



Title	Nostosin G and Spiroidesin B from the Cyanobacterium Dolichospermum sp. NIES-1697
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Citation	Journal of Natural Products, 85(8), 2000-2005 https://doi.org/10.1021/acs.jnatprod.2c00382
Issue Date	2022-08-10
Doc URL	http://hdl.handle.net/2115/90284
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File Information	np2c00382_si_001-1.pdf (Supporting Information)



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Supporting Information

Nostosin G and Spiroidesin B from the Cyanobacterium *Dolichospermum* sp. NIES-1697

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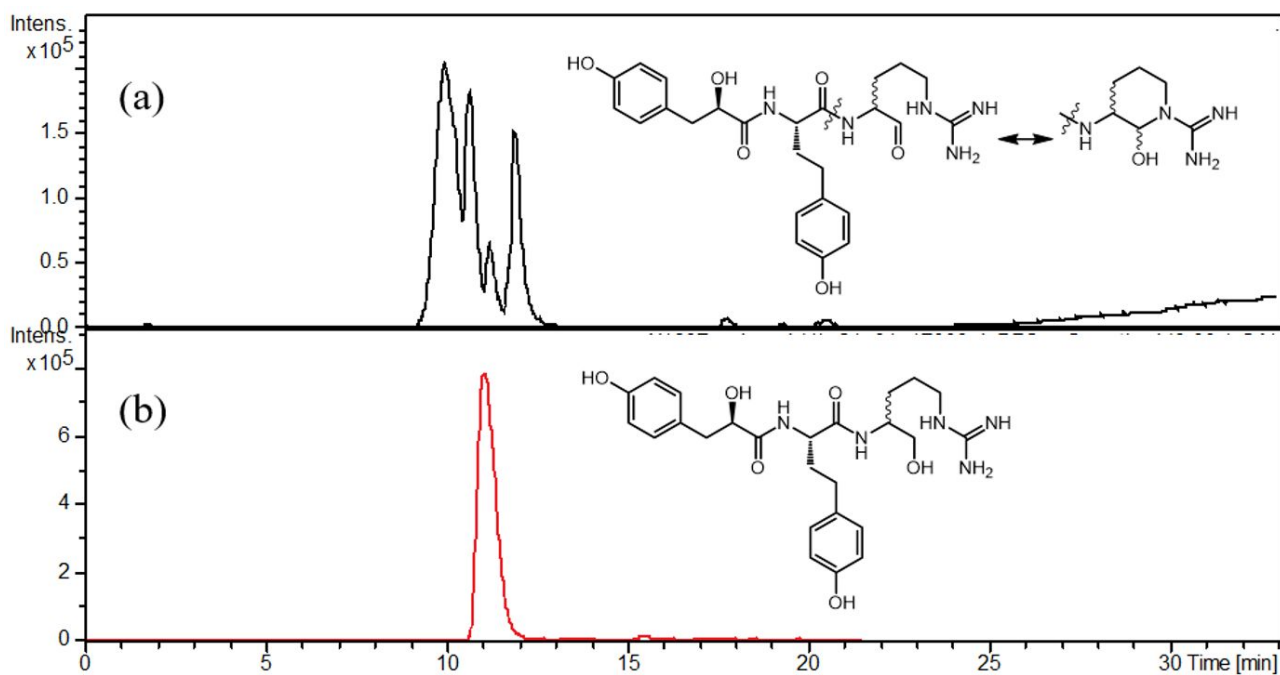


Figure S1. LC-MS chromatogram of **1** before NaBH₄ reduction (a) and **5** after NaBH₄ reduction (b)

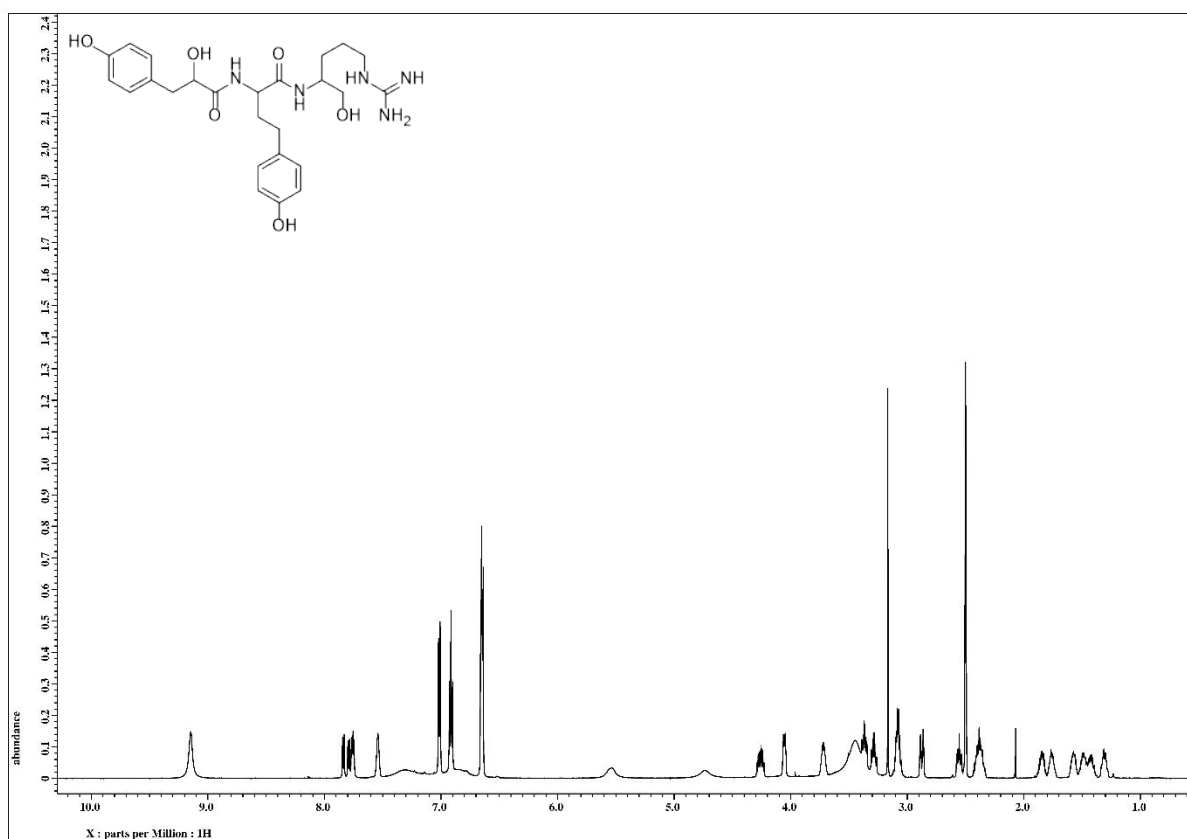


Figure S2. ¹H NMR (600 MHz) spectrum of **5** (reduced form of **1**) in DMSO-*d*₆

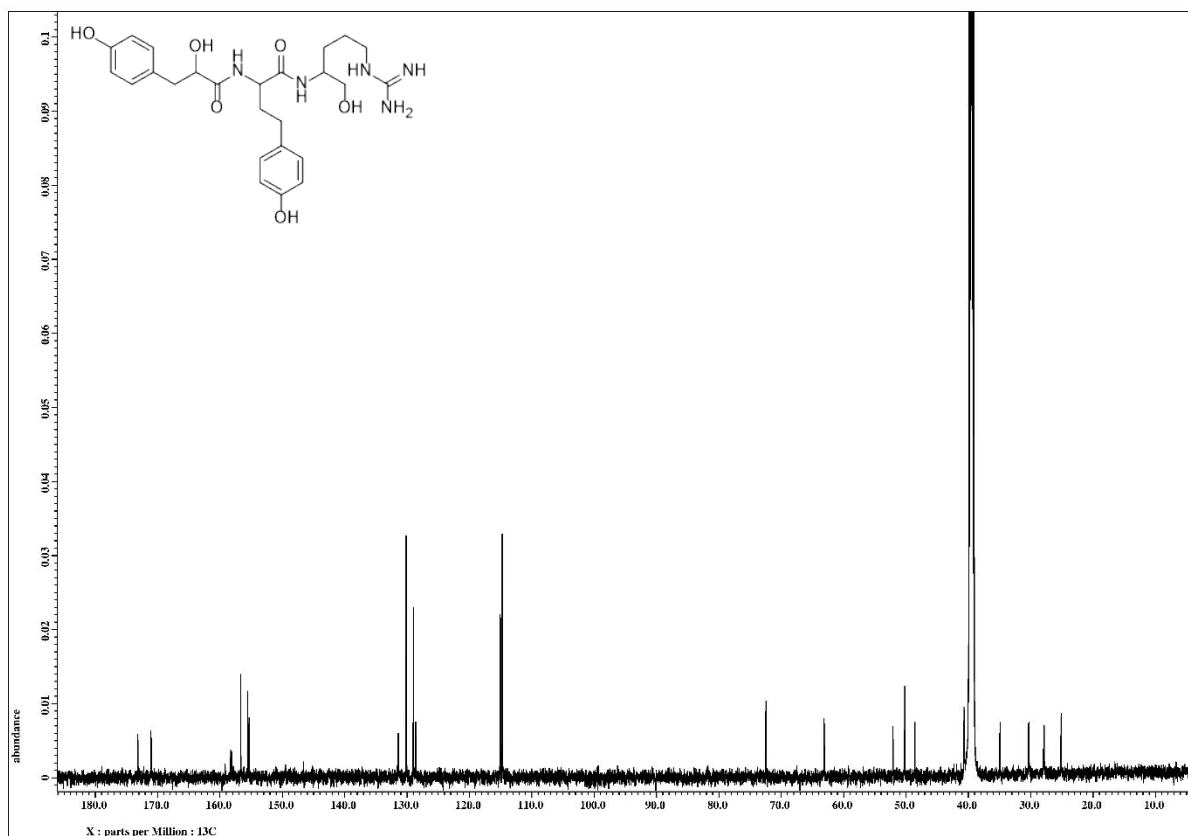


Figure S3. ^{13}C NMR (150 MHz) spectrum of **5** (reduced form of **1**) in $\text{DMSO-}d_6$

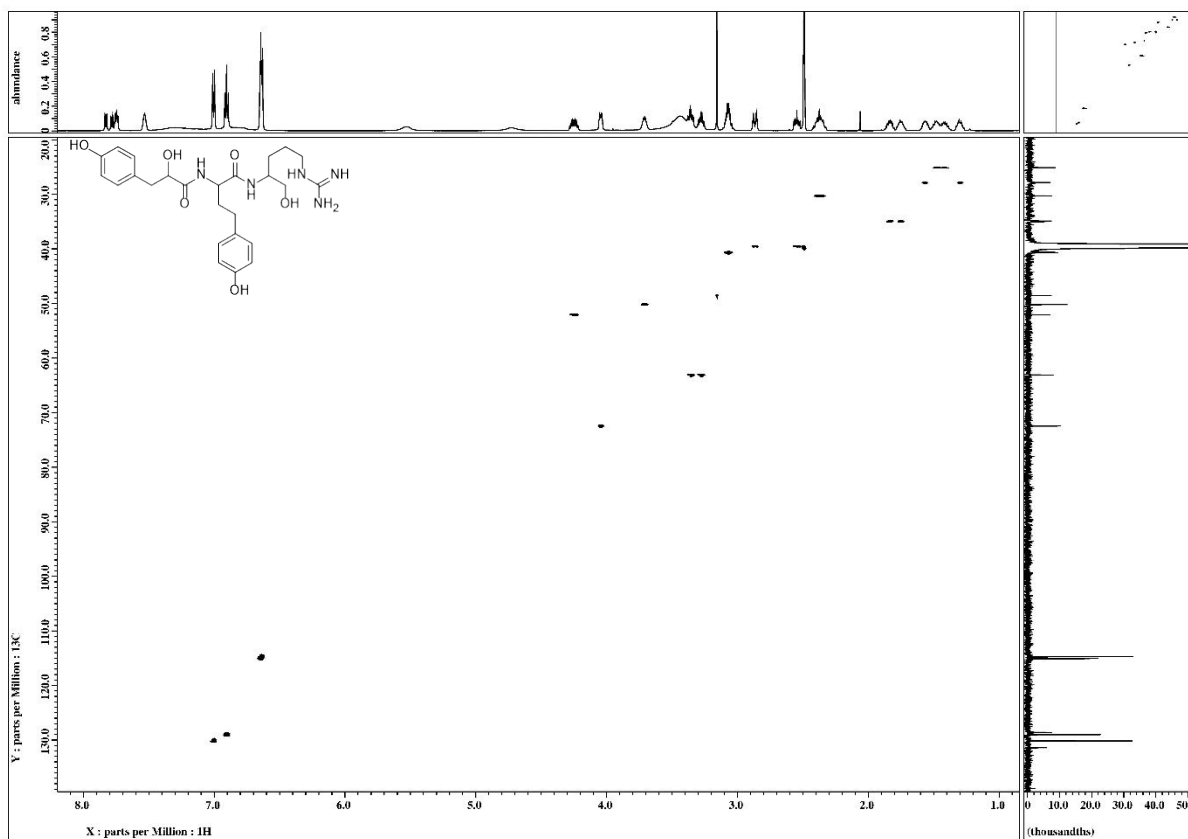


Figure S4. HSQC spectrum of **5** (reduced form of **1**) in $\text{DMSO-}d_6$

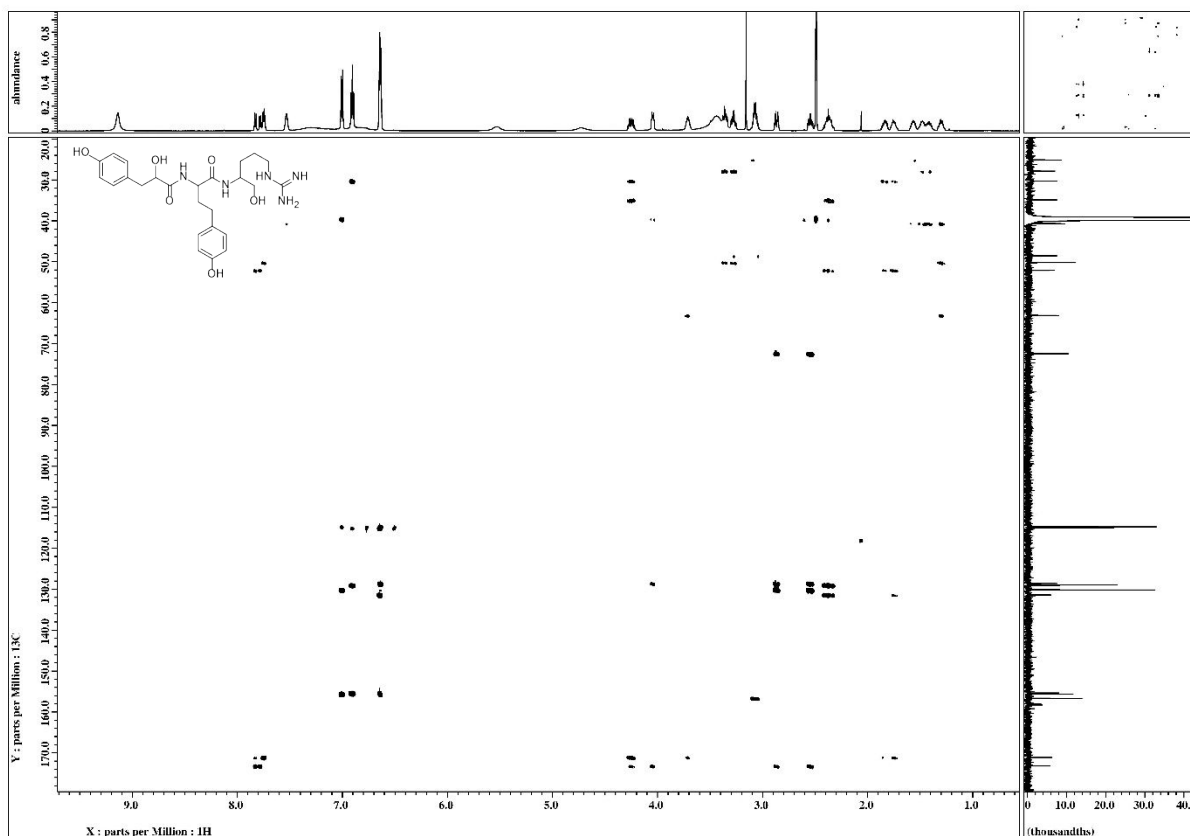


Figure S5. HMBC spectrum of **5** (reduced form of **1**) in DMSO- d_6

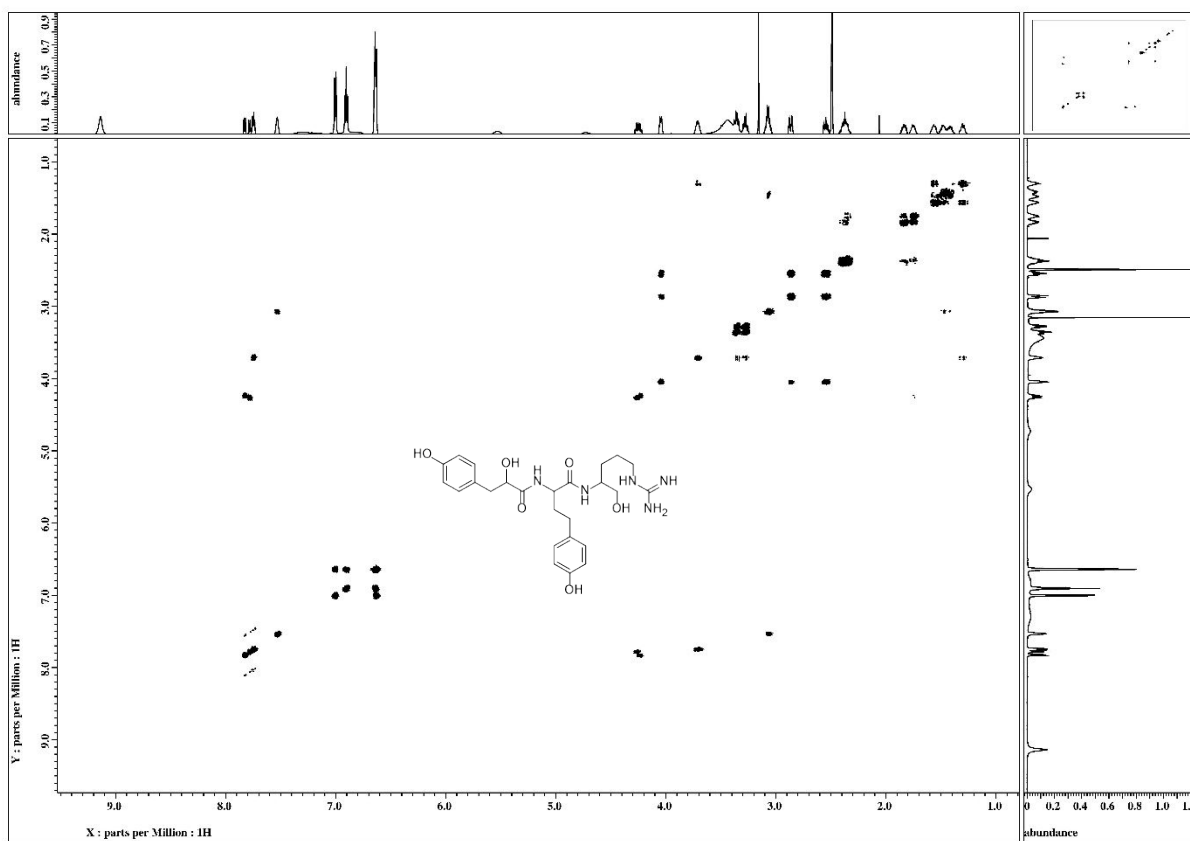


Figure S6. ^1H - ^1H DQF COSY spectrum of **5** (reduced form of **1**) in DMSO- d_6

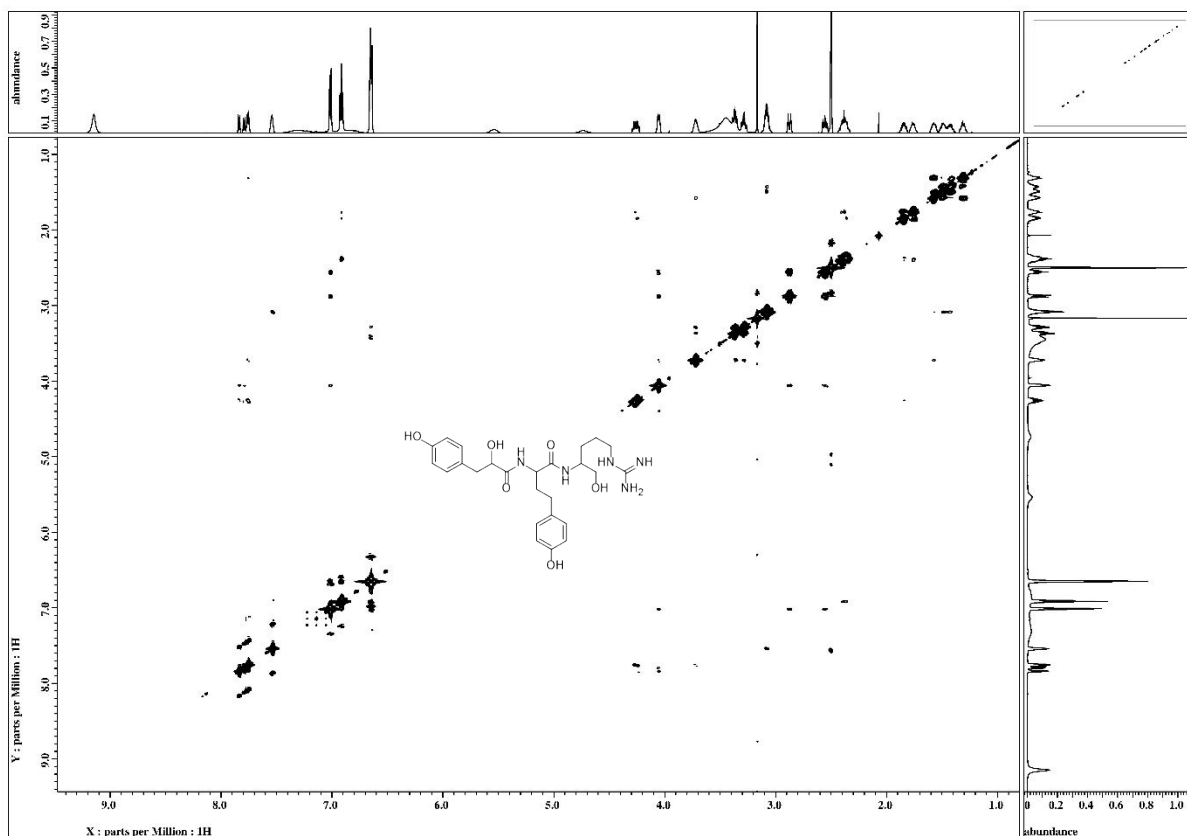


Figure S7. ROESY spectrum of **5** (reduced form of **1**) in DMSO- d_6

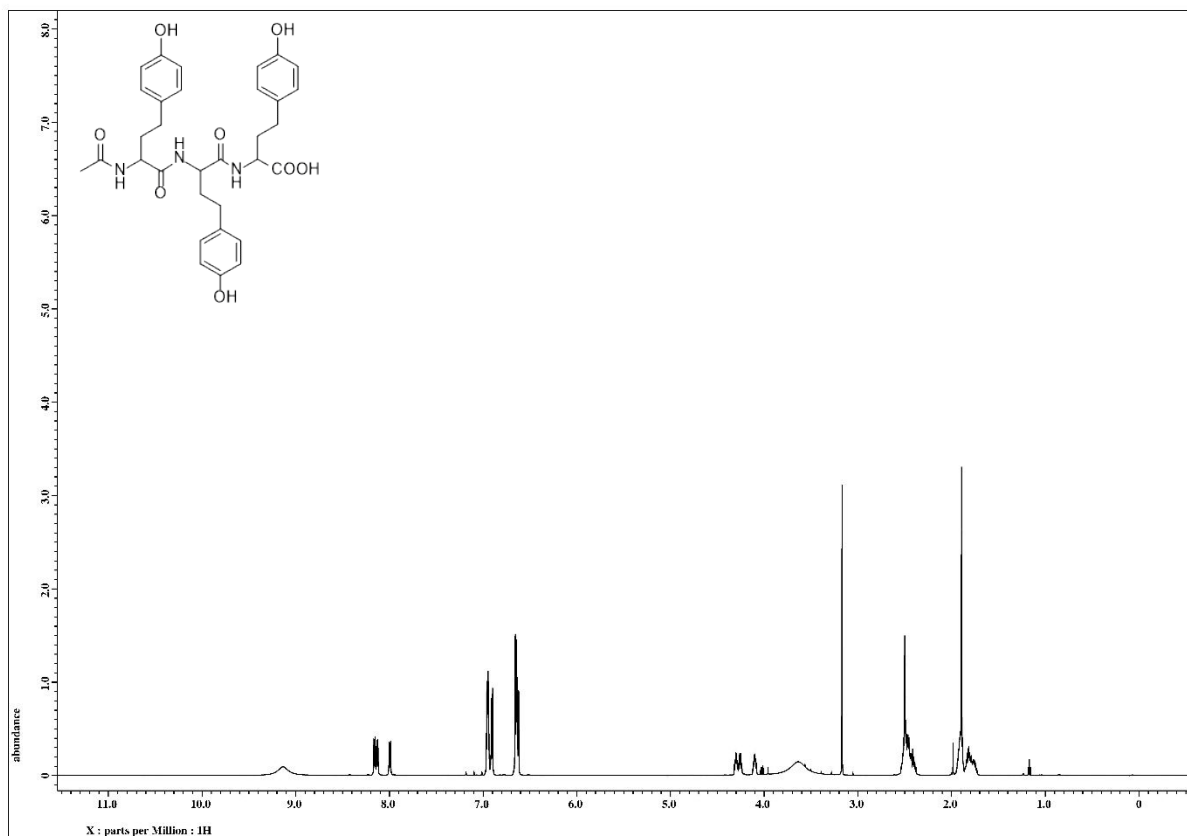


Figure S8. ^1H NMR (600 MHz) spectrum of **2** in DMSO- d_6

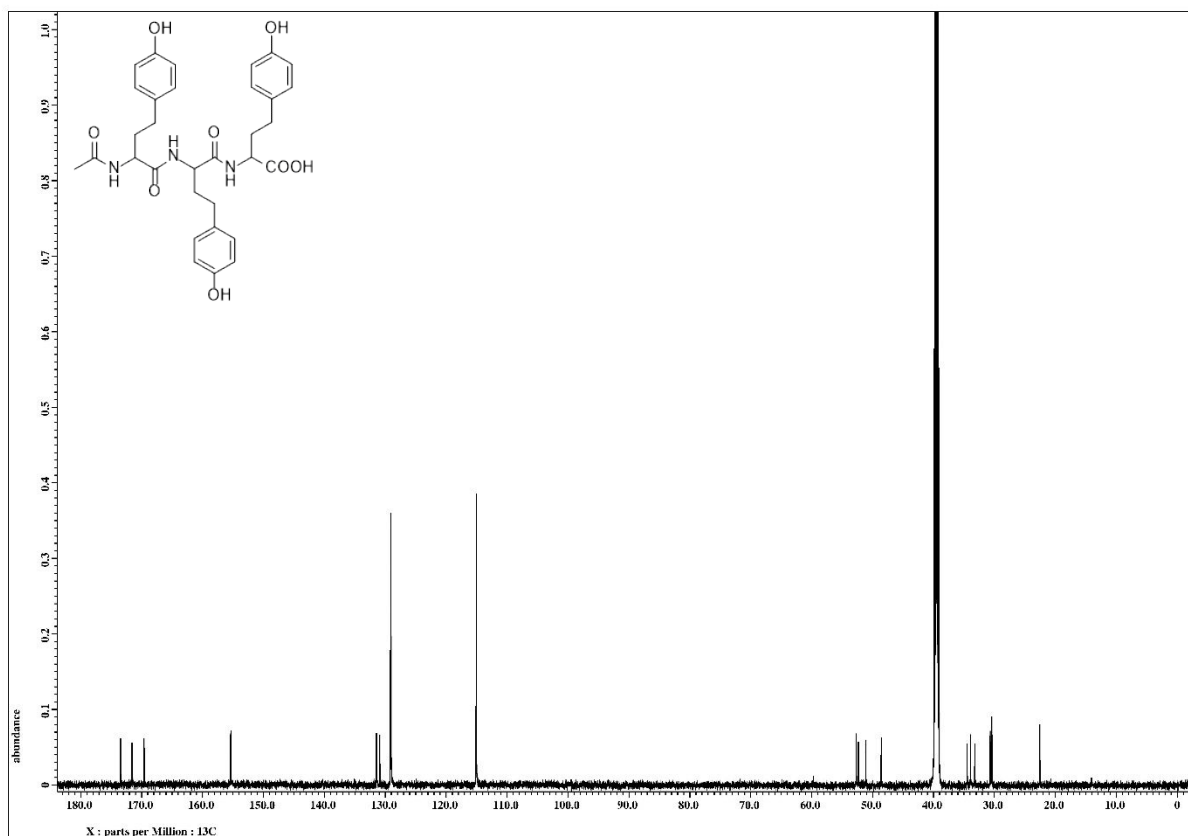


Figure S9. ^{13}C NMR (150 MHz) spectrum of **2** in $\text{DMSO-}d_6$

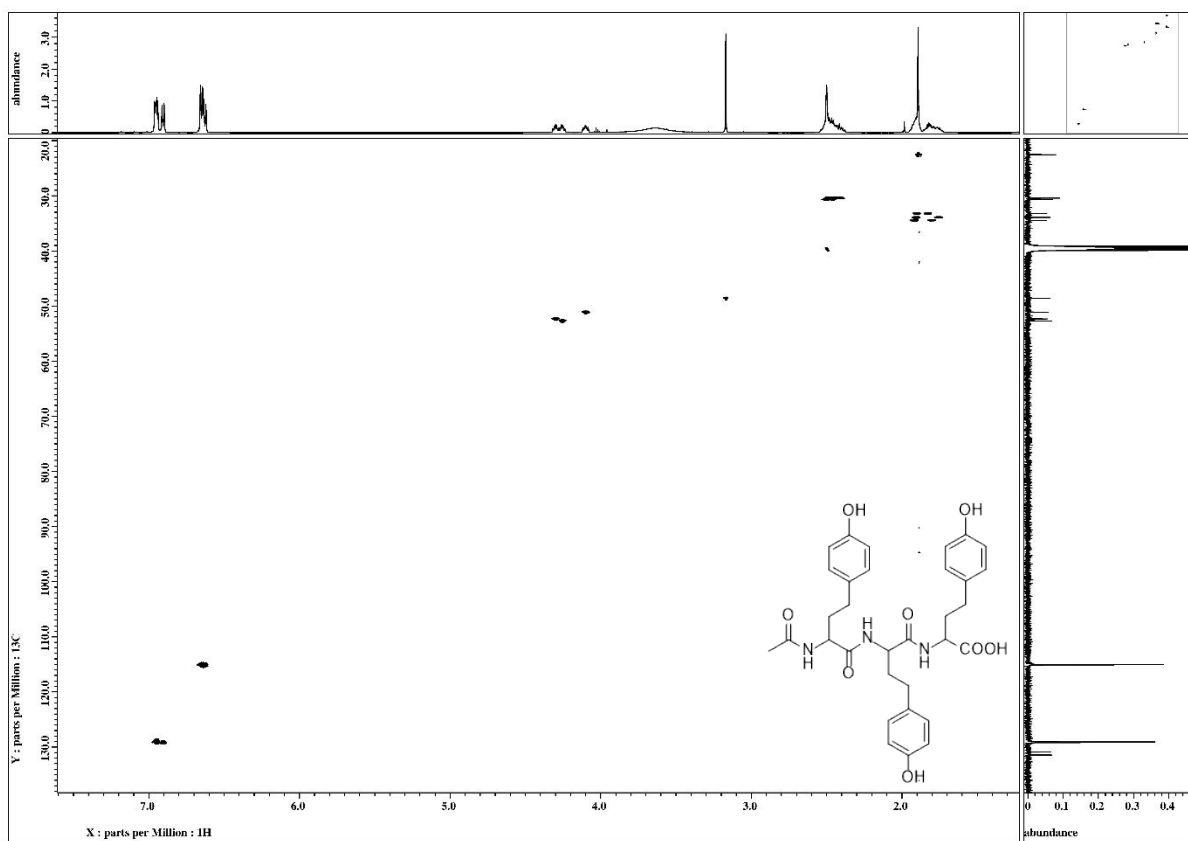


Figure S10. HSQC spectrum of **2** in $\text{DMSO-}d_6$

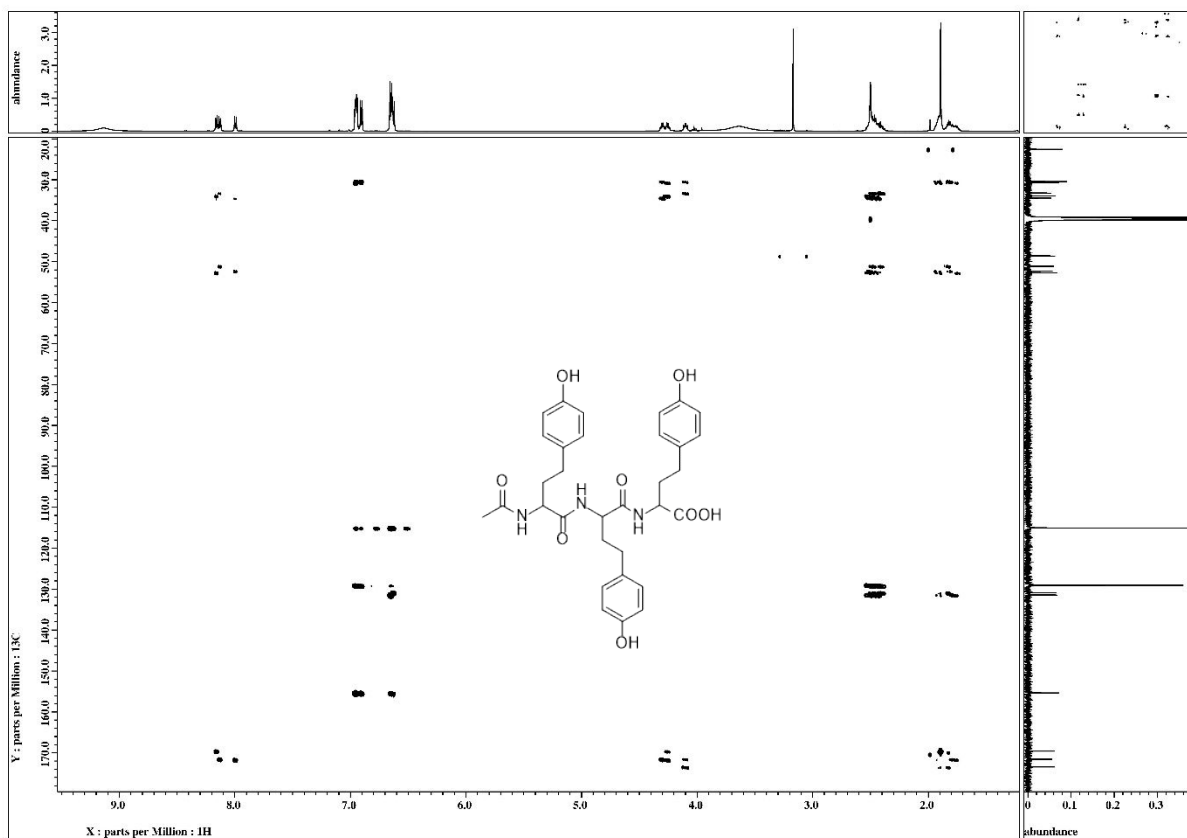


Figure S11. HMBC spectrum of **2** in DMSO- d_6

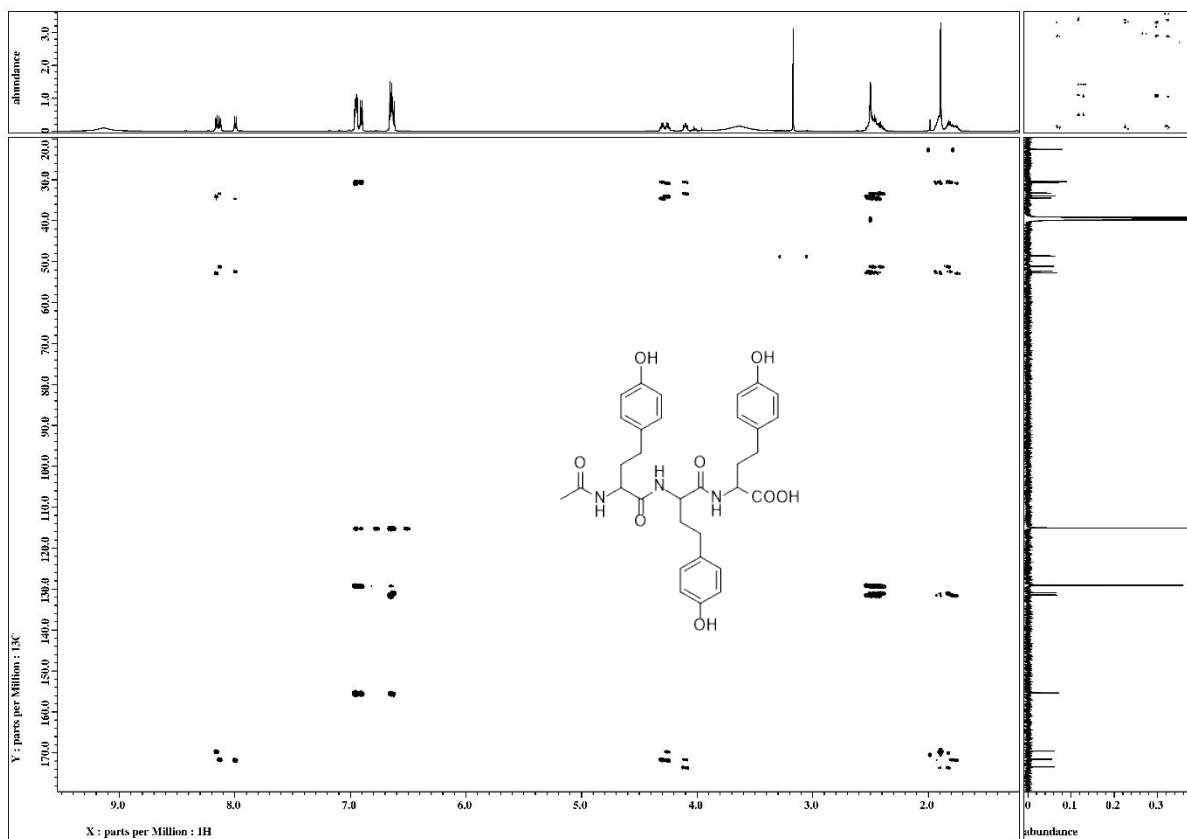


Figure S12. ^1H - ^1H DQF COSY spectrum of **2** in DMSO- d_6

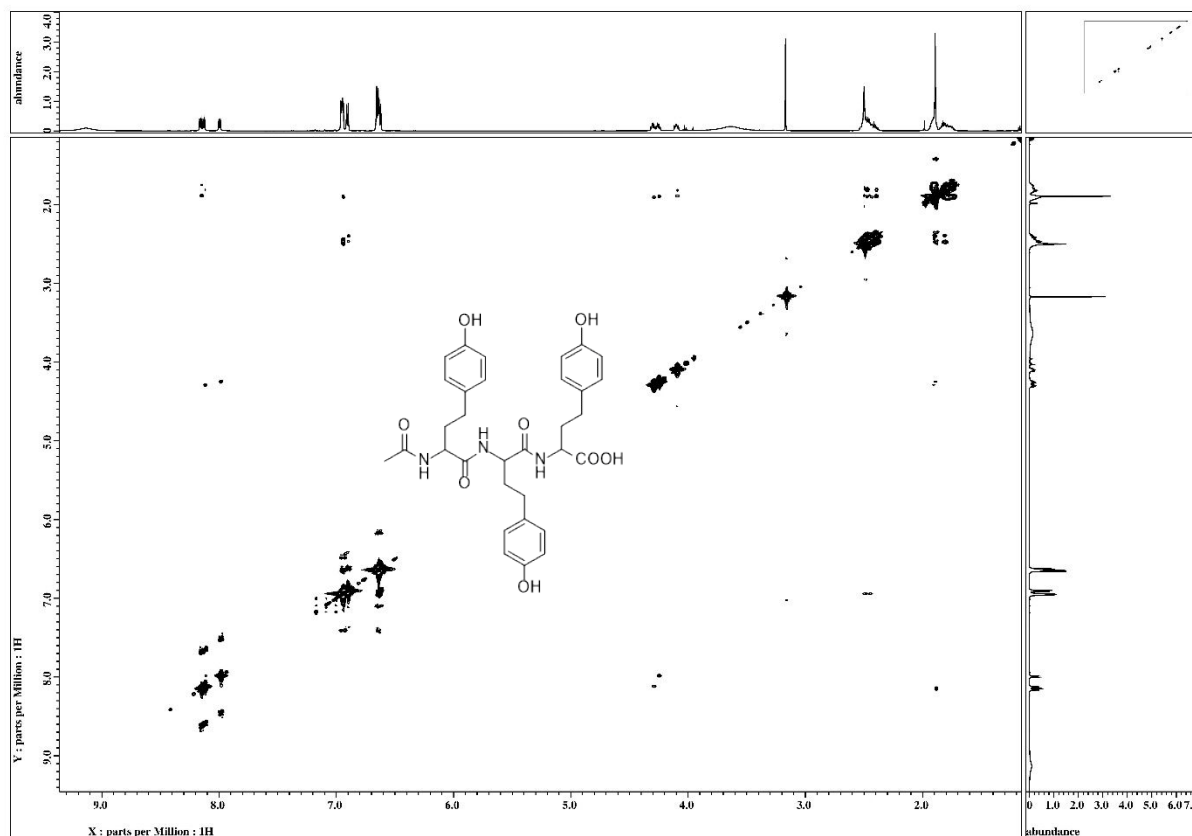


Figure S13. ROESY spectrum of **2** in DMSO- d_6

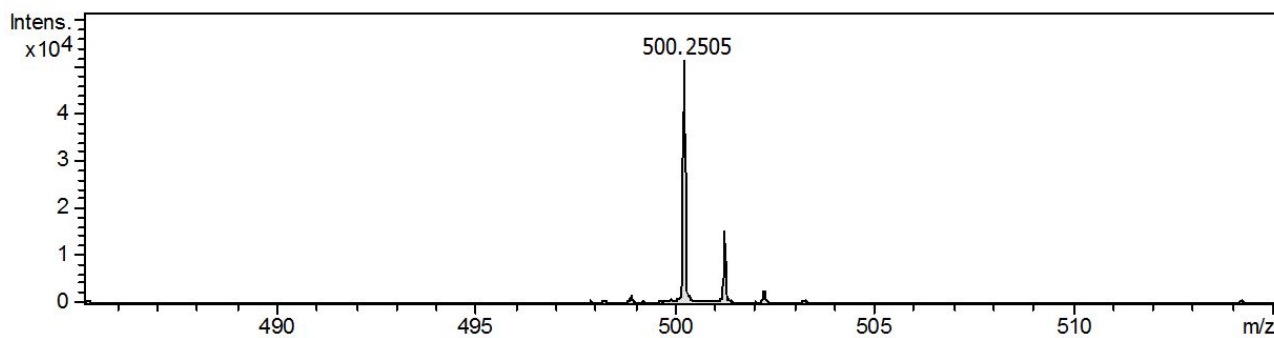


Figure S14. HRESI(-) mass spectrum of **5** (reduced form of **1**)

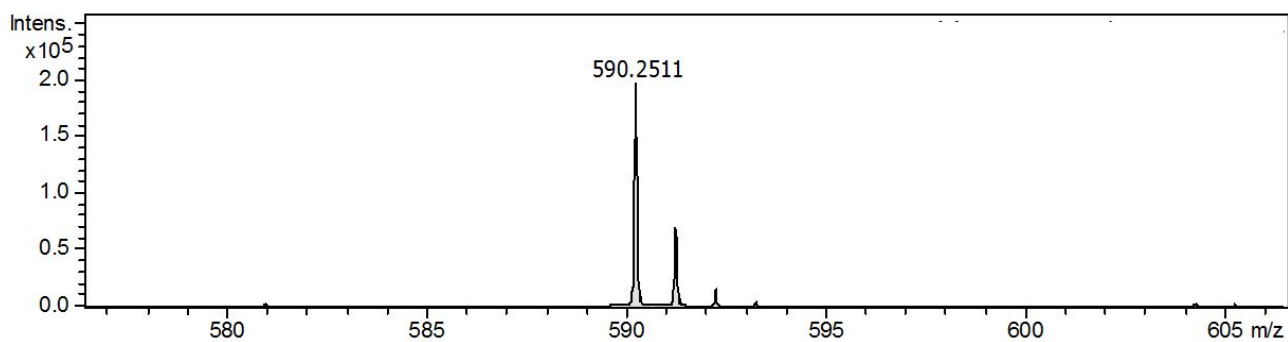


Figure S15. HRESI(-) mass spectrum of **2**

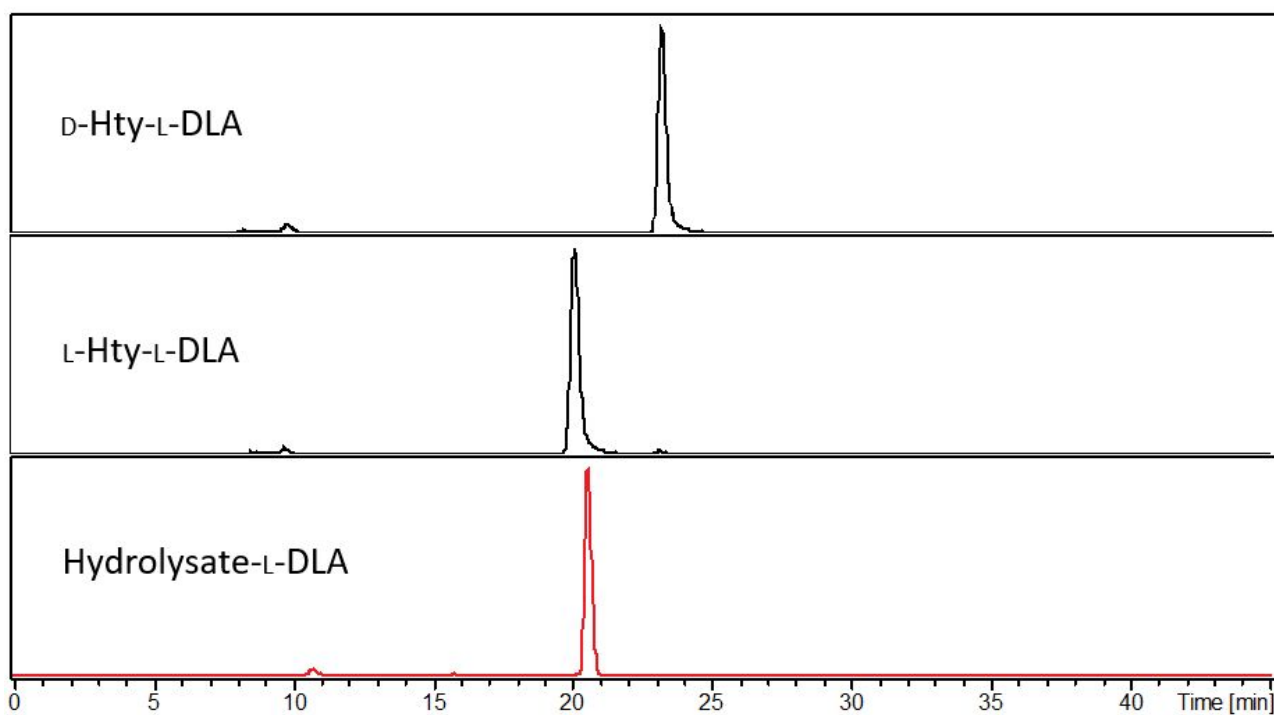


Figure S16. LC-MS chromatogram of Marfey's analysis of hydrolysate **1** (non-reduced), and standards D -Hty and L -Hty.

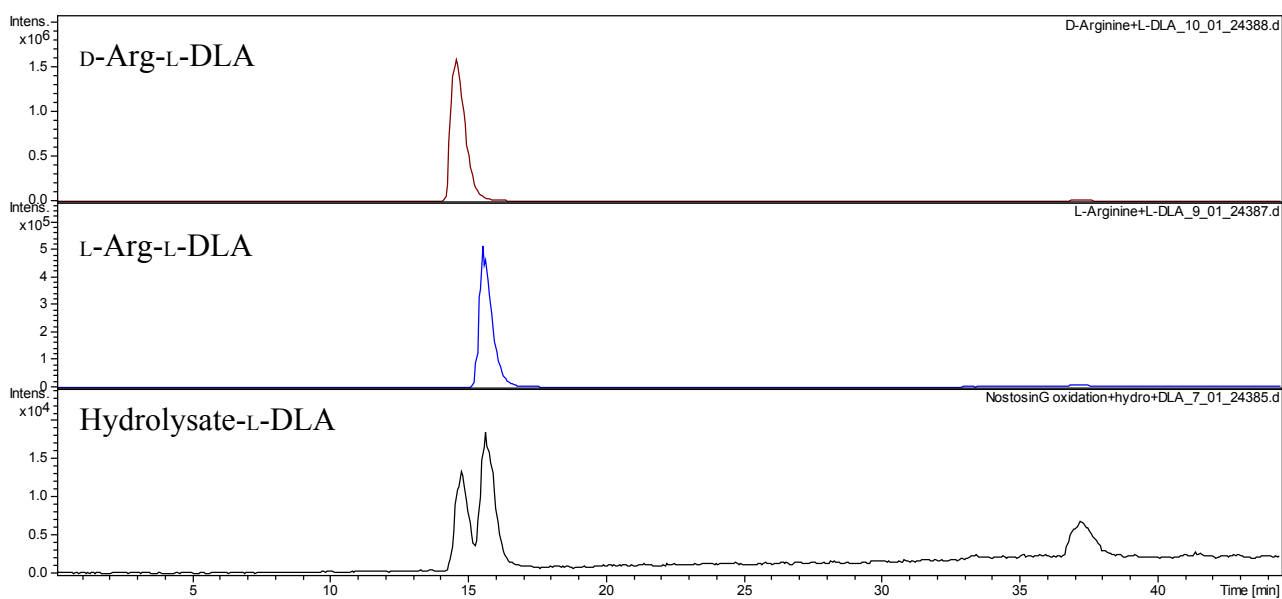


Figure S17. LC-MS chromatogram of Marfey's analysis of hydrolysate **1** (non-reduced), and standards D -Arg and L -Arg.

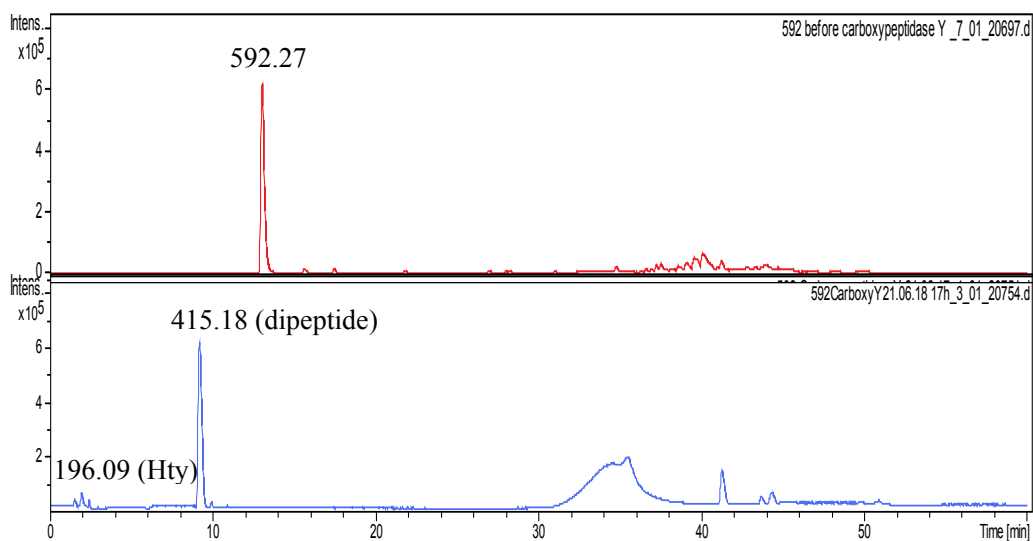


Figure S18. Enzymatic hydrolysis of **2** using carboxypeptidase Y.

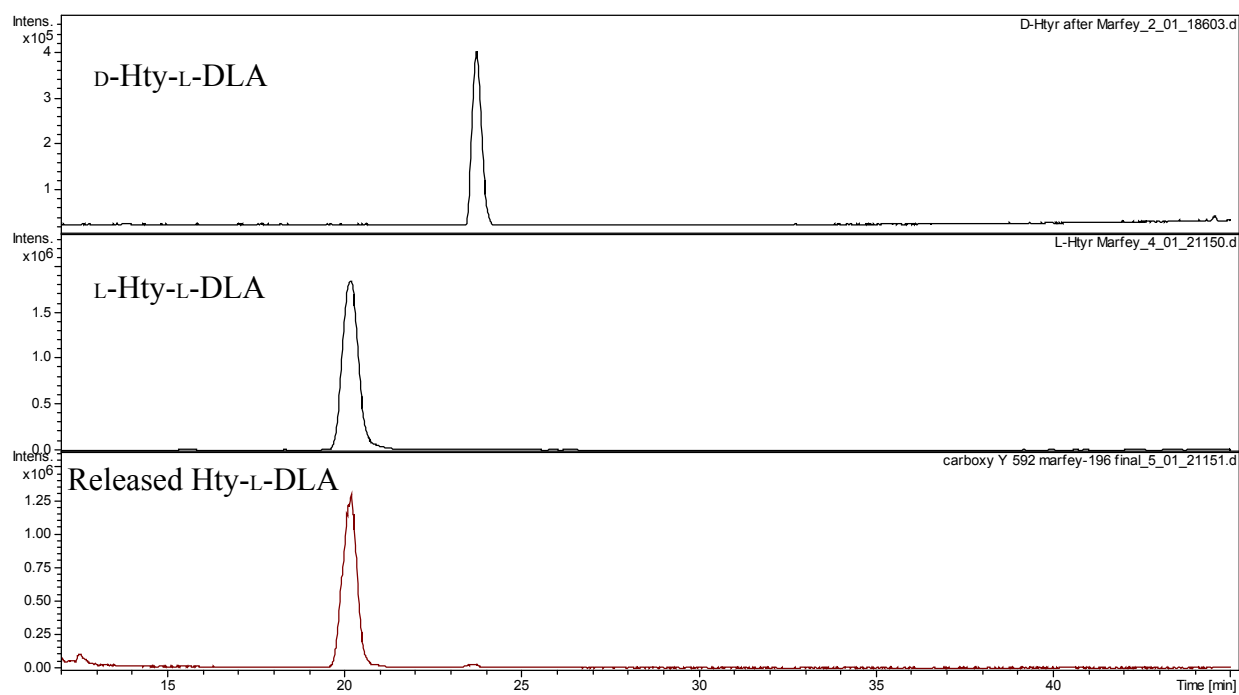


Figure S19. LC-MS chromatogram of Marfey's analysis of released Hty from **2** after hydrolysis with carboxypeptidase Y.

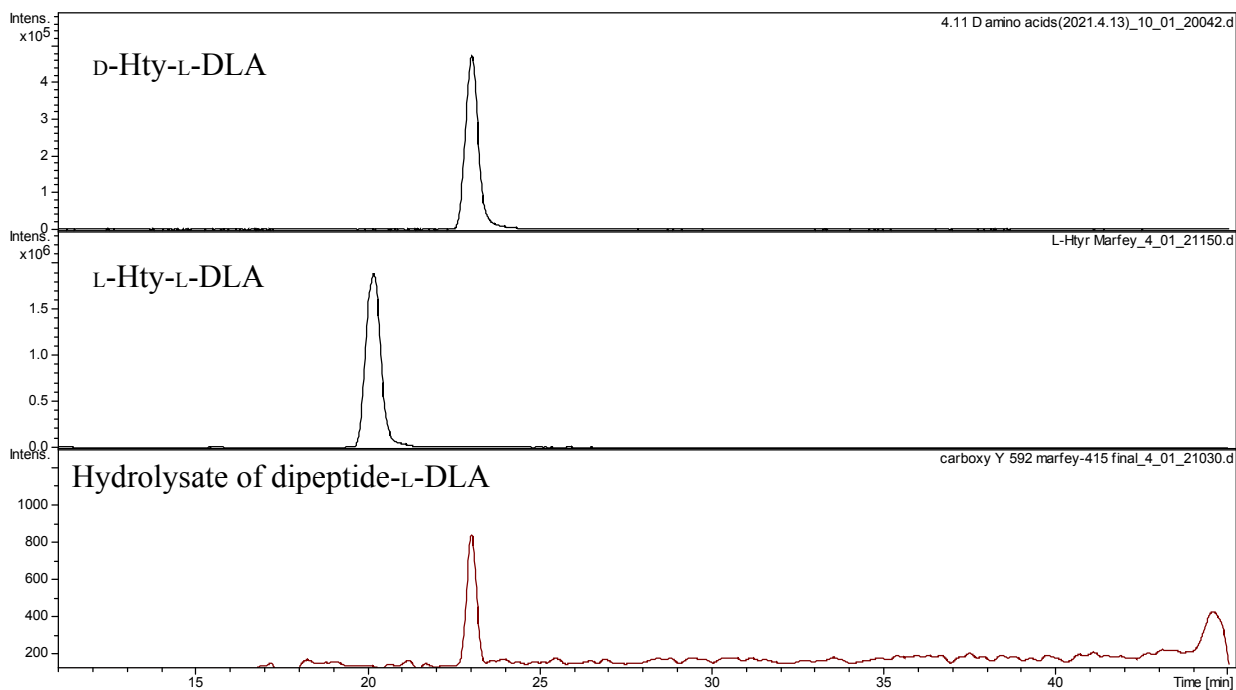


Figure S20. LC-MS chromatogram of Marfey's analysis of hydrolysates of dipeptide from **2** after hydrolysis with carboxypeptidase Y.

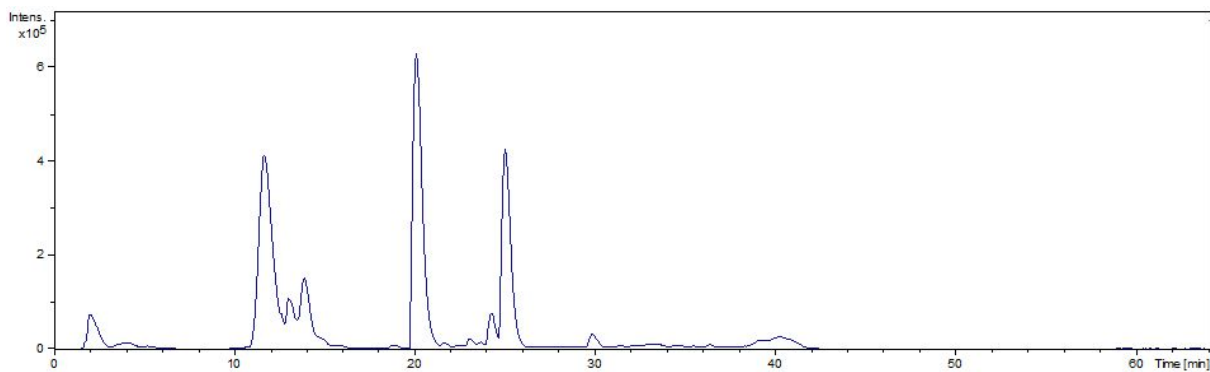


Figure S21. LC-MS chromatogram of water fraction of *Dolichospermum* sp. NIES-1697.

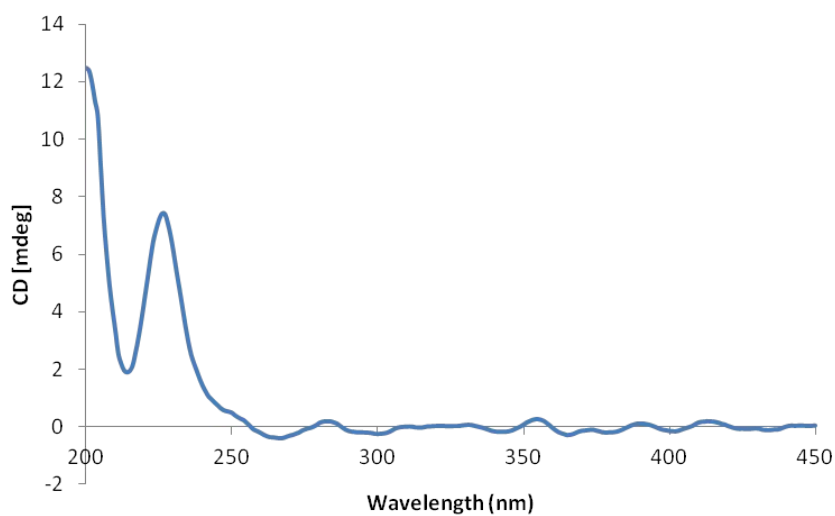


Figure S22. ECD spectrum of reduced form of nostosin G (**5**).

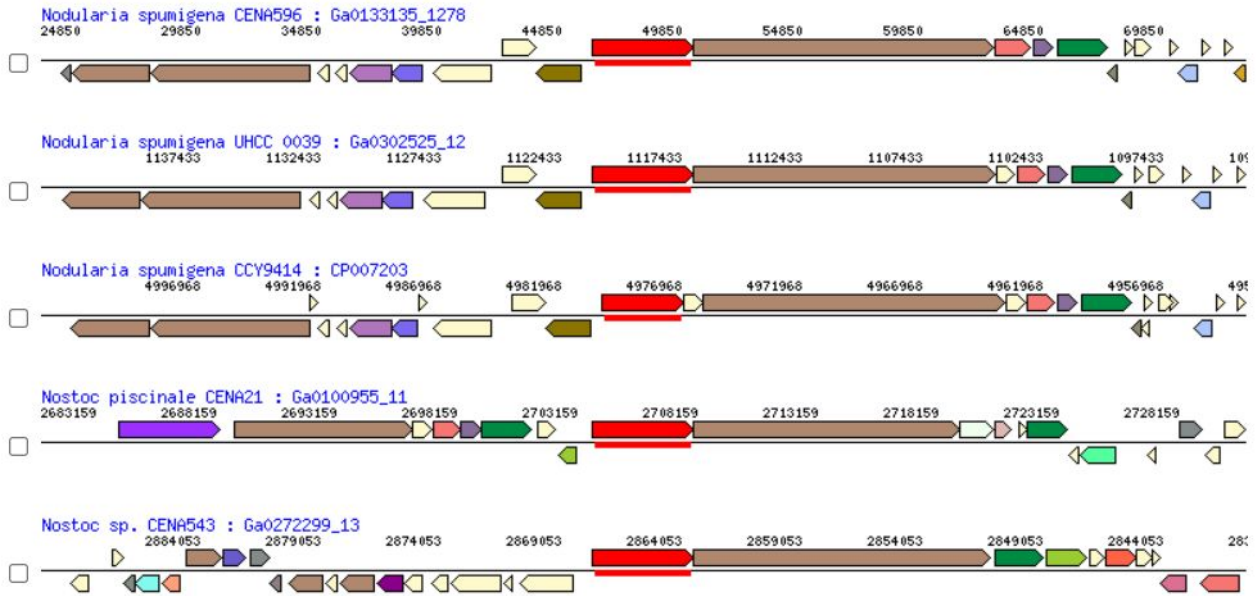


Figure S23. The homolog analysis was carried out on the Integrated Microbial Genomes and Microbiomes (IMG/M) System using NstA as query found five homolog genes (indicated as red color).

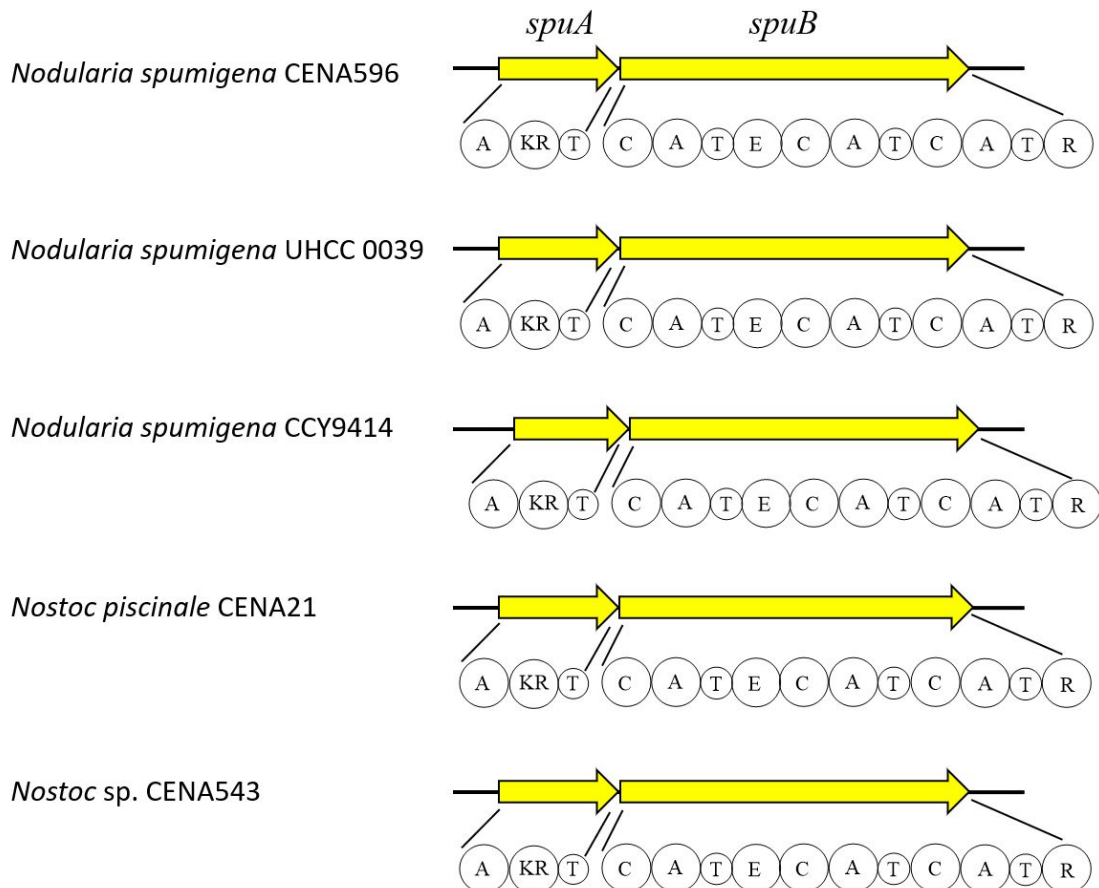


Figure S24. NstA shares homology 89.2% to SpuA (IMG Gene ID: 2745817816, *Nodularia spumigena* CENA596), 90.2% to SpuA (IMG Gene ID: 2813300686, *Nodularia spumigena* UHCC 0039), 89.4% to SpuA (IMG Gene ID: 2563163931 and 2563163930, *Nodularia spumigena* CCY9414), 75.9% to SpuA (IMG Gene ID: 2651623386, *Nostoc piscinale* CENA21) and 76.6% to SpuA (IMG Gene ID: 2791298690, *Nostoc sp.* CENA543). The domain structures of SpuA and SpuB (four residues spumigin biosynthesis) shared similarity to NstA and NstB except their genetic organization.

Table S1. Comparison of the AptC A₂ domain binding pockets for Hty or Hph residue in cyanobacteria.

Cyanobacteria	AptC A ₂	Activated residue (peptide)
<i>Sphaerospermopsis torquesreginae</i> ITEP-024	DLGFTGCV	Hty (anabaenopeptin 808)
<i>Anabaena</i> sp. 90	DLGFTGCV	Hty (anabaenopeptin A)
<i>Nodularia spumigena</i> CCY 9414	DLGAIGCV	Hph (nodulapeptin B)
<i>Nostoc punctiforme</i> PCC 73102	DLGTIGCV	Hty (anabaenopeptin NZ 857) Hph (anabaenopeptin NZ 825)
<i>Planktothrix agardhii</i> CYA126/8	DLGFTGCV	Hty (anabaenopeptin 908) Hty (anabaenopeptin 915)

Note: AptC A₂ domain is the adenylation domain of module 4 in anabaenopeptin biosynthetic gene clusters; Hty is homotyrosine; Hph is homophenylalanine; references.¹⁻³

Table S2. NstA, SprA, NstB and their amino acid sequences

Genes	Amino acid sequences
NstA	MNLDNHNQILATSTNENFTYQKIEVAIRSSLTVDDCVVIKQRQTEKVKQELIAY IVPSGLFAPEQLLSHLQITLPSLELTPTAFVPVSTIPLTETGQVDEVALASLEVIKS DLIRDLEKQLESLSEIDQVAVVVEPVVKSISIPPVHLEDLLGETPANPYENNQQ EIQVSTHSQNIENKNYYLSKCLAISHSEPLPYSPPDAPKNLVEVLQRASENSNK GIIYIKSDGSETFQSYRKLWQDAQIILAGLRKTGLKPQDKVIFQLEDNQDFICA FWGCVLGGFVPPVPSIAPIYEPANNTASKLKNTWEMLEKPLVLTSGSLAADI DDFARGLNLENFKIVTVDELQCEADLNIYENQPEDLAILLTSGSTGIPKCV MLNHRNLSMTTGLILMGHFSSQESVLNWMPLDHV GALVSLSIMAASLGCQ QIHVHTDLIVQKPLHWLDLIDKHQATISWSPNFAFSLICDRAVEINRQQWDL SMKFIINAGEPIVTKTARNFLKLLSHHGLPTNAIHPAFGM CETSSGITYSDSFSL ESSSDQTSFVELGLPIAGAALRIVDENEQIVTENTIGRLQVK GASVTIGYYQNP QANQEAFTTDGWFNTGDLGFLDQGRITITGRIKDVIIINGLNYYCHEIEAAVE EMTGVEVSYTGACAVRQPGSNTDKLAIFFNTYLNDDQSLTLLKEIRACVVN KVRINPDY LIPIDKDIIPKTAIGKIQRSQLSQRFQTGEFKSTIKRVDILLGNSNTIP NWFYRQVWPKSPITVNSSLTITNTTLVFLDDWGLGDYLCQTLSENKLSYIT VYPGKEFQKISSSHYVFNPEIAKDYQLLIESLAADKIIIGQILHLW TYDKYQEI NNIDSLEKAQAQGIYSLLFLVQALAKVQGTNNYIQLLFISSHIQSIASDDPIAYE KSTVLGLLKTIPQELPKLNCRHIDL PFAEVEKNGFYILQEMQISSKERELAYRN GQRLISRLEQVDFTNTPKSSITFQQEGTYLITGGLGGIGVEVARYLLKHYQAK LLL VGRTPLNSEKHIKLYQELAQLGGEVIYESVDICDLEQLQIIVEKAQFRWG ENLDGILHLAGTFHEQQVLEETQENLAAILCPKLLGAWVLHQLAKENQASIFI NFSSAHGFFGSTAVGGYAAANSFLDSFTHYQNSPNKLTNKLTSYCFWSMW DDTGMSQGYQMKNLIRAKGSYIMSCSQAISSMLASLHHQQHNL LIGLDGSN QNILRWQSSTFNLQKLTAYFTTNTGEVVKLPGLKVQDNFGNVCIYDSVQLPE MPCLENGVDRARLIKRSNNQENREQIEPRNEIELKIAQCWQQV LKVTLGLIH DNFFELGGNSLLAGQVISRLREDFSLQLRLLQTPTIVGLAQ TIAAIQTVTQ SQNTFTETSLQEYEEDYL
SprA	MWFIYQIAPESVAYNIFITVKIDYELKIDVVNRVWQKII EKHPILRTTYTNHEG KPVQQVNQQENFSVEVIDSKEWSEEQLAKKIYAIADRPFNLEQDSVLRVNL SRSAEKHILMLTMHHIAGDMWSFDLLSEFQTLYLREIEQISQEQTETVDYLS EKKS YADFVHWQSEMLSSSQGEKLWQHWQEQLAGELPILNLLPKPRPAVQ TYQGASYIVKLDEQLTEKLNHLAVASKTSLYQILLTAFYIQLYRYTNQTDILIS SPMRGRRGGNFEEIVGYFVNLTVLRVSVQENATFQEFLAQVSKTVKKAQYH

QDYPFGLLAQKLQPQKNPSRSPQSQVSFTWQRHRWCELTEKSSHIQEQVLQM
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	<p>LFESLYAPDSQAYFEQSIYNLSGNLNLSAFEKAWQQVLERHSILRTAFIWEQL AQPLQIVYRQAEVKLQTDWQHLSPPEAQKLELLLSQSRKQGFQLSAAPL MQWSVIQLGTDSYQFVWNFHLLLDGWSVPLLFQDLLYSYQAIKGENRILP PVLSYDNYIAWLQQQDLTKAQEFWREKLGFTAPTPLTVDKLSSNQKQLDS SYREQKIQLTREETSNLQTFARQHQLTMNNVVQGAWALLSRYSQESDIVFG ATVSGRPPSLMGVESMVGLFINSLPVRVKTCAETEVLTKDLQTTQVVESEQ YSYSSLADIQRLSDVPGGTSLFESLVVFENYPVNEAGEKTNYGFSIDNVQGIE QTNYPPLTVGVIPRKELLIISYDTSRFDDSAISRLLGHFQRLLSGIVTNPQESIAQ LSLLTEVEKHQLLTEWNTQVEYPVDKCIHELFEQVEKTPDAVAVVFENQQ LTYQQLNIRANQLANYLQTLGVKPDPLVVICVERSLMVLGILLGILKAGGAY VPLDPEYPQERLSFMLEDAQVSVLLTQKSLNLEPLDNREKPCQVICIDENTF NLELTENPSHQSPNNLAYVIYTSGSTGRPKGVMIEHSAIVNLSLTWAKTFQV ENHSRLLQFGSFSFDLSVGEISTALVTGACLYLGNKVTLLPSQSLVDFLTVNKI THSFLSPSALSVLPAKLPDLQYLTVGGEACTTELVNQWGTERNFYNCYGPT ESTVTATIFHCQPNGRKPPIGKPISNLRIYILDRNNQLPPGIPGELCIAGVGLA RGYLNRPQATTEKFMEIDICGQVERIYRTGDLARWGTGDNIEYLGRIDNQVKI RGFRIELGEIETVLNQHPIQTSCVIAREDTPTDKRLVGYIVPKKDVMPPESTAI RQFLVNKLPGYMIPSAIVMVEFLPLTPNGKIDHRALPKPELDSTILEKYVAPR NPIEELLTQTLWLQVLKIESVGVNDNFFELGGHSLAVKLLNHIQKVFNQKLA LSSLFDNPTIAQLALQISDHEVQQSHPHLLTLQSQGNATPIFCLPGTNGHGFYF KDLAINLENHPVYSLETPGRNGFGKVPDSVELHSSQMIDLLREQQPHGPPYVL AGYSGGSVVAFEMACQLENQGEKVELLAILDAGLVIHPEYSSKMTDIDWMW QLLQHIENVERISLGLDYADLAAQPDELARWDLVAEYLYKHDVLPENSSLSL LKTNMQVMKQMAINYDNYRPSHQISAPIGLFRAEEVDEVALQQLRAISNYDL PDWGWQDYTENSVKVISVPGNHHRMLYEPNVKTLASHLRMMMTYPINSQA ISSLTTTFLNWX</p>
NstB	<p>MAIFEFLSLLNNDIKIWVEDGQLRYRAPKGAMTDEIKQQIKERKAEIIAFLQE AQTATQINYSSLVPVVRDKDLPLSFAQQRMWFLSQLDGESTSYNESFQLRIV GKLSLTALEQSINEIIRRHVLRNTNFPTVEGVFPFQVIRPNLTLIPVINVQEFTEI AVQEIIINQEVNKSFDLGTPELIRATLLQQDPESHLLLITMHIIIDGWSMGVFF KELEALYPAFIQKPSPLPELTIQYADFALWQREWLTKEVQDKQLEYWKQQ LAGTPPILLEPTDYPRPPEQSFAGASIEFNIDADLTSQLVTLQKSGVTLFMTL LTAFAVVLHRYSGQDDICIGSPFANRNRREIDSLIGFFVNTLVLRQMEGNPR FSQLEKVRVVDAAHAHQDIPFEQVVEALKPERSLGYNPLFQVMFVLENFS LDTLELPGISLTPDIVDRGTAKFDLGLSMWQTQQGLIGSWEYNSDIFAPDTIAR MINHFQTLLAGIVKNPEQRIGELPLLTESEKHQLLTEWNTQVEYPVDKCIHQ LFEEQVEKTPNAIAVVFENEQLTYQQLNSTRANQLAHLQSLGVKPEPLVVIC VERSLMVLGILLGILKAGGAYVPLDPEYPTERLSFMLEDAQVSVLLTQKSLN QLPLDNREKPCQVICLEQLVSEVEVNTFNLELTENPNHQNPENLAYVIYTS GSTGKPKGVITIEHRAIVNLSLTWGQTFQVQNHRSRLLQFGSFSFDLSVGEITTA LVTGACLYLGNKVTLLPSQSLVDFLTVNKITHSFLSPSALSVLPAKLPDLQCI TVGGEACTTELVNQWGTERKFYNCYGPTTESTVTATIFHCQPNPKPAIGKAI SNIRTYILDKNNQLPPGIPGELCIAGVGLARGYLNRPQATGEKFIEIDINGQV ERIKYTGDLARYLADGNIEYIGRIDNQVKIRGFRIELGEIEAVLNQHPIQTSC VIVREDNPGQKQLVSYLIPHQHSTVTISEMRQYLKETLPEYMPHVSFVTLETL PITPNGKIDRRALPKPELESTLLEKYVAARNPIEELLTQTLWLQVLKVESVGIN NFFELGGHSLIATQIVSRIRNIFQVELPLNKLFAAPTRELAAHITQLQQDKLEL SADKAPLLLPRAKDADLPMSFAQQRLWFLDQFEANSVVYNMPTALRLLGKL QIAALEQSLQTIHRHEALRTNFTTIDGQPIQVIREQKAGNKEQGIVSIVDLQHL PKTEQEIAVEQLTQQMMTGFDLETEALIRVQLILLNETEQVLLVCMHHIVSD GWSMGVVFVQELTQLYNAYCQNQPLPLTPLPIQYSDFAIWQRQWLQGDVLQN</p>

QITYWEQQLKDAPTLTLLSLPTDRPRPAVQTLVGATHEFALSVELTDKLIKLSQN
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TEQMVFENMTN

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