–**o** in good yield. The alkylating agent 2-(phenylethynyl)benzyl bromide required for the synthesis of **6o** was prepared by coupling 2-bromobenzaldehyde with phenylacetylene under Pd(0) catalysis



Scheme 1. Some typical ligands exhibiting mGluR activity.



Compound 9	R	Compound 9	R
a	2-hydroxybenzyl	**i**	2-nitrobenzyl
b	3-hydroxybenzyl	**j**	3-nitrobenzyl
c	4-hydroxybenzyl	**k**	4-nitrobenzyl
d	4-methoxybenzyl	**l**	3,5-dinitrobenzyl
e	2-bromobenzyl	**m**	2-aminobenzyl
f	2-carboxybenzyl	**n**	4-aminobenzyl
g	3-carboxybenzyl	**o**	2-(phenylethynyl)benzyl
h	4-carboxybenzyl		

Scheme 2. Synthesis of N^1-substituted analogues of (2*R*,4*R*)-4-aminopyrrolidine-2,4-dicarboxylic acid. Reagents and conditions: (i) see refs 11 and 12; (ii) $Pd(OH)_2/C$, H_2 (1 bar), MeOH, 4 h; (iii) RCH(Br)COOMe, *i*-Pr_2NEt, CH_2Cl_2, rt; (iv) RBr, *i*-Pr_2NEt, CH_2Cl_2, rt (examples **6a**–**b**, **6e**, **6g**–**k**, and **6o**) or RCl, *i*-Pr_2NEt, Bu_4NI (20 mol%), CH_2Cl_2, rt (examples **6c**–**d**, **6f**, and **6l**); (v) 6 N HCl, reflux, 2–3 h; (vi) 1 N NaOH, THF, rt; (vii) 6 N HCl, rt; (viii) NH_2NH_2, Raney-Ni, H_2O, 35–40 °C, 1 N HCl, for the conversion of **9i** and **9k** to **9m** and **9n**, respectively: (ix) 2-pyridylmethyl mesylate, *i*-Pr_2NEt, CH_2Cl_2, rt.

followed by sequential reduction of the aldehyde functionality with sodium borohydride and conversion of the alcohol to bromide with phosphorus tribromide. For the synthesis of compound **5**, the required alkylating agent, 2-pyridylmethyl mesylate, was prepared from 2-pyridylcarbinol by reaction with methanesulfonyl chloride in the presence of triethylamine. Other alkylating agents that are not commercially available were prepared from the corresponding alcohols with phosphorus tribromide. The target amino acids **7b**, **9a**–**b**, and **9e**–**l** were isolated as their hydrochloride salts in quantitative yield from **4b**, **6a**–**b**, and **6e**–**l** by direct hydrolysis with 6 N HCl under reflux conditions. Direct acidic hydrolysis of compounds **4a**, **5**, **6c**, **6d**, and **6o** afforded inseparable mixtures of the desired compounds and the corresponding debenzylated products. To avoid this problem, these intermediates were subjected to sequential hydrolysis of the ester and Boc protecting groups with 1 N NaOH in THF followed by treatment with dilute HCl (pH 1) to produce **7a**, **8**, **9c**, **9d**, and **9o**. The nitro derivatives **9i** and **9k** were additionally converted to the corresponding amines **9m** and **9n** using the Raney nickel–hydrazine system followed by acidification with dilute HCl. All final products and synthetic intermediates[13] exhibited satisfactory spectral and analytical (C, H, N) data.

The activity of these new APDC derivatives was tested in seven cell lines with stable expression of the individual subtypes of metabotropic glutamate receptors. mGluR1a and mGluR5a (group I) receptors were expressed in Chinese hamster ovary (CHO) cells,[14] and activity at these receptors was measured by the assay of agonist-stimulated phosphoinositide (PI) hydrolysis.[9] mGluR2 (group II) and mGluR6 (group III) receptors were expressed in CHO cells, while the mGluR4 receptor (group III) was expressed in baby hamster kidney (BHK) cells.[14] Activity at these receptors was determined by measurement of their ability to decrease the forskolin-induced elevation of cAMP formation.[9] Receptors mGluR3 (group II) and mGluR8 (group III) were expressed in CHO cells in the presence of a chimeric G protein (G_{qi9}) which allows the coupling of these receptors to phospholipase C and, hence, activity measurement by determination of PI hydrolysis.[15] Within each experiment, the results were normalized to the maximal response induced by 1 mM glutamate for mGluR1 and mGluR5, 100 μM glutamate for mGluR2, 100 μM APDC for mGluR3, and by 100 μM 4-aminophosphonobutyrate (AP4) for mGluR4, mGluR6, and mGluR8, and are expressed as percent of the maximal response. Antagonist activity was measured in the presence of the above agonists used at concentrations yielding half-maximal stimulation (EC_{50}). The evaluation of the relative potencies of the tested compounds and the determination of their EC_{50} or IC_{50} values was performed by fitting the normalized data to the logistic equation by nonlinear regression.

Initially, all compounds were tested at 100 μM concentrations for agonist and antagonist activity at all seven mGluRs. None of the compounds showed significant activity (>20%) at mGluR1, mGluR5, mGluR4, or mGluR8 receptors. In contrast, many of the APDC derivatives were active as agonists or antagonists at both group II receptors: mGluR2 (Fig. 1) and mGluR3 (Fig. 2). Several compounds showed also agonist activity at the mGluR6 receptor (Fig. 3). Results from the dose–response experiments are summarized in Table 1. As will be noted, agonist, antagonist, and partial agonist activity is shown by some of these ligands, and such activity is dependent upon subtle variations in the nature of the functionality present in the N^1-substituent.

We have previously reported on the pharmacology of 1-amino-APDC[11] and 1-benzyl-APDC.[10] The former ligand is a partial agonist of group II receptors. At both receptors, the maximal stimulation reaches about 50% of the stimulation obtained with full agonists. The EC_{50} values were found to be 5.5±2.1 μM for mGluR2 and 39±11 μM for mGluR3. On the other hand, the 1-benzyl derivative of APDC showed selectivity very different from that reported for APDC. Tested on CHO cells expressing the various mGluRs, 1-benzyl-APDC was an agonist of mGluR6 with an EC_{50} of 20 μM. In contrast to APDC, 1-benzyl-APDC was not an agonist of mGluR2, in fact it behaved as a weak antagonist with an IC_{50} of 200 μM. Additionally, and subsequent to our

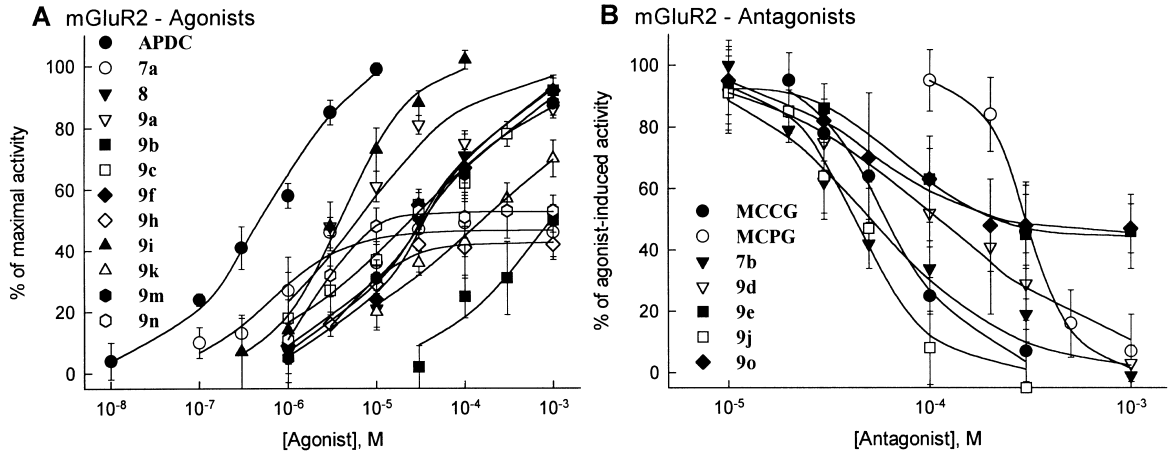


Figure 1. Activity of APDC analogues acting as agonists (A) and antagonists (B) in cells expressing mGluR2 receptors. Activity of antagonists was tested in the presence of 2 μM glutamate. Points represent means and error bars±SEM from at least three experiments performed in triplicate.

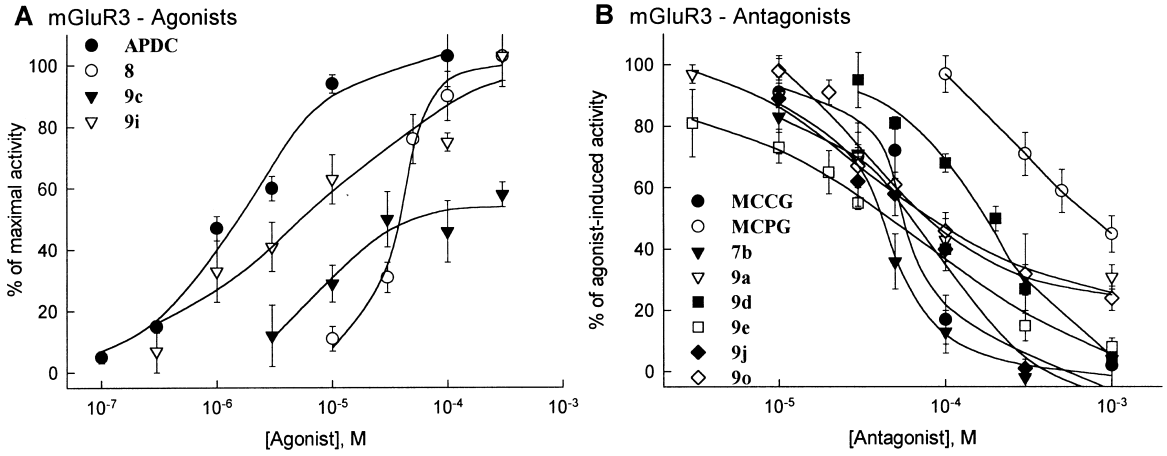


Figure 2. Activity of APDC analogues acting as agonists (A) and antagonists (B) in cells expressing mGluR3 receptors. Activity of antagonists was tested in the presence of 2 μM APDC. Points represent means and error bars ± SEM from at least three experiments performed in triplicate.

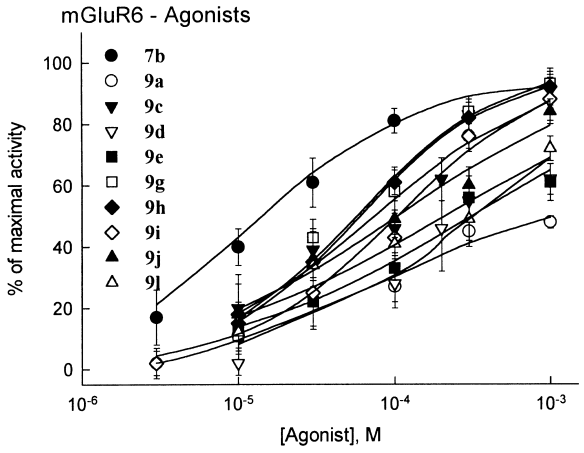


Figure 3. Activity of APDC analogues acting as agonists in cells expressing mGluR6 receptors. Points represent means and error bars ± SEM from at least three experiments performed in triplicate.

report on 1-benzyl-APDC, researchers at the Lilly Company published accounts of their own studies on the activity of a number of monochlorobenzyl, dichlorobenzyl, diphenylalkyl, naphthylmethyl, and phenylalkyl derivatives of APDC, showing that some of these ligands were capable of acting as reasonably potent group II mGluR antagonists.[16] Interestingly, none of the compounds in the Lilly series showed any agonist activity. Thus, in the present study, we chose to extend the SAR of these 1-substituted derivatives of APDC, with the idea to further explore the dependence of their biological activity on the nature of the N^1-substituent. We considered the possibility that an improved potency as well as selectivity might be achieved through variations of the electronic character of the benzene ring by the attachment of appropriate functionality. Additionally, we considered the possibility that the aryl ring substituent might influence agonist versus antagonist activity, based upon the idea that the substituent may either prevent or promote closure of the two lobes that make up the amino terminal domain of the binding site for the mGluRs.[17]

As can be noted from Table 1, all of the newly synthesized compounds are less potent than APDC at group II mGluRs. Of the compounds presented, analogue **7a**, which contains a carboxymethyl group on the ring nitrogen, is particularly interesting, as this compound behaves as a partial agonist at mGluR2 with an apparent EC_{50} value of 610 nM, while being inactive at all other mGluRs. Curiously, the only other compounds to show this partial agonistic character at mGluR2 are the 4-carboxybenzyl derivative **9h** and the 4-aminobenzyl derivative **9n**. Like **7a**, **9h** is also a triacid, but with a larger spacer between the ring nitrogen and the CO_2H group. Its potency is lower than that of **7a**, with an EC_{50} value of 4.7 μM. Compound **9h** also shows weak agonist activity at mGluR6 with an EC_{50} of 58 μM.

Of the various derivatives showing full agonist activity, the most potent of these at mGluR2 are **9a** and **9i**, which bear a 2-hydroxybenzyl ($EC_{50} = 6.1$ μM) or 2-nitrobenzyl group ($EC_{50} = 4$ μM), respectively. Compound **9i** also shows agonist activity at mGluR3. The 2-aminobenzyl and 2-carboxybenzyl derivatives (**9m** and **9f**) also function as agonists, but they are much less potent. However, in contrast to these results, it is interesting to find that compounds **9d**, **9e**, **9j**, and **9o** all act as antagonists or partial antagonists at mGluR2 and mGluR3. Thus, while the 4-hydroxybenzyl derivative **9c**, which contains an extra H-bond donor group, behaves as a weak agonist, the corresponding methoxy derivative is an antagonist. Of the two *ortho*-substituted antagonists, **9o** bearing the larger phenylethynyl group is slightly more potent than the 2-bromo derivative **9e**. The pyridylmethyl analogue **8**, which can be considered to be a bioisostere of **9a**, functions as an agonist at mGluR2, albeit of lower potency. Lastly, compound **7b,** while acting as an antagonist of mGluR2 and mGluR3, shows the best mGluR6 agonist activity.

In summary, as is apparent from the new analogues reported herein, it is possible to alter the pharmacological profile of APDC to arrive at structures capable of functioning as agonists, partial agonists, or antagonists at mGluR2. This alteration of activity is effected through the choice of the substituent borne by the ring

Table 1. Pharmacological characterization of N^1-substituted analogues of APDC at mGluR2, mGluR3, and mGluR6

Compound	MGluR2 (μM[a])		mGluR3 (μM[a])		mGluR6 (μM[a])
	Agonists EC_{50}	Antagonists IC_{50}	Agonists EC_{50}	Antagonists IC_{50}	Agonists EC_{50}
APDC	0.48		1.7		na
7a	0.61[b]		na	na	na
7b		51		21	22
8	37		37		na
9a	6.1			27	88[b]
9b	950		na	na	na
9c	24		7.5[b]		74
9d		110		190	320
9e		77		77	310
9f	33		na	na	na
9g	na	na	na	na	57
9h	4.7[b]		na	na	58
9i	4.0		6.8		110
9j		45		93	100
9k	160		na	na	na
9l	na	na	na	na	210
9m	35		na	na	na
9n	2.3[b]		na	na	na
9o		45[c]		23	na
MCCG[d]		62		68	na
MCPG[d]		290		270	na

[a]Values were calculated by nonlinear regression from data shown in Figures 1–3 (na = not active).
[b]Partial agonist.
[c]Maximal inhibition reached only 50%.
[d]Two antagonists, α-methyl-2-(carboxycyclopropyl)glycine (MCCG) and α-methyl-4-carboxyphenylglycine (MCPG), were used for comparison.

nitrogen of APDC. Based upon the recently published X-ray structure of the extracellular ligand-binding region of mGluR1,[18] one may suggest that these substituents are capable of altering the dynamic equilibrium among the various conformational states of the receptor, so as to favor the open, closed (active), or intermediate conformational states. Homology-based modeling methods may allow for a more precise understanding of these substituent effects.[19]

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13. Representative procedure: To a solution of **3** (170 mg, 0.562 mmol) and 2-nitrobenzyl bromide (134 mg, 0.618 mmol) in dichloromethane (4 mL) was added *N*,*N*-diisopropylethylamine (0.25 mL, 1.41 mmol), and the mixture was stirred under an argon atmosphere at room temperature for 30 h. The reaction mixture was diluted with dichloromethane (20 mL), washed with H_2O (10 mL), dried (Na_2SO_4), and concentrated. The crude residue was chromatographed on silica gel with AcOEt–hexane (1:3) to afford compound **6i** (230 mg, 93%) as a light-yellow solid: mp 98–99 °C; ^{1}H NMR (300 MHz, $CDCl_3$, referenced to TMS) δ 1.42 (9H, s), 2.25 (1H, dd, J = 5.1 and 13.8 Hz), 2.78 (1H, dd, J = 9.9 and 13.8 Hz), 2.98 (1H, d, J = 9.9 Hz), 3.09 (1H, d, J = 10.2 Hz), 3.58 (1H, dd, J = 5.1 and 9.2 Hz), 3.66 (3H, s), 3.74 (3H, s), 4.05 (1H, d, J = 14.7 Hz), 4.28 (1H, d, J = 14.7 Hz), 5.47 (1H, br s), 7.40 (1H, t, J = 6.0 Hz), 7.54 (1H, t, J = 7.5 Hz), 7.65 (1H, d, J = 6.9 Hz), 7.84 (1H, d, J = 8.1 Hz); ^{13}C NMR (75 MHz, $CDCl_3$, referenced to central peak of solvent (δ = 77.0)] δ 28.06, 39.34, 52.00, 52.57, 54.52, 61.67, 63.43, 64.13, 79.92, 124.24, 128.05, 130.64, 132.63, 133.49, 149.17, 155.02, 172.22, 173.04. Anal. calcd for $C_{20}H_{27}N_3O_8$: C, 54.91; H, 6.22; N, 9.61. Found: C, 54.83; H, 6.14; N, 9.37. The compound **6i** (125 mg, 0.286 mmol) was dissolved in 12 mL of aqueous 6 N HCl, and the solution was refluxed for 2.5 h. Most of the solvent was removed under reduced pressure, and the residue was lyophilized to give **9i** (103 mg, 94%) as a white solid. Mp 178–180 °C; ^{1}H NMR (300 MHz, CD_3OD, referenced to central peak of solvent (δ = 3.31)] δ 2.65 (1H, dd, J = 9.9 and 14.4 Hz),

3.16 (1H, dd, $J=8.1$ and 14.4 Hz), 3.80–3.93 (2H, m), 4.54 (1H, t, $J=9.0$ Hz), 4.73 (1H, d, $J=13.8$ Hz), 4.90 (1H, d, $J=13.5$ Hz), 7.69 (1H, t, $J=7.8$ Hz), 7.79 (1H, t, $J=7.5$ Hz), 7.91 (1H, d, $J=7.5$ Hz), 8.14 (1H, d, $J=7.8$ Hz); ^{13}C NMR (75 MHz, CD_3OD, referenced to central peak of solvent ($\delta=49.15$)] δ 39.49, 57.14, 61.53, 62.57, 67.53, 126.71, 128.62, 132.26, 134.90, 135.40, 150.69, 170.76, 171.01.

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