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A Novel NADH Model: Design, Synthesis, and its Chiral Reduction and Fluorescent Emission

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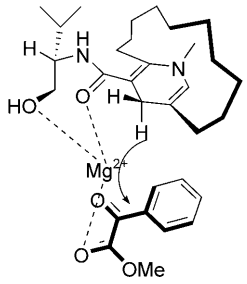
Abstract: A novel chiral nicotinamide adenine dinucleotide hydrogen (NADH) model with C_3 symmetry was designed and synthesized. Hydrogens at the C-4 position of all dihydropyridine rings in the inner part of the bowl could transfer to the substrate with powerful enantioselectivity. This novel C_3 symmetrical NADH model is capable of fluorescence emission at 455 nm when excited at 390 nm.

Keywords: chiral NADH model; enantioselectivity; fluorescence; magnesium(II); synthesis



The coenzyme nicotinamide adenine dinucleotide hydrogen (NADH) is an important molecule in nature. Over the past several decades, NADH mimics have become one of the highlights of biochemistry and organic chemistry.[1] To the best of our knowledge, the dihydropyridine amido group is the key structure for NADH models.

Kanomata[1f] designed and synthesized a bridged NADH model with an [n](2,5)pyridinophane (parapyridinophane) skeleton as a sterically-demanding side chain that induces high enantioselectivity. Kanomata believed that the oligomethylene chain bridging the 2- and 5-positions of a dihydronicotinoyl ring would behave as an "enzyme wall" that allows the substrates to approach from the opposite side exclusively. In the asymmetric reduction of methyl benzoylformate, mandelate was obtained with a high enantiomeric excess when a 1 molar ratio of magnesium was used.

Generally, chemists had formerly designed NADH models according to three aspects: the first one was to control the stereoselectivity by changing the substitutent of the dihydronicotinamide with a view to form a remote sterically-demanding side chain.[2] The second aspect was to incorporate a substituent at the reaction center: the C-4 position of the dihydropyridine ring.[3] The third one was to design the specific conformation to obtain high stereoselectivity.[4] However, in the first situation the dihydronicotinamide unit needs to be modified significantly by introducing big chiral auxiliaries, a complicated ring or by changing the amide group to a chiral sulfinyl group. The second one suffered from loss of chirality at the C-4 position during the course of the model reduction reaction. The third one was particular and by far fewer models have been studied. Herein, a novel chiral NADH model compound **1** with C_3-symmetry was designed and synthesized.

Our model **1** has six chiral carbon centers. (*1R*,*2R*)-Diaminocyclohexane is introduced as chiral source to connect three identical pyridine-3,5-dicarbonyl groups into a large ring. Then three identical 1,4-dihydropyridine units are connected by the phenyl-1,3,5-trimethylene group to form model **1**. Molecular modeling *via* molecular dynamics followed by energy minimization with Gaussian 03 shows that the bowl-shaped conformation shown in Figure 1 is the most stable one. The phenyl-1,3,5-trimethylene group is the "bottom" of the "bowl" and the rigidly defined concave cavity of the bowl can hold a metal ion in a fixed position relative to the 1,4-dihydropyridine. As a result, regulation of the stereoselective approach of the substrates for accomplishment of their biomimetic reduction with

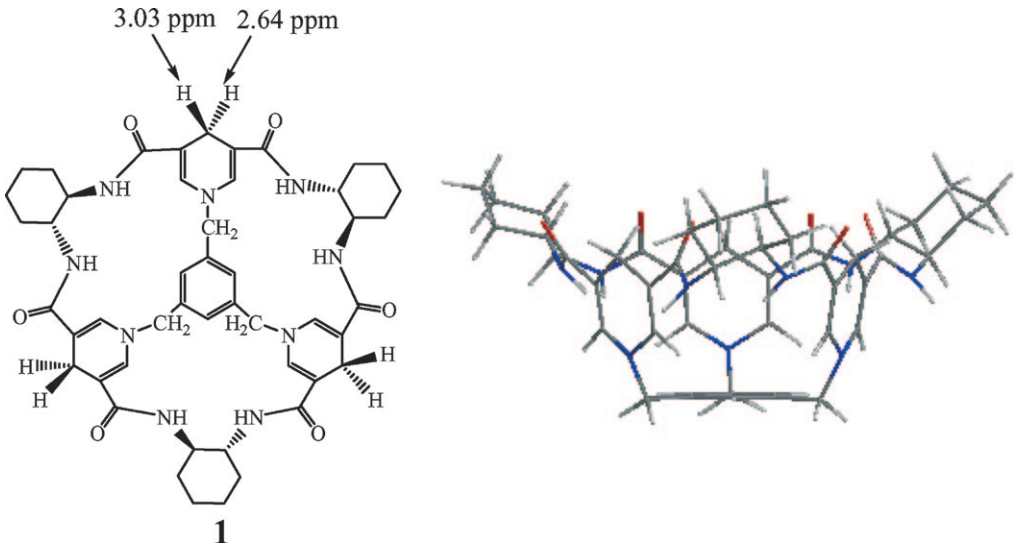


Figure 1. The structure of model **1** and its conformation.

high enantioselectivity could be realized. Besides, three identical dihydropyridine units are arranged in a large ring system in such way that all of the three hydrogens in the C_3 symmetrical model can transfer to the substrate. Our NADH model should be interesting for the research on biosimulation. This C_3 symmetrical NADH model should also be a new fluorescent probe in the course of the signal transduction and for some research on the reaction mechanism.

We originally attempt to synthesize model **1** by the tri-directional synthetic method outlined in Scheme 1. Since three identical 3,5-diethoxycarbonylpyridine units were fixed by a phenyl-1,3,5-trimethylene group, the most difficult ring-closing reaction in this route would be more feasible.

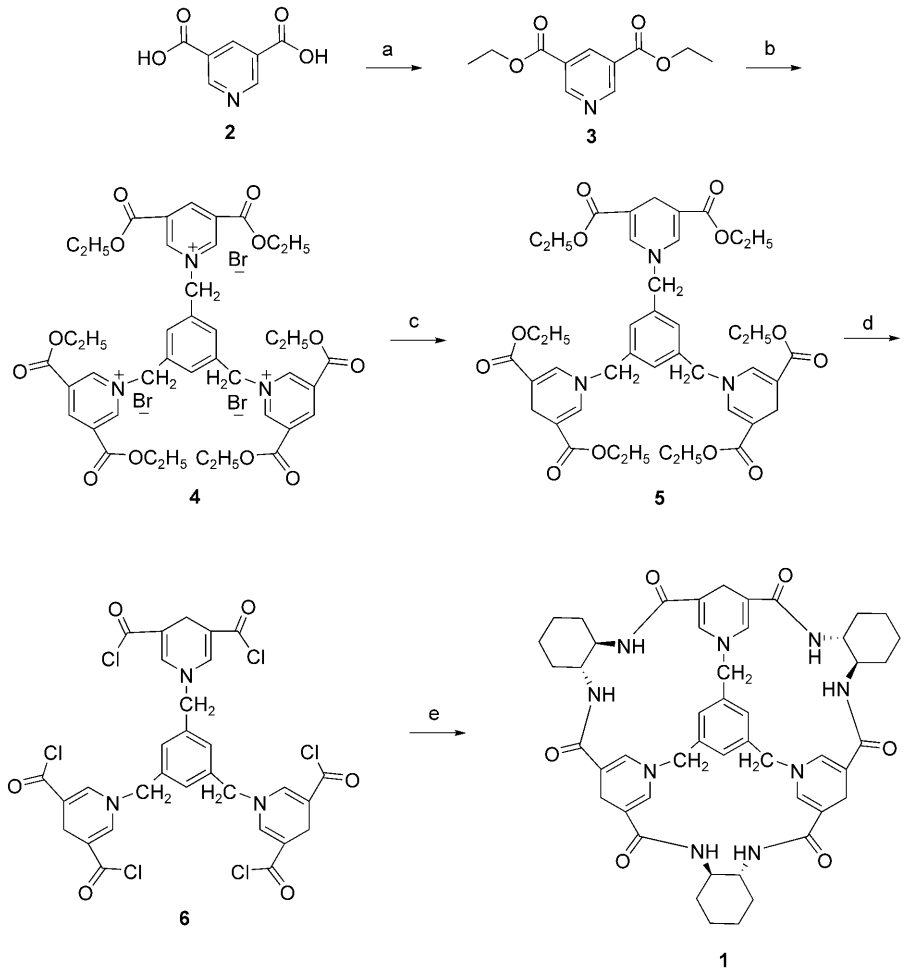
It seems that this synthetic route should have been available.[5–7] Unfortunately, we found that our intermediate **5** was very sensitive to temperature, light and air. Compound **5** was very difficult to keep its stability in the following procedures. Thus the route shown in Scheme 1 failed.

A practical and efficient synthetic route is outlined in Scheme 2. The key method in Scheme 2 was the reduction reaction which occurred in the last step. At first, the large heterocycle compound **8** was expected to be obtained by the reaction of 3,5-dichlororocarbonylpyridine with (1*R*,2*R*)-diaminocyclohexane.[8] However, we found that the products were very complicated and some linear polymer also was formed. It seemed that the acyl chloride was too active to make the reaction controllable. Then we tried to look for a suitable reagent which was a little bit less active than the acyl chloride. Pentafluorophenol was found to be a good reagent and the pentafluorophenoxy moiety was a good leaving group. Compound **7** was prepared by reaction of **2** with pentafluorophenol. It was easy to obtain **8** by treatment of **7** with excess (1*R*,2*R*)-diaminocyclohexane under mild conditions. 1,3,5-Tris-(bromomethylene)benzene was added into a DMF solution of **8** and the resulting reaction mixture was heated at 85 °C, **9** was obtained and used directly in the next procedure without further purification. Crude **9** was reduced by sodium dithionite to afford crude product **1**. After separation on Sephadex LH-20 with methanol as the eluent, the pure model compound **1** was obtained.

It is interesting that there is an obvious difference of the chemical shifts between the two prochiral C-4 protons in the ^{1}H NMR spectrum of model **1** ($\Delta\delta =$ 0.4 ppm). As shown in Figure 1, this significant feature suggests that the carbonyl groups in the C-3 and C-5 positions of the dihydropyridine rings are greatly restricted by the rigidly defined concave cavity and have an intilted orientation. Thereby the prochiral C-4 protons are highly diastereotopically distinguished by the special bowl-shaped conformation. Three hydrogens at the C-4 positions of all dihydropyridine rings in the inner part of the bowl could transfer to the substrate in the reduction reactions of our novel NADH model with powerful enantioselectivity.

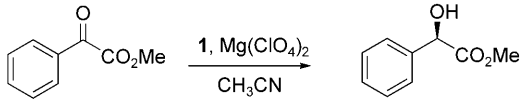
The C_3 symmetrical NADH model **1** was found to effect a good biomimetic reduction simply, and this is not in prevailing organocatalyzed asymmetric reduction. The reaction of **1** with methyl benzoylformate was carried out in the presence of magnesium perchlorate in acetonitrile (Table 1).

Enantioselective reduction is one of the key reactions to produce chiral compounds in academia and in the chemical industry.[9] Recently the Hantzsch dihydropyridine (not a chiral hydride source) has been used as a transfer-hydrogenation reagent for organocatalytic asymmetric reactions.[10–13] However, enantio-



Scheme 1. Attempted synthesis of model **1**. (a) C_2H_5OH, H_2SO_4, reflux, 69%; (b) 1,3,5-tris(bromomethyl)benzene, CH_3OH, reflux, 45%; (c) $Na_2S_2O_4$, Na_2CO_3, H_2O, room temperature, 26%; (d) 1. NaOH, H_2O, room temperature, 5 h; 2. $SOCl_2$, reflux, 8 h; (e) (1*R*,2*R*)-diaminocyclohexane, THF, room temperature, 12 h.

Table 1. Asymmetric reduction reactions of methyl benzoylformate with **1**. (The ratio of NADH model to methyl benzoylformate is 1:1).



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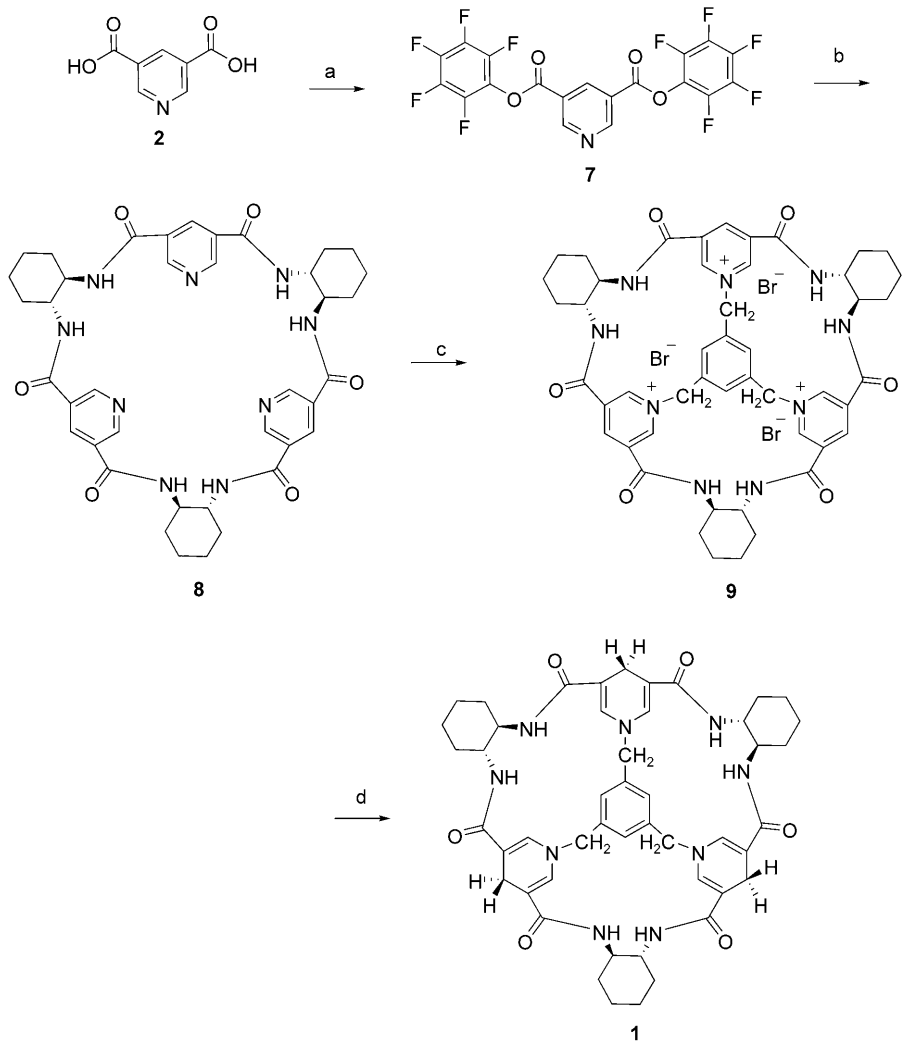
Entry	*T* [°C]	*t*	Ratio $Mg(ClO_4)_2$/model **1**	**10**		
				ee [%][a,b]	Configuration	Yield [%][c]
1	r.t.	3 d	3.0	88	*R*	96
2	50	18 h	3.0	82	*R*	92

[a] Determined by HPLC.
[b] No 2-propanol present.
[c] Isolated yields.

selective hydrogenation with Hantzsch dihydropyridine has to use costly chiral organocatalysts. To the best of our knowledge, most of the Hantzsch dihydropyridine compounds are neither chiral molecules nor chiral hydride sources, thus, asymmetric reduction with Hantzsch dihydropyridine needs chiral organocatalysts. NADH is a coenzyme molecule in nature, Hantzsch ester compounds would not be subject to



Scheme 2. Successful synthesis of model **1**. Reagents and conditions: (a) pentafluorophenol, DMF, room temperature, 6 h, 92%; (b) (*1R,2R*)-diaminocyclohexane, THF, room temperature, 4 h, 21%; (c) 1,3,5-tris(bromomethyl)benzene, DMF, 85 °C, 12 h, 35%; (d) $Na_2S_2O_4$, Na_2CO_3, H_2O, room temperature, 10 h, 42%.

NADH models.[1e] Researches on NADH models are mainly useful for some of the biosimulating and life sciences in the future.

Nicotinamide adenine dinucleotide hydrogen (NADH) in nature is capable of fluorescent emission at 430–445 nm when excited at 340 nm ($\lambda_{exc}=340$ nm, $\lambda_{em}=430$–445 nm), while the oxidized forms (NAD^+) are not.[14a] Blomquist reported that the fluorescence emission intensity at 460 nm of reduced NADH in solution is increased in the presence of deuterium oxide over that observed in water.[14b] We found that our model **1** is capable of fluorescent emission at 455 nm when excited at 390 nm ($\lambda_{exc}=390$ nm, $\lambda_{em}=455$ nm). The dihydropyridine rings in the model **1** could act similarly as pH-sensitive fluorophores, this is a unexpected new finding in our model **1**. Figure 2 shows the pH dependence on the emission spectrum of **1** that displays fluorescent pH-sensing activity in the physiological pH range.

On the basis of the results described, we believe that the C_3 symmetrical NADH model **1** should be a new pH-sensing fluorescent probe, which should have some good potential as a small probe molecule for the research on atherosclerosis in the course of signal transduction.[15a] Plenio reported the use of fluorescence signal molecules to follow the course of a Suzuki coupling reaction, and the fluorescence signal changes at each stage of the reaction.[15b]

In summary, we have designed and synthesized the first C_3 symmetrical NADH model with a special bowl-shaped conformation. Three identical dihydropyridine units were connected to form a rigidly defined concave cavity which could encase and fix some substrates to accomplish the biomimetic reduction

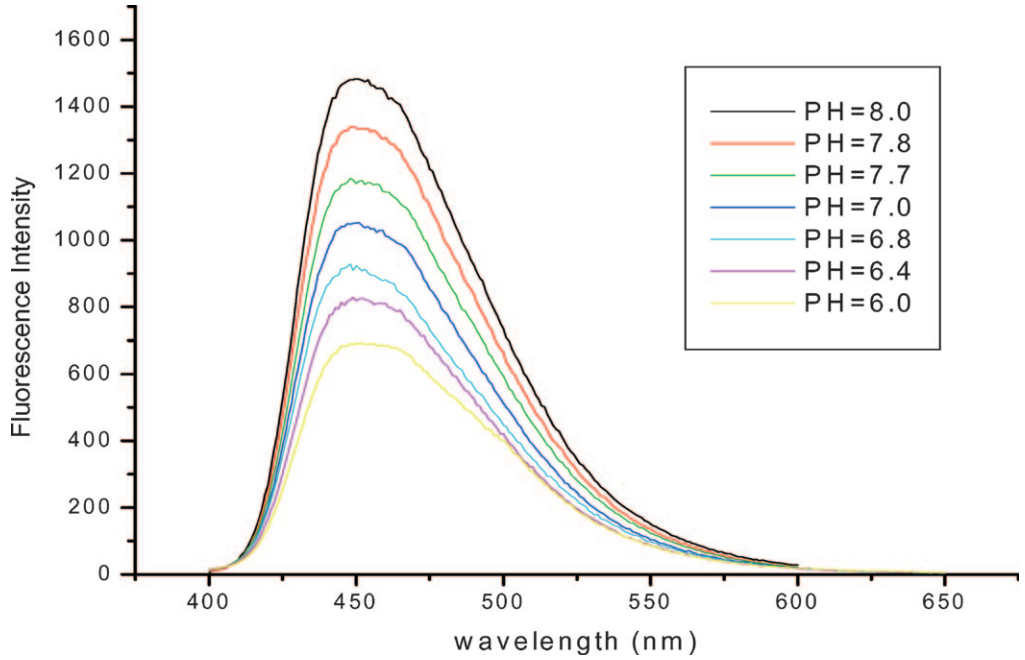


Figure 2. Emission spectra of **1** in methanol as a function of pH. Spectra were recorded with excitation at 390 nm at the different pH values.

with high enantioselectivity. Meanwhile, all three hydrogens at the C-4 positions of three dihydropyridine rings in the inner part of the bowl could transfer to the substrate in the reduction reactions of NADH model **1** with powerful enantioselectivity. The C_3 symmetrical NADH model should also be a new fluorescent probe for the research on atherosclerosis in the course of signal transduction[15a] and some reaction mechanisms.[15b]

Experimental Section

Preparation of Compound 7

1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (6.4 g, 33.2 mmol) was added to a solution of 3,5-pyridinedicarboxylic acid (2.8 g, 16.8 mmol) and pentafluorophenol (6.2 g, 33.2 mmol) in 150 mL of dry DMF. The resulting solution was stirred at room temperature for 6 h, water (200 mL) was added and a white precipitate formed. The product was collected by filtration and purified by flash column chromatography (silica gel, CH_2Cl_2) to give the white solid **7**; yield: 7.80 g (93%); mp 154–157 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.20 (s, 1H, pyr-4-C*H*), 9.68 (s, 2H, pyr-2,6-C*H*); IR (KBr): v = 3061, 2670, 2465, 1773, 1655, 1602, 1516, 1471, 1430, 1321, 1294, 1223, 1154, 1095, 996, 731 cm^{-1}; EI-MS: *m*/*z* (%) = 316 (100), 260 (23.8), 183 (6.6), 105 (10.8).

Preparation of Compound 8

A solution of (1*R*,2*R*)-diaminocyclohexane (1.3 g, 11.4 mmol) in THF (30 mL) was added dropwise to a mixture of the bis(pentafluorophenyl) ester of 3,5-pyridinedicarboxylic acid **7** (5.0 g, 10.0 mmol) in THF at 0 °C (ice bath), The resulting reaction mixture was stirred at room temperature for 4 h. The solvent was removed to give the crude product. The product was purified by column chromatography, ethanol and ethyl acetate (1: 9) was used as eluent to give the white solid **8**; yield: 0.5 g (21%); ^{1}H NMR [400 MHz, $(CD_3)_2SO$]: δ = 1.32 (m, 6H, C*H*H′CH_2CHNH), 1.52–1.55 (m, 6H, CH*H*′CH_2CHNH), 1.76–1.78 (m, 6H, C*H*H′CHNH), 1.88–1.92 (m, 6H, CH*H*′CHNH), 3.93 (m, 6H, C*H*NH), 8.38 (s, 3H, pyr-4-C*H*), 8.66 (d, *J* = 7.56 Hz, 6H, N*H*), 8.87 (s, 6H, pyr-2,6-C*H*); ^{13}C NMR [100 MHz, $(CD_3)_2SO$]: δ = 24.9 (CH_2CH_2CHNH), 31.5 (CH_2CHNH), 53.5 (*C*HNH), 130.3 (pyr-3,5-*C*),134.8 (pyr-4-*C*H), 150.1 (pyr-2,6-*C*H), 165.0, 165.1 (*C*=O); IR (KBr): v = 3252, 3065, 2938, 2860, 1635, 1541, 1298, 705 cm^{-1}; ESI-MS *m*/*z* = 736.5 $[M+H]^+$, 774.5 $[M+K]^+$, 734.3 $[M-H]^-$, 770.4 $[M+Cl]^-$; $[\alpha]_D$: −177.4° (*c* 0.002, DMSO).

Preparation of Compound 9

Compound **8** (0.4 g, 0.54 mmol) and 1,3,5-tris(bromomethyl)benzene (0.2 g, 0.56 mmol) were added to dry DMF (5 mL), and the resulting solution was stirred under nitrogen at 85 °C for 12 h, then cooled and ether was added. The precipitate was collected by filtration and washed with ether to give the crude product as a white solid (yield: 0.53 g, 89%) which was used without further work-up.

Preparation of Compound 1

To an aqueous solution of compound **9** (0.5 g, 0.46 mmol), an aqueous solution of sodium dithionite (1.9 g, 10.9 mmol) and sodium carbonate (0.73 g, 6.5 mmol) were added dropwise at room temperature under nitrogen. The mixture was

left to stir overnight at room temperature, during the time a yellow precipitate formed. The product was collected by filtration and purified by gel chromatography (Sephadex LH-20, CH_3OH) to give **1** as a yellow solid; yield: 0.16 g (yield 42%); ^{1}H NMR [400 MHz, $(CD_3)_2SO$]: $\delta = 1.20$ (m, 12H, CH_2CH$_2$CHNH), 1.52–1.54 (m, 6H, CH$_2$CHH′CHNH), 1.78 (m, 6H, CH$_2$CHH′CHNH), 2.64 (d, $J = 18.3$ Hz, 3H, pyr-4-CHH′), 3.03 (d, $J = 18.3$ Hz, 3H; pyr-4-CHH′), 3.63 (m, 3H, CHNH), 3.78–3.80 (m, 3H, CHNH), 3.95 (d, $J = 14.1$ Hz, 3H, ArCHH′), 4.56 (d, $J = 14.1$, 3H, ArCHH′), 6.44 (s, 3H, C$_6H_3$), 6.92 (d, $J = 8.4$ Hz, 3H, CHNH), 7.06 (d, $J = 7.6$ Hz, 3H, CHNH), 7.16 (s, 3H, pyr-2,6-CHCH′), 7.21 (s, 3H, pyr-2,6-CHCH′); ^{13}C NMR [150 MHz, $(CD_3)_2SO$]: $\delta = 25.2$ (CH_2CH$_2$CHNH), 25.6 (C′H$_2$CH$_2$CHNH), 32.0 (CH$_2$CHNH), 32.9 (C′H$_2$CHNH), 40.5 (pyr-4-CH_2), 52.0 (CHNH), 53.0 (C′HNH), 57.3 (ArCH$_2$), 107.3 (pyr-3,5-CC′), 109.1 (pyr-3,5-CC′), 127.7 (Ar-2,4,6-CH), 132.6 (Ar-1,3,5-C), 136.2 (pyr-2,6-CHC′H), 138.1 (pyr-2,6-CHC′H), 167.0, 167.1 (C=O); ESI-MS: $m/z = 856.7$ [M+H]$^+$, 873.8 [M+Na]$^+$; HR-MS (ESI): $m/z = 854.43633$, calcd. for [M−H]: 854.43590; $[\alpha]_D$: −238.2° (c 0.002, DMSO).

Acknowledgements

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