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STEREOCHEMISTRY OF FULLY ACETYLATED TETRAHYDROPTERINS AND TETRAHYDROQUINOXALINES

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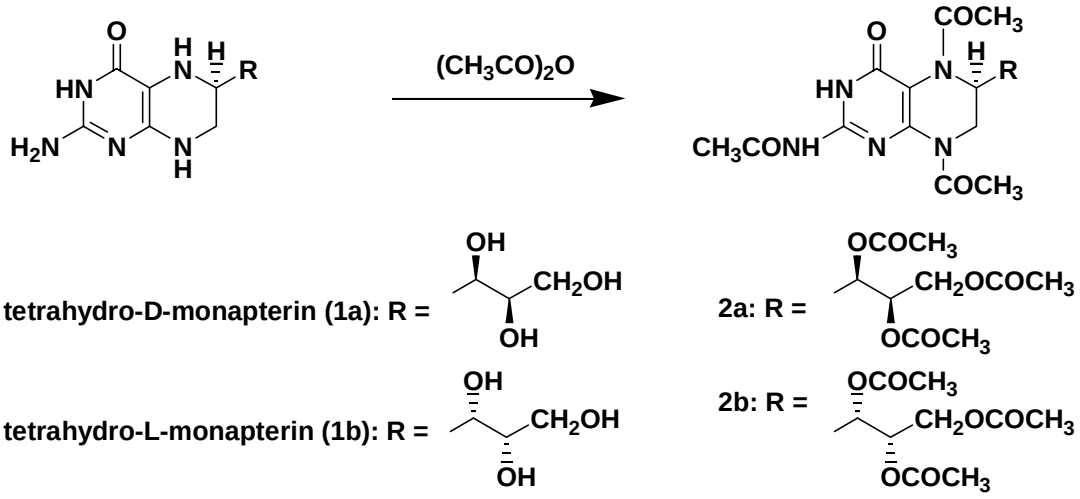
Abstract – Completely acetylated derivatives of naturally occurring tetrahydro-D-monapterin, (6*R*)-2-acetamido-6-[(1*R*,2*R*)-1,2,3-triacetoxypropyl]-5,8-diacetyl-5,6,7,8-tetrahydropteridin-4(3*H*)-one, show plus and minus CD signs at λ 280 and 240 nm, respectively. Similar CD spectra are obtained on (2*S*)-1,4-diacetyl-1,2,3,4-tetrahydro-2-isopropylquinoxaline and (2*S*)-1,4-diacetyl-1,2,3,4-tetrahydro-2-isobutylquinoxaline but not on (2*S*)-1,4-diacetyl-1,2,3,4-tetrahydro-2-methylquinoxaline nor on the 2-ethyl derivative. The similarity of their CD spectra is represented the same stereogenic structure based on the boat conformation confirmed by X-Ray crystallographic analyses.

INTRODUCTION

Tetrahydropteridines are biologically important nitrogen heterocycles, since some of them are operating in various metabolic systems. For example, biopterin cofactor, *i.e.* (6*R*)-tetrahydrobiopterin, is known to work in biosynthesis of nitrogen oxide (NO) as well as metabolic transformation of aromatic amino acids to cathecholamine.[1, 2] Folic acids, like (6*R*)-tetrahydrofolate, belonging to the other series of biologically active pteridines play essential roles in biosyntheses of nucleosides and methionine.[1] Recently different (6*R*)-tetrahydropterin (**1a** and **1b**) have been isolated from microorganisms known not to produce biopterin cofactor and are supposed to replace biopterin cofactor in the enzymatic reactions.[3, 4] The importance of such tetrahydropteridine derivatives and the pteridine-depending metabolisms for controlling homeostasis would increase in chemical biology. Since such biologically active tetrahydropteridines generally adopt the 6*R* configuration, the stereochemistry of synthetic as well as

naturally occurring pteridine derivatives is one of most fundamental subjects in biochemical and pharmaceutical studies.[5-10] A facile CD spectroscopic technique has been employed for determination of the absolute configuration, and, indeed, it is easy to distinguish the (6*R*)-tetrahydropteridine from the (6*S*)-isomer in comparison with their CD signs at λ_{max} 265 nm in acidic solutions, *e.g.* the 6*R* and 6*S* isomers afford the minus and plus signs, respectively.[11] The CD spectroscopic rule has been rationalized by the molecular distortion based on a stable twisted-chair conformation of the tetrahydropyrazine part. On the contrary, the configuration of completely acetylated (6*R*)-tetrahydropterins (**2a** and **2b**) isolated from cell extracts of a protozoa and bacteria has not been discussed based on the regularity of CD spectra due to different CD patterns.[3, 4] Complete acetylation is only one reliable technique to isolate and analyze a small amount of tetrahydropterin derivatives in complex mixtures of biological samples. In order to understand the stereochemistry of such acetylated tetrahydropteridine derivatives, CD spectra and X-Ray crystallographic analyses were investigated on completely acylated and optically active tetrahydroquinoxaline derivatives which have been considered to be model compounds of **2a** and **2b**.



Scheme 1. Biologically Active Tetrahydropterins and Peracetylated Derivatives

RESULTS AND DISCUSSION

CD Spectra. The (6*R*)-hexaacetyltetrahydro-D-monapterin (**2a)** and (6*R*)-hexaacetyltetrahydro-L-monapterin (**2b**) afforded the similar CD spectra illustrated in Figure 1, where + (λ_{max} 322 nm), – (248 nm), + (223 nm), and – (216 nm) signs are recommended. The similarity of the +/–/+/– figures clearly indicated that the CD spectra were affected not by the absolute configuration of stereogenic centers on the side chain but by the 6R chirality of the heterocyclic structure. Therefore, optically active 2-alkylated (2*S*)- and (2*R*)-tetrahydroquinoxaline derivatives, (*S*)- and (*R*)- **3** – **7**, illustrated in Scheme 2

were employable to CD studies as the model compounds, the 2*S* configuration of which means the same stereogenic structure as (6*R*)-tetrahydropterin derivatives. CD spectra of methyl and ethyl substituted derivatives, (*R*)-**3** and (*R*)-**4** in Figure 2, did not show a significant deflection in the whole wave length (λ = 200 – 400 nm). Although their absolute configurations are same, the CD spectra look like those of an enantiomeric pair. On the contrary, **5** and **6** with rather bulky groups (R = *i*Pr and 2-methylpropyl, respectively) indicated obvious CD curves illustrated in Figure 3 and 4, and the CD spectra of (*S*)-**5** and

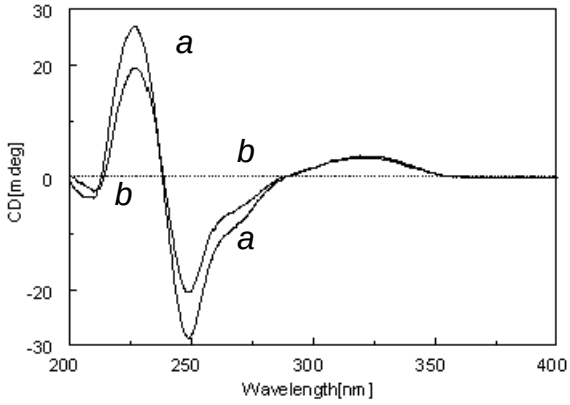
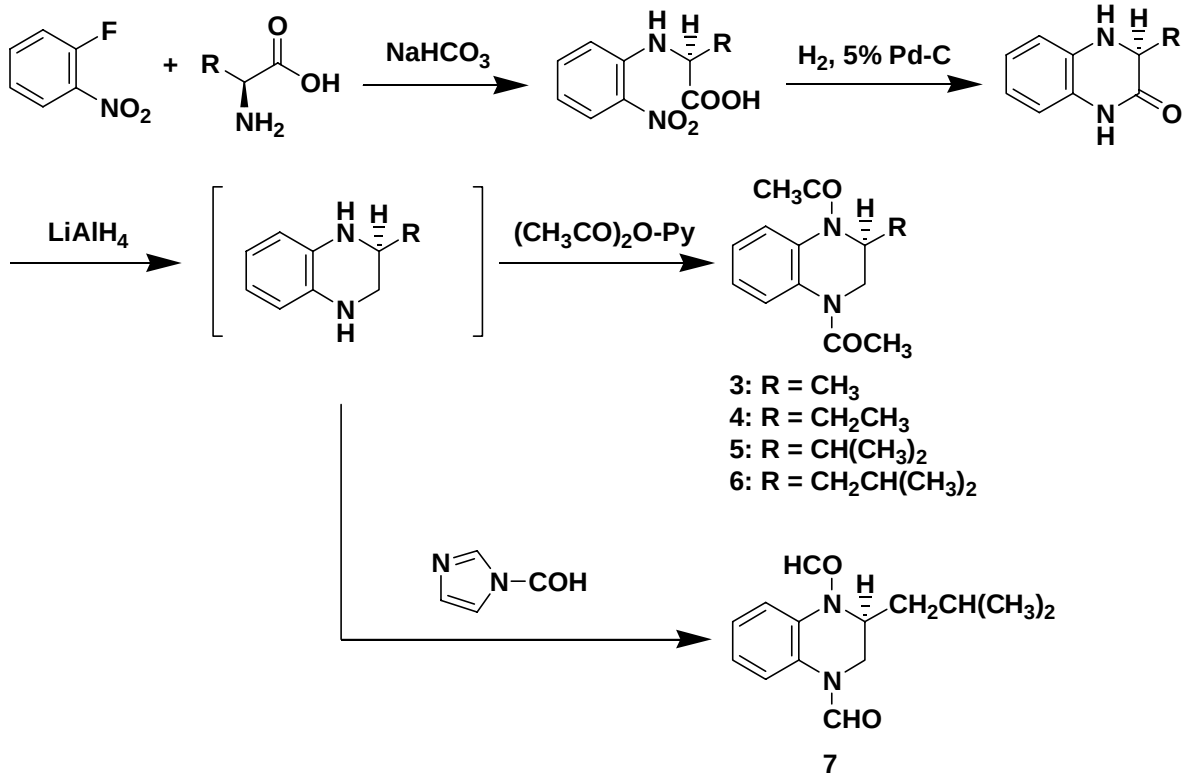


Figure 1. CD spectra of **2a** (*a*) and **2b** (*b*) in CH_3CN (C = 1.95 x 10^{-5} M).



Scheme 2. Preparation of Optically Active Peracetylated Tetrahydroquinoxaline ((*S*)-**3** – **7**).

(*S*)-**6** (Figure 3 (*b*) and Figure 4, respectively) changing the +/–/+/– fashion at λ = 320 – 200 nm are similar in each other. The similarity of the CD spectra of the (6*R*)-pterin (Figure 1) and (2*S*)-quinoxaline derivatives (Figure 3 (*a*)) suggested the conformational similarity in these compounds. Armarego and coworkers have reported based on a ^{1}H NMR spectroscopic study that (6*R*)-tetrahydrobiopterin tetraacetate preferred a half-chair conformation in which the bulky side chain occupied an axial geometry.[8] However, ^{1}H and ^{13}C NMR spectra of the tetrahydroquinoxaline derivatives indicating peak broadening and missing due to conformational flexibility were not useful to discuss their conformation. For example, only 7 signals were detected in a ^{13}C NMR spectrum of (*S*)-**5** at room temperature, while all 15 signals were recommended at –30 ºC.

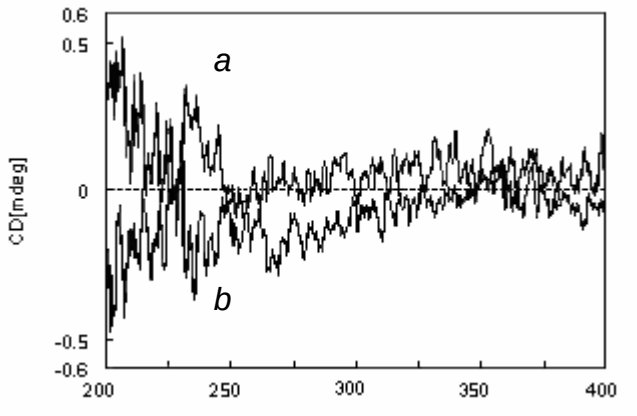


Figure 2. CD spectra of (*R*)-**3** (*a*) and (*R*)-**4** (*b*) in CH_3CN (C = 1.95 x 10^{-5} M).

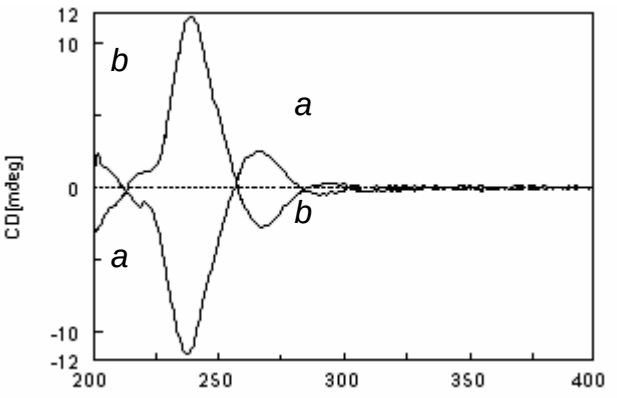


Figure 3. CD spectra of (*R*)- **5** (*a*) and (*S*)-**5**(*b*) in CH_3CN (C = 1.95 x 10^{-5} M).

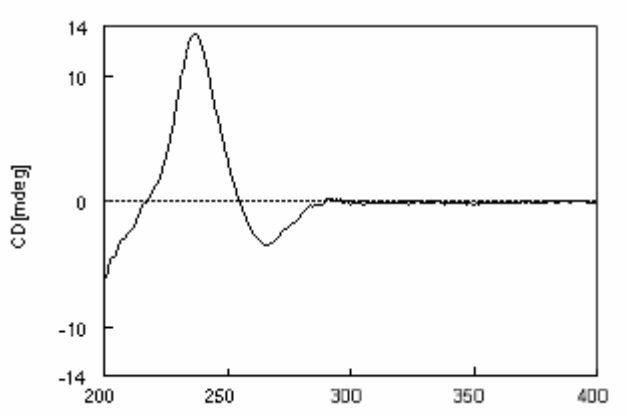


Figure 4. CD spectra of (*S*)- **6** in CH_3CN (C = 1.95 x 10^{-5} M).

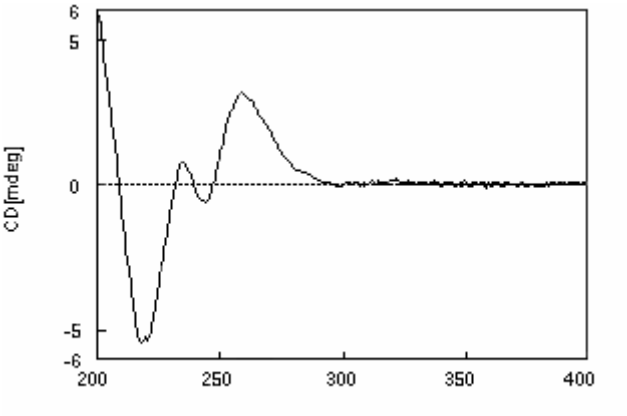


Figure 5. CD spectra of (*S*)-**7** in CH_3CN (C = 1.95 x 10^{-5} M).

X-Ray Crystallographic Analysis. Although Viscontini and coworkers determined the 6R absolute configuration of biopterin cofactor based on the X-Ray crystallographic analysis of its peracetylated derivative, they did not mention about the conformation and CD spectra.[6, 7] Based on 2D NMR study and X-Ray crystallographic analysis, one of us proved that the preferable conformation of **2a** was the boat form.[12] If the stable boat conformation is recommended in **5** and **6**, the similarity of their CD spectra could be rationalized by the conformational similarity. The X-Ray crystallographic analysis was carried out on (*S*)-**5**,[13] and the molecular structure is illustrated in Figure 6. Here, it is obvious that the tetrahydropyrazine moiety of (*S*)-**5** adopts the typical boat conformation in which the N(1) acetyl group ($CH_3CON(1)$) and the isopropyl group are located in an anti geometry to avoid steric repulsion. Three typical structures (**8** – **10**) of (*S*)-**5** are illustrated in Figure 7. In the half-chair conformation (**8**) with the equatorial isopropyl group (R), the existence of major steric interactions between R and $CH_3CON(1)$ can be recommended. The half-chair conformation (**9**) is the similar structure as that was mentioned by Armarego,[8] and the quasi 1,3-diaxial relationship of R and $CH_3CON(4)$ is significant in this structure. On the contrary, no significant interaction caused by R is recommended in the boat structure (**10**). However the stability is mainly depending on the relative bulkiness of R and the acetyl group in **10**. Because of general disadvantages of the boat form 10 might become less favorable than the half-chair forms, when the steric repulsion is smaller. The fact that CD spectra of (*S*)-**7** (Figure 5) did not obey the similarity to *N*-acetyl derivatives is rationalized by good stability of the half-chair conformation like **9** due to the small *N*-formyl group. In the cases of tetrahydroquinoxaline derivatives with smaller R (**3**: CH_3, **4**: C_2H_5), the repulsion in **8** would be reduced very much, and the small interaction allowed the

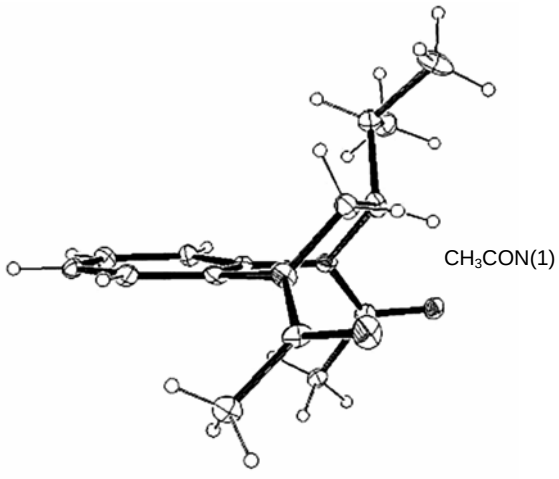


Figure 6. ORTEP drawing of (*S*)-**5**.

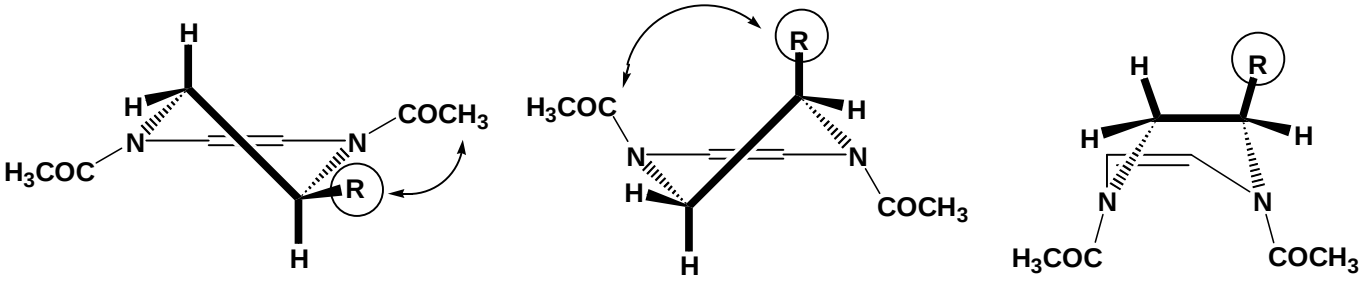


Figure 7. Major conformations of diacetyltetrahydroquinoxaline.

rapid interconversion of **8** and **9**. Based on the quadrant rule[14] employed to the theoretical explanation on the CD spectra of tetrahydropterins,[11] the two half-chair conformations could afford opposite CD signs in each other. The obscure CD spectra of **3** and **4** shown in Figure 2 might be caused by the conformational flexibility.

EXPERIMENTAL

*(*R*)-1,4-Diacetyl-1,2,3,4-tetrahydro-2-methylquinoxaline ((R)-**3**)*. A mixture of D-alanine (2.0 g, 22 mmol), *o*-fluoronitrobenzene (3.5 g 25 mmol), and $NaHCO_3$ (9.0 g) in 50% aq. ethanol (180 mL) was heated under reflux for 6 h. The mixture was concentrated on a rotary evaporater (20 mmHg, 50 °C) to half of the volume, and the residue was washed by ether (30 mL x 2). The aqueous solution was acidified (pH 1) with HCl, and crude *N*-(2-nitrophenyl)-D-alanine (3.3 g) was obtained as pale yellow precipitates. A mixture of the crude product (2.0 g, 9.5 mmol) and 5% Pd-C in 50% aq. ethanol (50 mL) was stirred vigorously at room temperature under atmospheric pressure of H_2. Catalysts were removed by filtration, and solvents were removed by evaporation. The resulting crude product (1.2 g) was dissolved in anhydrous ether (100 mL), and to this was added $LiAlH_4$ (2.5 g). The mixture was heated under reflux for 5 h, and the reaction was quenched by addition of water (10 mL) and 3M aq. NaOH (2.5 mL). Precipitates were filtered off, and the filtrate was concentrated. To the obtained oily compound were added pyridine (8 mL) and acetic anhydride (24 mL), and the mixture was stirred at rt for 18 h. Then, the excess reagents were decomposed by the addition of methanol (30 mL), and solvents were removed. Column chromatography on silica gel eluting with a mixture of toluene and ethyl acetate (7:3, v/v) gave pure (*R*)-**3** (1.16 g). [15, 16] Colorless needles (recrystallized from toluene), mp 138 – 140 °C; TLC (silica gel, toluene:ethyl acetate = 1:5): R_f = 0.26; ^{1}H NMR ($CDCl_3$, 17 °C): δ 1.15 (d, 3H, J = 6.0 Hz, CH_3), 2.18 (s, 3H, CH_3CO), 2.22 (s, 3H, CH_3CO), 2.80 (br s, 1H), 5.00 (br s, 2H), 7.24–7.30 (m, 4H, Ar); ^{13}C NMR ($CDCl_3$, -30 ºC): δ 18.59 (CH_3), 22.20 (CH_3CO), 23.32 (CH_3CO), 49.92 (C(3)), 52.38 (C(2)), 124.32 (Ar), 126.35 (Ar), 126.39 (Ar), 126.52 (Ar), 133.51 (Ar), 136.10 (Ar), 168.48 (CO),

169.46 (CO); UV (CH_3CN): λmax 228 (ε 27,300), 253 nm (ε 14,200). *Anal*. Calcd for $C_{13}H_{16}N_2O_2$: C, 67.22; H, 6.94; N, 12.06. Found: C, 67.08; H, 6.95; N, 11.93.

*(*R*)-1,4-Dacetyl-2-ethyl-1,2,3,4-tetrahydroquinoxaline ((R)-**4**)*. Pale brown oil TLC (silica gel, toluene:ethyl acetate = 1:5): R_f = 0.29; ^{1}H NMR ($CDCl_3$, 17 °C): δ 0.85 (t, 3H, *J* = 7.6 Hz, CH_3), 1.36–1.50 (m, 2H), 2.18(s, 3H, CH_3CO), 2.22 (s, 3H, CH_3CO), 3.02 (br s,1H), 4.93 (br s, 2H), 7.25 (br s, 4H, Ar); ^{13}C NMR ($CDCl_3$, -30 ºC): δ 9.78 (CH_3), 22.54 (CH_2), 23.13 (CH_3CO), 26.27 (CH_3CO), 48.95 (C(3)), 55.59 (C(2)), 124.34 (Ar), 126.35 (Ar), 126.41 (Ar), 126.54 (Ar), 133.54 (Ar), 135.81 (Ar), 168.96 (CO), 169.42 (CO).

*(*S*)-1,4-Diacetyl-1,2,3,4-tetrahydro-2-isopropylquinoxaline ((S)-**5**)*. Colorless prisms (recrystallized from toluene), mp 133 – 134 °C; TLC (silica gel, toluene:ethyl acetate = 1:5): R_f = 0.36; ^{1}H NMR ($CDCl_3$, 17 °C): δ 0.85 (d, 3H, *J* = 6.9 Hz, CH_3), 0.87 (d, 3H, *J* = 6.9 Hz, CH_3), 1.68 (m, 1H), 2.15 (s, 3H, CH_3CO), 2.21 (s, 3H, CH_3CO), 3.19 (br s, 1H), 4.80 (br s, 2H), 7.27 (s, 4H, Ar); ^{13}C NMR ($CDCl_3$, -30 ºC): δ 17.04 (CH_3), 19.12 (CH_3), 22.52 (CH_3CO), 23.04 (CH_3CO), 30.70 (CH), 46.12 (C(3)), 59.26 (C(2)), 124.27 (Ar), 126.37 (Ar), 126.43 (Ar), 126.65 (Ar), 134.26 (Ar), 136.00 (Ar), 169.07 (CO), 169.60 (CO); UV (CH_3CN): λ_{max} 228 (ε 21,200), 253 nm (ε 10,700). *Anal*. Calcd for $C_{15}H_{20}N_2O_2$: C, 69.20; H, 7.74; N, 10.76. Found: C, 69.24; H, 7.87; N, 10.62.

*(R)-1,4-Diacetyl-1,2,3,4-tetrahydo-2-isopropyloquinoxaline ((R)-**5**)*. Colorless prisms (recrystalized from toluene), mp 134 – 135 °C. *Anal*. Calcd for $C_{15}H_{20}N_2O_2$: C, 69.20; H, 7.74; N, 10.76. Found: C, 69.29; H, 7.86; N, 10.65.

*(S)-1,4-Diacetyl-1,2,3,4-tetrahydro-2-(2-methylpropyl)quinoxaline ((S)-**6**)*. Colorless oil, TLC (silica gel, toluene:ethyl acetate = 1:5): R_f = 0.41; ^{1}H NMR ($CDCl_3$, 17 °C): δ 0.86 (d, 3H, *J* = 6.8 Hz, CH_3), 0.93 (d, 3H, *J* = 6.3 Hz, CH_3), 1.06–1.23 (m, 1H), 1.29–1.36 (m, 1H), 1.46–1.56 (m, 1H), 2.16 (s, 3H, CH_3CO), 2.22 (s, 3H, CH_3CO), 3.05 (br s, 1H), 4.71 (br s, 1H), 5.14 (br s, 1H), 7.21–7.29 (br s, 4H, Ar); ^{13}C NMR ($CDCl_3$, -30 ºC): δ 22.19 (CH_3), 22.64 (CH_3), 22.98 (CH_3CO), 23.82 (CH_3CO), 24.51 (CH_2), 42.38 (CH), 49.65 (C(3)), 51.89 (C(2)), 124.32 (Ar), 126.28 (Ar), 126.49 (Ar), 126.66 (Ar), 133.16 (Ar), 135.68 (Ar), 169.05 (CO), 169.08 (CO); UV (CH_3CN): λ_{max} 228 (ε 20,800), 254 nm (ε 10,900).

*(S)-1,4-Diformyl-1,2,3,4-tetrahydro-2-(2-methylpropyl)quinoxaline (**7**)*. Colorless oil, TLC (silica gel, toluene:ethyl acetate = 1:5): R_f = 0.72; ^{1}H NMR: δ ($CDCl_3$, 17 °C): 0.89 (d, 3H, *J* = 6.8 Hz, CH_3), 0.96 (d, 3H, *J* = 6.8 Hz, CH_3), 1.16–1.33 (m, 3H), 3.25 (dd, 1H, *J* = 13.2 and 3.9 Hz), 4.58 (dd, 1H, J = 13.2 and 2.0 Hz), 4.98–5.04 (m, 1H), 7.18–7.35 (m, 4H), 8.78 (s, 1H, CHO), 8.96 (s, 1H, CHO); ^{13}C NMR ($CDCl_3$, 17 ºC) δ: 22.49 (CH_3), 22.53 (CH_3), 24.85 (CH_2), 38.69 (CH), 41.99 (C(3)), 44.34 (C(2)), 116.52 (Ar), 118.42 (Ar), 125.49 (Ar), 125.63 (Ar), 160.15 (CHO), 160.39 (CHO).

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