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Synthesis of (*S*)- and (*R*)-5-Oxo-piperazine-2-Carboxylic Acid and Its Application to Peptidomimetics

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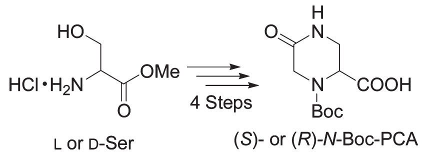
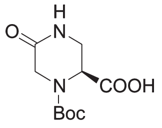


A straightforward synthesis of (*S*)- and (*R*)-*N*-Boc-5-oxo-piperazine-2-carboxylic acid is reported starting from L- or D-serine and ethyl glyoxylate. Those were evaluated as constituents in two tetrapeptides by studying their secondary structure by ^{1}H NMR spectroscopy. In the case of Boc-Val-(*S*)-PCA-Gly-Leu-OMe, two readily interconverting conformations (in a 40%:60% ratio) were observed, differing for the cis−trans isomerizaton of the tertiary amide bond, while Boc-Val-(*R*)-PCA-Gly-Leu-OMe displayed an equilibrium between a γ-turn and a type II β-turn conformation.

The design and synthesis of conformationally restricted amino acids has been the focus of extensive research because these compounds mimic or induce specific secondary structural features of peptides and proteins.[1] Since the discovery of the crucial role of proline in protein structures, cyclic α-amino acids containing a heterocyclic ring have attracted considerable attention from both synthetic and medicinal chemists.[2] In particular several six-membered-ring heterocyclic amino acids have been synthesized, comprising derivatives of pipecolic,[3] piperazine-2-carboxylic,[4] 1,4-thiazine-3-carboxylic,[5] 1,3-thiazine-4-carboxylic,[6] and morpholine-3-carboxylic acid.[7] 5-Oxo-piperazine-2-carboxylic acid (PCA, Figure 1), on the other hand, has received much less attention, and only a few reports deal with its synthesis and structural and biological properties.[8]



(*S*)-*N*-Boc-PCA **1**

FIGURE 1. (*S*)-*N*-Boc-5-oxo-piperazine-2-carboxylic acid **1**.

In the frame of our studies directed toward the synthesis of peptidomimetics with well-defined conformational properties,[9] we became interested in the synthesis of 5-oxo-piperazinone-2-carboxylic acid (PCA), and its introduction into peptide sequences as an inducer of secondary structures.

Herein we report a practical synthesis of (*S*)- and (*R*)-*N*-Boc-5-oxo-piperazine-2-carboxylic acid **1** (Figure 1), its introduction into peptides by a solution-phase peptide-synthesis strategy, and a conformational analysis of two tetrapeptide mimics incorporating a PCA residue.

Our synthetic approach (Scheme 1) started from L-serine methyl ester hydrochloride, which was *N*-alkylated by using ethyl glyoxylate in the presence of Pd/C and under an atmosphere of H_2. Following a procedure that has been reported for the synthesis of 2,3-diamino propionic acid starting from serine derivatives,[10] the resulting alcohol **2** was transformed into the azide **3** by a Mitsunobu reaction, using a solution of hydrazoic acid in toluene. Other methodologies involving the activation of the hydroxyl group of

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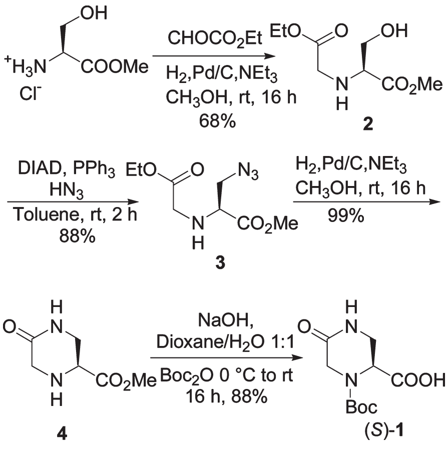
SCHEME 1. Synthesis of (*S*)-*N*-Boc-5-oxo-piperazine-2-carboxylic Acid 1



serine were hampered by a concurrent elimination reaction leading to the corresponding dehydroalanine derivative as the major reaction product.

Catalytic hydrogenation of **3** with Pd/C caused reduction of the azide to the primary amine with concurrent cyclization to methyl 5-oxo-piperazinone-2-carboxylate **4** in quantitative yield. Finally, a one-pot hydrolysis of the methyl ester and *N*-Boc protection afforded *N*-Boc-5-oxo-piperazine-2-carboxylic acid **1** in pure form. The synthesis of (*R*)-**1** was analogously achieved by starting from D-serine. The protection of the amino group of methyl (*S*)-5-oxo-piperazinone-2-carboxylate **4** was then realized by using Fmoc-Cl in a biphasic aqueous $NaHCO_3$ dioxane medium in good yield. (*S*)-*N*-Fmoc-5-oxo-piperazine-2-carboxylic acid methyl ester **5** (Figure 2) could be crystallized from a mixture of methanol and diethyl ether to allow an X-ray structure analysis: the piperazinone ring adopts a half-chair conformation with the methoxycarbonyl group in a pseudoaxial and the *N*-protecting group in an equatorial orientation. This arrangement, which has been reported for pipecolic acid containing peptides,[11] was found also in solution as judged by the coupling constants for the protons on C2/C3 being smaller than 5 Hz (typically 4.4 Hz). The amide group displays a planar geometry as expected with the N−H bond bisecting the angle of the vicinal CH_2 group.

To evaluate the enantiomeric purity of these derivatives, (*S*)-**1** and (*R*)-**1** were then coupled to (*R*)-1-phenylethylamine.[7,12] The coupling was achieved in good yield with EDC (*N*-ethyl,*N*′-[3′-(dimethylamino)propyl]carbodiimide)/HOBT (1-hydroxybenzotriazole)/*i*Pr_2NEt; however, the optical purity of the resulting *N*-Boc amides could not be established either by HPLC or by NMR, the latter being complicated by doubling of the signals due to rotamers caused by the *N*-Boc group. Removal of the Boc protecting group revealed a single set of NMR signals, whereas HPLC analysis of the single amides and of the 1:1 mixture showed that in both (*S*)-**1** and (*R*)-**1** cases the amides were obtained with a de greater than 95%.

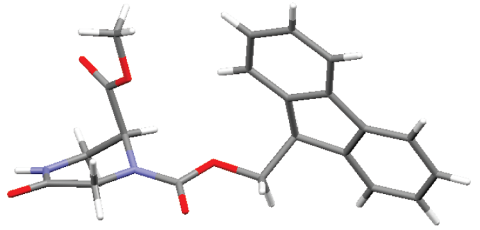


FIGURE 2. X-ray structure of methyl (*S*)-*N*-Fmoc-5-oxo-piperazine-2-carboxylate **5**.

The ability of both (*S*)- and (*R*)-PCA to act as proline mimics in inducing turn structures when inserted into peptide sequences was then studied. Two tetrapeptide sequences (Boc-Val-(*S*)-PCA-Gly-Leu-OMe **6** and Boc-Val-(*R*)-PCA-Gly-Leu-OMe **7**) were prepared by solution phase peptide synthesis (Boc strategy), using HATU (*O*-(7-azobenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate) to couple Boc-PCA to the Gly-Leu-OMe dipeptide and Boc-Val to the PCA moiety. Similar sequences containing L- or D-proline,[13] and other proline mimics such as (*S*)- (*R*)-pipecolic acid,[11] have been studied and their preferred conformations in solutions were determined by ^1H NMR. In the latter case both thermodynamic and kinetic aspects of the cis−trans isomerization about pipecolic peptide bonds have been studied.

A conformational study of **6** and **7** was performed in $CDCl_3$ solution by ^1H NMR. In the case of Boc-Val-(*S*)-PCA-Gly-Leu-OMe **6**, the ^1H NMR spectrum (5 mM) showed two sets of signals in a 1.7:1 ratio, which were attributed to the trans and cis tertiary amide isomers, respectively (37% of the cis-conformer). It has been reported that the presence of a pipecolic acid residue leads to a significant increase in population of the cis conformer (35−50%),[11] whereas the cis amide-content in tetrapeptides containing L-proline appears to be definitely lower (up to 21% if an aromatic amino acid, Phe or Tyr, is preceding proline in the sequence).[14] Analogously, the introduction of a morpholine-3-carboxylic acid (Mor) in the tetrapeptide sequence Ac-Val-(*S*)-Mor-Gly-Leu-OMe resulted in a 2:3 mixture of cis and trans rotamers (40% of the cis-conformer) at the Val-Mor amide bond.[7b] NOESY experiments conducted at 298 K revealed the presence of intense exchange cross peaks (EXSY) between the cis and trans PCA containing peptides, which hampered the correct attribution of the signals to the cis and trans conformers. This is indicative of a rather low barrier to isomerization, as can also be inferred from the coalescence of several backbone (and amide) signals, which is observed by heating the sample to 313 K. The presence of the pipecolic acid ring is reported to increase the kinetics of cis−trans isomerizaton with respect to proline, but with PCA an even faster isomerization is observed. When a NOESY experiment was conduced at 273 K the exchange peaks were far less intense and contacts for the two conformers could be assigned. The trans conformer did not show the presence of relevant cross-peaks indicative of a well-organized structure. This fact, together with the relatively low chemical shift value of the NH signals ($\delta \leq 7.0$ ppm) and their high temperature coefficients (see the Supporting Information for the ^1H NMR spectra and the determination of the $\Delta\delta/\Delta T$ values), is indicative of a

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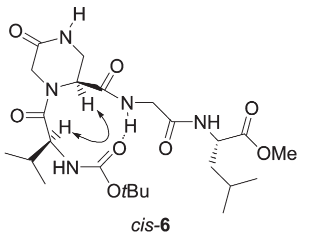

cis-**6**

FIGURE 3. Hypothesized conformation of tetrapeptide Boc-Val-(*S*)-PCA-Gly-Leu-OMe **6**. The arrow indicates the NOE contact between Val H-α and H-2 of the PCA moiety.

TABLE 1. ^{1}H NMR Chemical Shifts at Room Temperature and Temperature Coefficients for Tetrapeptide 7

NH	δ (ppm)	$\Delta\delta/\Delta T$ (ppb/K)
Val-NH	5.13	−1.82
PCA-NH	6.38	−10.25
Gly-NH	7.79	−4.59
Leu-NH	6.53	−2.78

random coil structure. In the case of the cis-conformer (Figure 3), the Gly-NH appears at a remarkably deshielded chemical shift value ($\delta = 8.27$ ppm) and with a temperature coefficient indicative of an equilibrium between an intramolecularly bonded and a nonbonded status. The NOESY experiments showed a rather strong contact between Val H-α and H-2 of the PCA moiety, indicating both the cis-conformation of the amide bond and the presence of a β-turn structure stabilized by a 10-membered cyclic hydrogen bond.

The ^{1}H NMR spectrum of the heterochiral Boc-Val-(*R*)-PCA-Gly-Leu-OMe (**7**) showed a single set of signals indicating the existence of a single rotamer in solution, which, owing to a strong NOE contact between the Val H-α and the H-6 protons of the PCA residue, was assigned to the trans isomer. A study of the chemical shift of the amide protons and their temperature coefficients determination (Table 1) indicated for Gly-NH an equilibrium between hydrogen-bonded and nonhydrogen-bonded states; a similar behavior could be assumed for Leu-NH, although with a distinctively lower chemical shift.

The NOESY experiments showed strong contacts of Gly NH with H-2 and on H-6 of the PCA moiety as well as with Leu-NH. These findings are consistent with an equilibrium between a γ-turn and a type II β-turn conformation (Figure 4).

A ^{1}H NMR study in DMSO-d_6 revealed two sets of signals in a 1.7:1 ratio, which were attributed to the trans and cis tertiary amide isomers, respectively. The NOESY experiment showed intense exchange peaks and the absence of significant long-range interactions. This behavior is in agreement with other similar cases.[13,15]

Conformational preferences of compounds **6** and **7** were investigated by Monte Carlo simulations without imposing any constraint, using Spartan 06 version 1.03. The lowest energy conformations calculated substantially reflect the experimentally observed structures (see the Supporting Information for a more detailed description of these results).

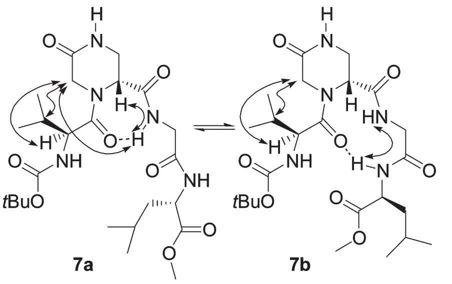

FIGURE 4. Hydrogen-bonded structures for tetrapeptide Boc-Val-(*R*)-PCA-Gly-Leu-OMe **7**. The arrows indicate significant NOE contacts.

In conclusion we have reported a straightforward synthesis of both (*S*)- and (*R*)-*N*-Boc-5-oxo-piperazine-2-carboxylic acid **1**, starting from serine and ethyl glyoxylate. The incorporation of both enantiomeric tertiary amino acids into a model tetrapeptide was obtained by solution phase peptide synthesis and their turn inducing abilities studied by ^{1}H NMR spectroscopy. Interestingly Boc-Val-(*S*)-PCA-Gly-Leu-OMe **6** showed two readily interconverting conformations (in a 40%:60% ratio), differing in the cis and trans configuration of the tertiary amide bond. The barrier to isomerization of the tertiary amide is unusually low with a coalescence temperature of 313 K. On the other hand, Boc-Val-(*R*)-PCA-Gly-Leu-OMe **7**, which contains the *R*-PCA enantiomer, showed a more defined turn conformation.

Experimental Section

(*S*)-2-((Ethoxycarbonylmethyl)amino)-3-hydroxypropionic Acid Methyl Ester (2). L-Serine methyl ester hydrochloride (1.0 g, 6.45 mmol) was dissolved in methanol, then triethylamine (902 μL, 6.48 mmol), a 50% solution of ethyl glyoxalate in toluene, and 10% Pd/C (90 mg), were successively added, and the resulting mixture was stirred overnight under a hydrogen atmosphere. The suspension was filtered over a pad of Celite, and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH, 98:2) to afford the desired product as a colorless oil (902 mg, 68%). $[\alpha]^{20}_D$ −27.8 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 4.21−4.15 (q, 2H, J = 8.7 Hz), 3.79 (dd, 1H, J_1 = 11.1 Hz, J_2 = 4.5 Hz), 3.75 (s, 3H), 3.69 (dd, 1H, J_1 = 5.8 Hz, J_2 = 11.1 Hz), 3.52 (d, 1H, J = 17.4 Hz), 3.40 (d, 1H, J = 17.5 Hz), 2.76 (br, 1H), 1.26 (t, 3H, J = 7.1 Hz); ^{13}C NMR δ 172.5, 172.0, 62.4, 62.3, 61.1, 52.3, 48.9, 14.1; IR (nujol) ν_{max} 3329, 3184, 1723, 1377, 1201, 1068. Anal. Calcd for $C_8H_{15}NO_5$: C 46.82, H 7.37, N 6.83. Found: C 46.74, H 7.32, N 6.46.

(*S*)-3-Azido-2-((ethoxycarbonylmethyl)amino)propionic Acid Methyl Ester (3). Triphenylphosphine (1.44 g, 5.50 mmol) was added to a solution of **2** (807 mg, 3.93 mmol) in dry toluene (30.0 mL) under nitrogen at room temperature. After complete dissolution of the phosphine, HN_3 (0.5 M in toluene; 15.70 mL, 7,86 mmol) was added, followed by diisopropylazodicarboxylate (DIAD, 1.10 mL, 5.50 mmol). After 2 h the mixture was directly purified by flash chromatography (CH_2Cl_2/MeOH, 99:1) to give the desired product as a colorless oil (796 mg, 88%). $[\alpha]^{20}_D$ −37.6 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 4.19 (q, 2H, J = 7.1 Hz), 3.77 (s, 3H), 3.58−3.56 (m, 2H), 3.52−3.46 (m, 3H), 2.33 (br, 1H), 1.25 (t, 3H, J = 7,1 Hz); ^{13}C NMR ($CDCl_3$) δ 171.9, 171.5, 60.9, 60.2, 53.0, 52.3, 49.0, 14.1; IR (CH_2Cl_2) ν_{max} 3352, 3063, 2108, 1744, 1445; HRMS (ESI+) m/z calcd for $[C_8H_{14}N_4NaO_4]^+$ 253.O9073 [M + Na]$^+$, found 253.09097.

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(*S*)-5-Oxo-piperazine-2-carboxylic Acid Methyl Ester (4). Pd/C (0.112 mmol 0.1 equiv) was added to a solution of **3** (260 mg, 1.12 mmol) in MeOH (5 mL). The reaction was stirred overnight at rt under 1.0 atm of H_2. The solution medium was then filtrated through a pad of Celite and the solvent was evaporated under reduced pressure. The crude was purified by flash chromatography (DCM/MeOH, 92:8) to obtain the desired product as a white paste (176 mg, 99%). $[\alpha]^{20}_{D}$ −46.3 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 6.87 (br, 1H), 3.77 (s, 3H), 3.75 (dd, 1H, J_1 = 8.0 Hz, J_2 = 4.4 Hz), 3.66 (d, 1H, J = 17.1 Hz), 3.61 (ddd, 1H, J_1 = 11.8 Hz, J_2 = 4.4 Hz, J_3 = 3.1 Hz), 3.53 (d, 1H, J = 17.4 Hz), 3.52 (ddd, 1H, J_1 = 11.8 Hz, J_2 = 8.0 Hz, J_3 = 1.8 Hz), 2.15 (br, 1H); ^{13}C NMR ($CDCl_3$) δ 171.3, 169.8, 54.3, 53.0, 48.3, 44.2; IR (CH_2Cl_2) ν_{max} 3406, 3354, 3211, 1744, 1678; HRMS (EI) *m*/*z* calcd for $[C_6H_{10}N_2O_3]^+$ 158.06910 $[M]^+$, found 158.06920.

(*S*)-5-Oxopiperazine-1,2-dicarboxylic Acid 1-*tert*-Butyl Ester (1). Compound **4** (120 mg, 0.76 mmol) was dissolved in a 1:1 dioxane/NaOH (1.0 M) solution (2.0 mL) at rt. The mixture was cooled to 0 °C and Boc_2O (331 mg, 1.52 mmol) was added. After 15 min the ice bath was removed and the reaction was stirred for 10 h at rt. The reaction was quenched by addition of water (5.0 mL), and the aqueous phase was washed with EtOAc (2 × 3.0 mL). The aqueous solution was acidified until pH 3 mediating addition of a 1.0 M solution of $KHSO_4$. The acid phase was extracted with EtOAc (5 × 5.0 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and after purification by flash chromatography (CH_2Cl_2/MeOH, 97:3) the product (*S*)-**1** was collected as a white powder (163 mg, 88%). Mp 103−104 °C; $[\alpha]^{20}_{D}$ −33.8 (*c* 0.5, CH_3OH); two sets of signals were observed in the ^1H and ^{13}C NMR spectrum due to the presence of two rotational isomers A:B (2:1 ratio) ^1H NMR ($CDCl_3$) δ 8.35 (d, $1H_B$, J = 3.2 Hz), 8.20 (d, $1H_A$, J = 4.0 Hz), 4.99 (d, $1H_A$, J = 4.0 Hz), 4.79 (d, $1H_B$, J = 4.0 Hz), 4.23 (d, $1H_A$ and $1H_B$, J = 18.8 Hz), 4.07 (d, $1H_A$ and $1H_B$, J = 18.8 Hz), 3.88 (dd, $1H_A$, J_1 = 12.6 Hz, J_2 = 3.9 Hz), 3.84 (dd, $1H_B$, J_1 = 17.3 Hz, J_2 = 4.1 Hz), 3.65 (dd, $1H_A$ and $1H_B$, J_1 = 17.3 Hz, J_2 = 4.9 Hz), 1.50 (s, $9H_A$), 1.48 (s, $9H_B$); ^{13}C NMR ($CDCl_3$) δ 174.3 (A), 174.0 (B), 170.7 (A), 170.1 (B), 154.4 (A), 153.6 (B), 81.9 (A), 81.8 (B), 52.6 (A), 51.1 (B), 45.9 (A), 45.3 (B), 42.4 (A), 40.7 (B), 28.3 (A), 28.2 (B); IR (CH_2Cl_2) ν_{max} 3408, 3230, 1707, 1647; HRMS (ESI+) *m*/*z* calcd for $[C_{10}H_{16}N_2O_5]^+$ 244.1059 $[M + H]^+$, found 244.1060.

(*S*)-5-Oxopiperazine-1,2-dicarboxylic Acid 1-(Fluoren-9-yl) Ester 2-Methyl Ester (5). **4** (128 mg, 0.81 mmol) was dissolved in a 2:1 water:dioxane (3.0 mL) solution and $NaHCO_3$ (136 mg, 1.61 mmol) was added. The mixture was cooled to 0 °C and a solution of Fmoc-Cl (209 mg, 0.81 mmol) in dioxane (1.5 mL) was added dropwise over 15 min. The ice bath was removed and the reaction mixture was left stirring for 2.5 h at rt. Successively, the mixture was partitioned between EtOAc (14.0 mL) and water (7.0 mL), and the organic phase was washed with 1.0 M HCl and brine and dried over Na_2SO_4. The organic solvent was removed and the crude product was purified by flash chromatography (DCM/MeOH, 95:5) to afford the desired compound **5** (258 mg, 87%). Mp 143−144 °C; $[\alpha]^{20}_{D}$ −11.9 (*c* 1.0, $CHCl_3$); two sets of signals were observed in the ^1H and ^{13}C NMR spectrum due to the presence of two rotational isomers A:B (3:1 ratio) ^1H NMR ($CDCl_3$) δ 6.57 (br s,$1H_B$), 6,54 (br s, $1H_A$), 5,04 (t, $1H_A$, J = 2.0 Hz), 4.61 (br s, $1H_B$),4.60−4.49 (m, H_A and H_B), 4.44 (dd, $1H_A$, J_1 = 7.2 Hz, J_2 = 3.6 Hz), 4.28−4.15 (m, $2H_A$ and $1H_B$), 4.12 (d, $1H_A$, J = 18.0 Hz), 4.03 (d, $1H_B$, J = 18.4 Hz), 3.84 (dd, $1H_B$, J_1 = 5.2 Hz, J_2 = 1.6 Hz), 3.81 (dd, $1H_B$, J_1 = 3.6 Hz, J_2 = 1.6 Hz), 3.78 (s, $3H_A$), 3.71 (s, $3H_B$), 3.68 (dd, $1H_A$, J_1 = 13.2 Hz, J_2 = 4.8 Hz), 3.57 (dd, $1H_A$, J_1 = 12.8 Hz, J_2 = 4.4 Hz); ^{13}C NMR ($CDCl_3$) δ 169.7 (A), 169.6 (B), 167.4 (B), 166.9 (A), 155.2 (A), 154.3 (B), 143.6 (A and B), 143.5 (B), 143.4 (A), 141.4 (A and B), 141.3 (A and B), 127.92 (A), 127.88 (B), 127.82 (B), 127.22 (A), 127.20 (A), 127.15 (B), 127.1 (B), 124.9 (A), 124.7 (B), 124.6 (B), 120.1 (A), 120.0 (B), 68.4 (A), 67.8 (B), 53.1 (A and B), 52.5 (B), 52.0 (A), 47.2 (B), 47.1 (A), 46.0 (A and B), 42.3 (A and B); IR (nujol) ν_{max} 3195, 1745, 1694, 1410, 1315, 1232, 1099. Anal. Calcd for $C_{21}H_{20}N_2O_5$: C 66.29, H 5.30, N 7.37. Found: C 66.20, H 5.35, N 7.28.

X-ray Crystallographic Data of 5:. $C_{21}H_{20}N_2O_5$; MW = 380.39 g·mol^{-1}; T = 123 K; λ(Mo Kα) = 1.54184 A; orthorhombic, space group *P* 21 21 21, a = 6.5176(2) Å, b = 11.6690(5) Å, c = 23.9190(9) Å, α = 90°, β = 90°, γ = 90°, V = 1819.13(12) Å^3, ρ_{calc} = 1.389 mg·m^{-3}, Z = 4; μ(Mo Kα) = 0.828 mm^{-1}, R_1 = 0.0283, wR_2 = 0.0713, for 2679 unique data collected in the 3.70−66.5° θ ranges.

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Supporting Information Available: Synthetic schemes, experimental procedures, and characterization of compounds **6**−**7** and ^1H and ^{13}C NMR of all reported compounds, conformational studies of compounds **6**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.