BIOTINYLATED PRIMARY AMINES

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Abstract

In the frame of a biotinylation program of biologically active amines we synthesized two amides from 2-phenetylamine and 3,4-dimethoxybenzylamine, that were fully characterized by means of multinuclear NMR spectroscopy.

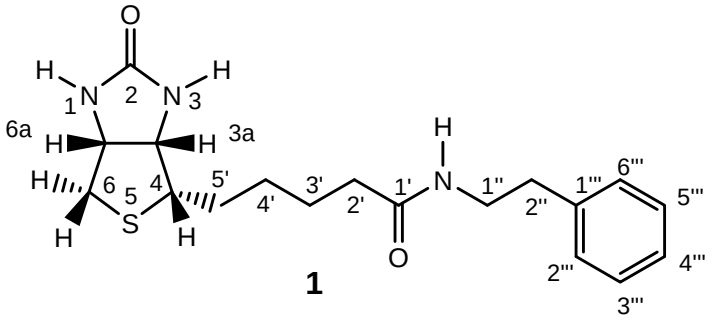
Keywords: primary amines, biotinylation, ^{1}H NMR, ^{13}C NMR, ^{15}N NMR

Introduction

The highly specific interaction of avidin with the small vitamin biotin can be a useful tool in assay systems designed to detect and target biological analytes.[1] The extraordinary affinity of avidin for biotin ($K_a = 10^{15}$ M^{-1}) allows biotin-containing molecules in a complex mixture to be discretely bound with avidin conjugates.[2,3]

In this context we have synthesized two new amides **1** and **2**, from 2-phenethylamine (an endogenous amine structurally and pharmacologically related to amphetamine, found in normal urine)[4] and veratrylamine or 3,4-dimethoxybenzylamine (a chemical repellent

presumed to activate trigeminal neurons)[5] and their fully characterization by multinuclear magnetic resonance spectroscopy (^{1}H, ^{13}C, and ^{15}N) in solution has been achieved.



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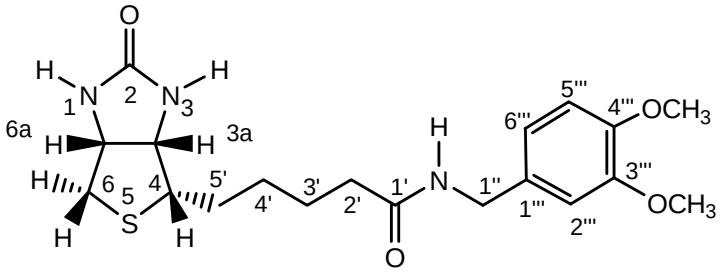

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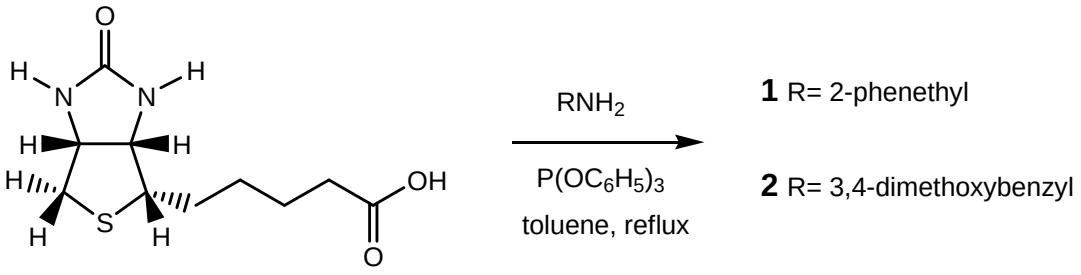
Figure 1. Structures of the amides

Results and discussion

Instead to start the preparation of the aforementioned amides **1** and **2** from (+)-biotin acid chloride or (+)-biotin methyl ester,[6] the synthesis was approached by treatment of the free acid, (+)-biotin, with the primary amines in presence of triphenylphosphite[7] with yields of 81% and 83%, respectively. Purification was achieved by successive wash with toluene and hexane.



The structural characterization of biotinylated amines, **1** and **2**, was achieved by ^{1}H, ^{13}C and ^{15}N NMR and the fully assignments are given in Tables 1 and 2. Multiplicity of the signals as well as 2D experiments (HMQC and HMBC) have been used.[8] The data here presented agree with those reported by us for related biotin derivatives.[9,10]

In the case of compound **1**, the correlations observed in the (^{1}H-^{1}H) COSY NMR spectra are depicted in Figure 2, being the signal that corresponds to H4, at 3.07 ppm, the key starting point to complete the assignments.

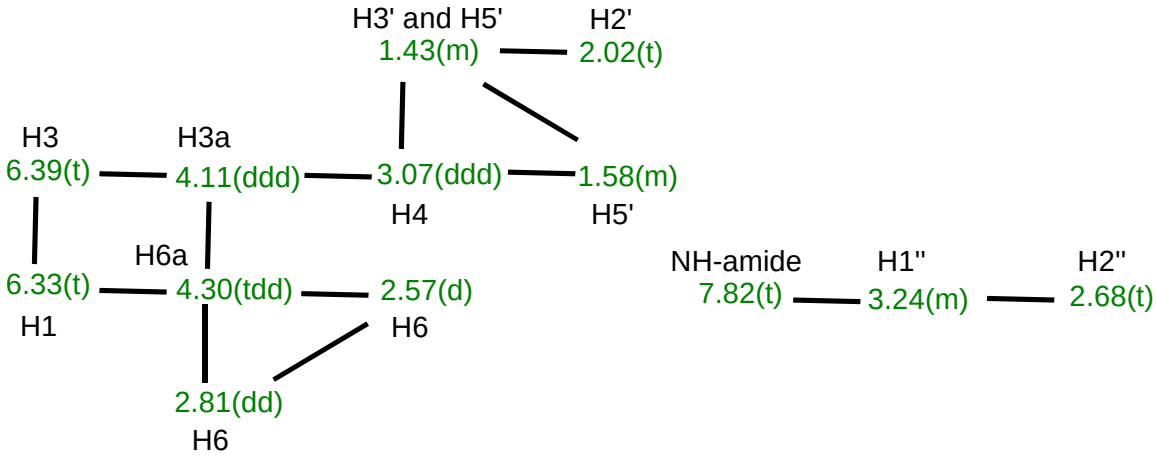


Figure 2. ^{1}H-^{1}H-COSY NMR spectra correlations for compound **1** in DMSO-*d*$_6$

The (^{1}H-^{15}N) HMBC spectra proved to be very useful not only to measure the chemical shifts of the different nitrogens for each amide, but to determine the NH coupling constants.

(a) (b)

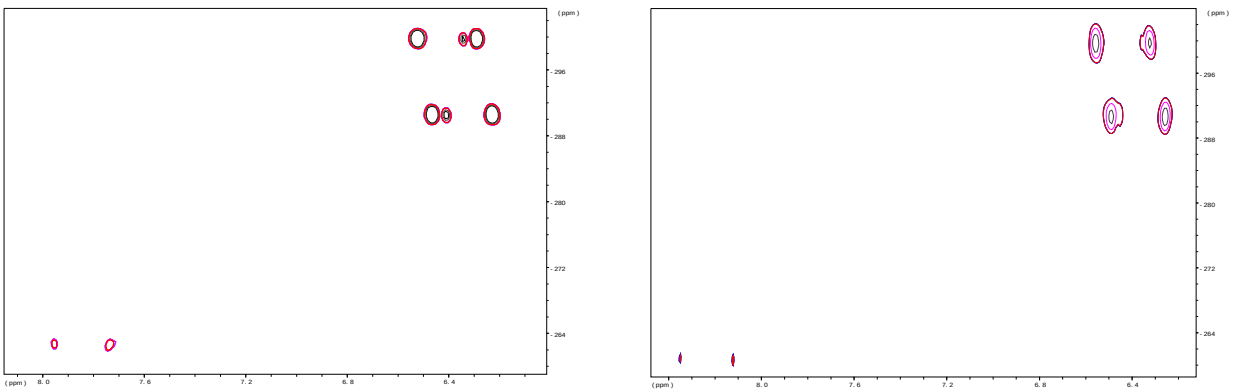


Figure 3. ^{1}H-^{15}N HMBC spectra in DMSO-d$_6$ of amides **1** (a) and **2** (b)

Table 1: Chemical shifts (δ in ppm) and coupling constants (J in Hz) of compound **1** in DMSO-d_6.

Chemical shifts	Nuclei	Coupling constants	HMQC correlations	HMBC correlations
7.82	H-amide	$^3J_{H1''}$= 5.7	N-amide	C1', C1''
7.27	H3'''	---	C3'''	C1''', C2''
7.18	H2''', H4'''	---	C2''', C4'''	C2'''
6.39	H3	$^3J_{H3a}$= $^4J_{H1}$= 1.8	N3	C2, C3a, C6a, N1
6.33	H1	---	N1	C2, C3a, C6a, N3
4.30	H6a	$^3J_{H3a}$= 7.7 $^3J_{H6}$= 5.2 $^3J_{H1}$= $^3J_{H6}$= 1.0	C6a	C2, C4
4.11	H3a	$^3J_{H6a}$= 7.7 $^3J_{H4}$= 4.4 J_{H3}= 1.9	C3a	C4
3.24	H1''	---	C1''	C1', C1'''
3.07	H4	$^3J_{H5}$= 8.5 $^3J_{H5}$= 6.1 $^3J_{H3a}$= 4.5	C4	
2.81	H6	$^2J_{gem}$= 12.4 $^3J_{H6a}$= 5.1	C6	N1
2.68	H2''	^3J= 7.7	C2''	C1''', C2''', C3''', N-amide
2.57	H6	$^2J_{gem}$= 12.3	C6	C3a, C62, C4, N1
2.02	H2'	^3J= 7.3	C2'	C1', C3', C5'
1.58	H5'		C5'	
1.43	H5', H3'		C5', C3'	
1.27	H4'		C4'	
171.9	C1'		---	NH-amide, H1'', H2', H3'
162.7	C2		---	H3, H1, H6a
139.5	C1'''		---	H3''', H1'', H2''
128.6	C3'''		7.27	H3'''
128.2	C2'''		7.18	H2''', H4'''
126.0	C4'''		7.18	H2'''
61.0	C3a		4.11	H3, H1, H6
59.2	C6a		4.30	H3, H1, H6
55.4	C4	^1J= 124.0	3.07	H6a, H3a, H6
40.0	C1''		3.24	H-amide
39.8	C6		2.81, 2.57	---
35.2	C2', C2''		2.02, 2.68	H3''', H1'', CH_2 multiplets
28.1	C4'		1.27	CH_2 multiplets
28.0	C5'		1.58, 1.43	CH_2 multiplets
25.3	C3'		1.43	H2'
-299.6	N3	^1J= 93.3	6.39	H1
-290.8	N1	^1J= 93.3	6.33	H3, H6
-262.5	N-amide	^1J= 89.4	7.82	H2''

Table 2: Chemical shifts (δ in ppm) and coupling constants (J in Hz) of compound **2** in DMSO-d_6.

Chemical shifts	Nuclei	Coupling constants	HMQC correlations	HMBC correlations
8.20	H-amide	$^3J_{H1''}$= 5.9	N-amide	C1', C1''
6.87	H5'''	$^3J_{H6'''}$= 8.2	C5'''	C1''', C3'''
6.86	H2'''	$^4J_{H6'''}$= 2.0	C2'''	C4''', C6''', C1''
6.74	H6'''	$^3J_{H5'''}$= 8.2 $^4J_{H2'''}$= 2.0	C6'''	C4''', C2''', C1'
6.40	H3		N3	C2, C6a, C3a
6.34	H1		N1	C2, C6a, C3a
4.29	H6a	$^3J_{H3a}$= 7.5 $^3J_{H6}$= 5.1 $^3J_{H1}$= $^3J_{H6}$= 1.0	C6a	C1
4.17	H1''	$^3J_{H\text{-amide}}$= 5.9	C1''	C1', C1''', C2''', C6'''
4.10	H3a	$^3J_{H6a}$= 7.7 $^3J_{H4}$= 4.4 $^3J_{H3}$= 1.9	C3a	
3.72	3'''-OCH_3	---	3'''-OCH_3	C3'''
3.71	4'''-OCH_3	---	4'''-OCH_3	C4'''
3.07	H4	$^3J_{H5}$= 8.6 $^3J_{H5}$= 6.2 $^3J_{H3a}$= 4.5	C4	
2.81	H6	$^2J_{gem}$= 12.4 $^3J_{H6a}$= 5.1	C6	C6a, C3a
2.57	H6	$^2J_{gem}$= 12.4	C6	C6a, C3a
2.11	H2'	^3J= 7.4	C2'	C1
1.31	H3'			
1.53	H4' H5'			
171.9	C1'			NH-amide, H1'', H2'
162.7	C2			H3, H1, H6a
148.6	C3'''			H5''', 3'''-OCH_3
147.7	C4'''			H6''', H2''', 4'''-OCH_3
132.1	C1'''			H5''', H1''
119.2	C6'''	^1J= 162.6	6.74	H2''', H1''
111.7	C5'''	^1J= 158.0	6.87	----
111.3	C2'''	^1J= 156.4	6.86	H6''', H1''
61.0	C3a	^1J= 147.2	4.10	H3, H1, H6
59.2	C6a	^1J= 150.3	4.29	H3, H1, H6
55.6	4-OCH_3	^1J= 144.2	3.71	---
55.4	3-OCH_3	^1J= 144.2	3.72	---
41.7	C1''		4.17	NH-amide, H2''', H6'''
39.8	C6		2.81, 2.57	
35.1	C2'	^1J= 125.7	2.11	CH_2 multiplets
28.2	C5'	^1J= 124.2	1.53	H4, CH_2 multiplets
28.0	C4'	^1J= 133.4	1.53	CH_2 multiplets
25.3	C3'	^1J= 127.3	1.31	H2'
-299.6	N3	^1J= 94.5	6.40	
-290.8	N1	^1J= 94.5	6.34	
-261.0	N-amide	^1J= 90.8	8.20	

Finally, the conformational changes in molecules **1** and **2**, induced by the solvent ($CDCl_3$, HMPA-d_{18}) or the phase (solid state), are now under investigation.

Experimental Procedure

Chemistry

N-phenethyl-5-{(3aS, 4S, 6aR)-2-oxo-hexahydro-1H-thieno[3,4-d]imidazol-4-yl} pentanamide (**1**) *and N-(3,4-dimethoxybenzyl)-5-{(3aS, 4S, 6aR)-2-oxo-hexahydro-1H-thieno[3,4-d]imidazol-4-yl}pentanamide* (**2**)

To a stirred suspension of (+)-biotin (2 mmol) and 2-phenethylamine or veratrylamine (3 mmol) in toluene (6 mL), a solution of triphenylphosphite (3 mmol) in toluene (6 mL) was gradually added at room temperature. The reaction mixture was stirred at toluene reflux (110°C) during 16 h. The solution was left to attain the room temperature and the product precipitated as white solid, then was filtered, washed with toluene (2 X 50 mL) and hexane (1 X 50 mL) and dried under vacuum affording the pure amides **1** and **2**. Melting points were determined in a hot-stage microscope being 194-196 °C and 153-155°C, respectively.

NMR spectroscopy

Spectra were recorded at 300 K on a Bruker DRX 400 (9.4 Tesla, 400.13 MHz for ^{1}H, 100.62 MHz for ^{13}C and 40.56 MHz for ^{15}N) spectrometer with a 5-mm inverse detection H–X probe equipped with a z-gradient coil for ^{1}H, ^{13}C and ^{15}N, save specified. Chemical shifts (δ in ppm) are given from internal solvent, DMSO-d_6 (2.49) for ^{1}H and (39.5) for ^{13}C. And external reference $CH_3{}^{15}NO_2$ (0.00) for ^{15}N NMR was used. 2D (^{1}H–^{1}H) gs-COSY and inverse proton detected heteronuclear shift correlation spectra, (^{1}H–^{13}C) gs-HMQC, (^{1}H–^{13}C) gs-HMBC, (^{1}H–^{15}N) gs-HMQC, and (^{1}H–^{15}N) gs-HMBC, were acquired and processed using standard Bruker NMR software and in non-phase-sensitive mode.[8] Gradient selection was achieved through a 5% sine truncated shaped pulse gradient of 1 ms.

Acknowledgments

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