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ORIGINAL ARTICLE

Dakin–West reaction on 1-thyminyl acetic acid for the synthesis of 1,3-bis(1-thyminyl)-2-propanone, a heteroaromatic compound with nucleopeptide-binding properties

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Abstract This work deals with the Dakin–West synthesis, starting from the nucleoamino acid 1-thyminyl acetic acid, as well the NMR, ESI MS, and X-ray characterization of a heteroaromatic compound denominated by us T_2CO, comprising two thymine moieties anchored to a 2-propanonic unit, the spectroscopic properties of which were studied by UV as a function of temperature and ionic strength. Preliminary binding-studies with molecules of biomedical interest such as nucleic acids and proteins, performed on samples containing T_2CO, suggested that this molecule is able to interact very weakly with double-stranded RNA, whereas it does not seem to bind other nucleic acids or proteins. Moreover, by studies with fresh human serum we found that T_2CO is resistant to enzymatic degradation till 24 h, whereas UV metal binding-studies, performed using solutions of copper (II) chloride dihydrate and nickel (II) chloride hexahydrate, revealed a certain ability of T_2CO to bind copper (II) cation. Finally, by CD spectroscopy we investigated the influence of T_2CO on the already described supramolecular networks based on L-serine-containing nucleopeptides. More particularly, we found that T_2CO is able to increase the level of structuration of the non-covalent supramolecular assembly of the chiral nucleopeptides, which is a feature of remarkable interest for the development of innovative drug delivery tools.

Keywords Thymine · 2-Propanone · Nucleopeptide

Introduction

Due to the importance that aromatic and heteroaromatic molecules have gained in biology and medicine thanks to their ability to interact with natural targets such as DNA and proteins, the realization of novel aromatic or heteroaromatic compounds with significant biological activity is clearly desirable in view of the realization of novel therapeutical strategies. Aim of this work was the synthesis from 1-thyminyl acetic acid, and the properties evaluation of a heteroaromatic compound, denominated by us T_2CO, the structure of which is characterized by two thymine moieties anchored to a 2-propanone unit.

T_2CO can be seen as a heteroaromatic analogue of the natural alkaloid 1,3-bis(2-piperidyl)-2-propanone, also called (−)-anaferine, which was extracted from *Withania somnifera* (Rother et al. 1962), a solanaceous plant from Asia known for its sedative-hypnotic as well as anticancer properties (Fig. 1). Moreover, T_2CO shares structural analogy with the 2-propanone-based antimuscarinic drugs realized by Kaiser et al. (1993) which are characterized by 2-propanone residues 1,3-disubstituted with two six-terms rings (phenyl and piperazine rings) as represented in Fig. 1. The spectroscopic properties of T_2CO were studied by UV spectroscopy as functions of temperature and ionic strength. Subsequently, several studies were conducted in order to investigate the interaction of the heteroaromatic

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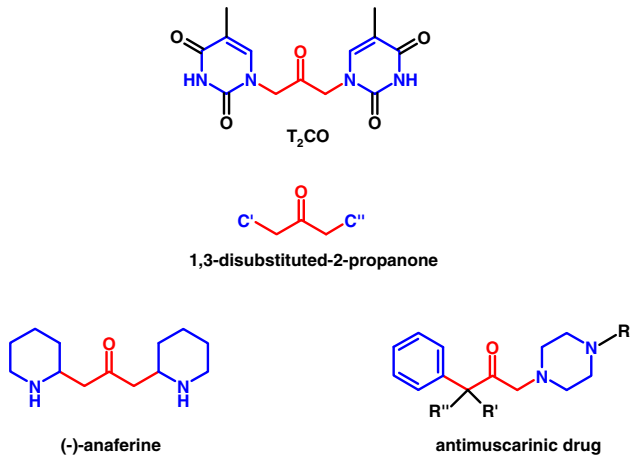


Fig. 1 Comparison of the molecular structure of T_2CO with those of bioactive 2-propanone molecules 1,3-disubstituted with six-terms rings

compound with metal cations and bioactive compounds such as nucleic acids, proteins and nucleopeptides. More particularly, nucleobase-containing peptides (also referred as nucleopeptides) represent a class of chimeric molecules some of which occur in nature (e.g. peptidyl nucleosides) or are synthetically produced in chemical laboratories. Nucleopeptides contain peptide moieties variously connected to the DNA nucleobases (Roviello et al. 2010, 2011) and in some cases present properties that render them molecules of great interest in biomedicine (Roviello et al. 2009, 2010, 2011). Recently, it was proven that chiral nucleopeptides are able to form supramolecular networks governed by non-covalent interactions involving the nucleopeptide units and able to host in their interior organic molecules, a feature of clear interest in view of the development of innovative drug delivery systems (Roviello et al. 2011). Since nucleobase-based supramolecular systems can be reinforced by means of the interaction of aromatic molecules with nucleopeptide units, in this work we also investigated the ability of T_2CO to influence the structure of an already described nucleopeptide-containing supramolecular system (Roviello et al. 2011) by performing CD experiments in the presence of different amounts of T_2CO as described below.

Material and methods

Chemicals and solvents

HATU was purchased from ABI. DMF, DMSO, DIEA and solvents for HPLC chromatography were purchased from Romil. Poly-A was purchased from Fluka; dA_{10} and dT_{10} were biomers; L-alanine, bovine serum albumin (BSA), poly-U, TCH_2COOH, copper(II) chloride dihydrate and nickel (II) chloride hexahydrate were purchased from Sigma.

Synthetic procedure

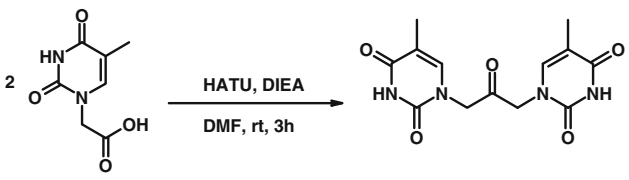
T_2CO was synthesized from thymine acetic acid (Scheme 1). More particularly, TCH_2COOH (0.77 g, 3.8 mmol) was activated with HATU (0.9 eq, 1.34 g, 3.5 mmol) and DIEA (2.5 eq, 1,690 µL, 9.7 mmol) in DMF (3.5 mL). The reaction mixture was stirred under Ar atmosphere for 3 h. Subsequently, it was treated with 500 mL of water and after freezing the sample was lyophilized. The crude product was then treated with water and the precipitation of a white compound was observed in the aqueous solution. After crystallization from water and acetone the desired product was obtained with a 45% yield (melting point for this compound was 345°C).

Characterization of T_2CO (^1H and ^{13}C NMR; HPLC; ESI MS, UV)

NMR: δ_H (200 MHz, DMSO-d_6) 11.35 (2H, s, CON*H*CO), 7.32 (2H, s, C6–H), 4.71 (4H, s, NCH_2CO), 1.73 (6H, s, CH_3); δ_C (50 MHz, DMSO-d_6) 199.1, 164.2, 150.8, 141.5, 108.5, 53.6, 11.9. Analytical RP-HPLC was performed on a C_{18} column (Phenomenex Jupiter C18 300 Å, 5 µm, 4.6 × 250 mm) using a flow rate of 4 mL/min and a linear gradient from 2% (for 5 min) to 80% B in A over 20 min: t_R = 12.1 min (A = 0.1% TFA in water; B = 0.1% TFA in acetonitrile). LC ESI MS (Fig. 3): *m/z* 138.40 (found), 139.13 (expected for $[M-TCH_2CO]^+$–$[C_6H_7N_2O_2]^+$); 181.18 (found), 181.17 (expected for $[M-T]^+$–$[C_8H_9N_2O_3]^+$); 306.62 (found), 307.29 (expected for $[C_{13}H_{14}N_4O_5 + H]^+$); 328.18 (found), 329.27 (expected for $[C_{13}H_{14}N_4O_5 + Na]^+$); 344.57 (found), 345.38 (expected for $[C_{13}H_{14}N_4O_5 + K]^+$). UV profile of T_2CO is reported in Fig. 2 and shows two absorbance maxima at 219 and 268 nm, respectively.

Single crystal X-ray crystallography

Single white crystals of T_2CO, suitable for X-Ray analysis were obtained at room temperature from an acetone/water solution. Data collection was performed at 293 K on a Bruker–Nonius kappaCCD diffractometer (MoKα radiation, CCD rotation images, thick slices, φ scans + ω scans



Scheme 1 Schematic representation of the synthesis of T_2CO

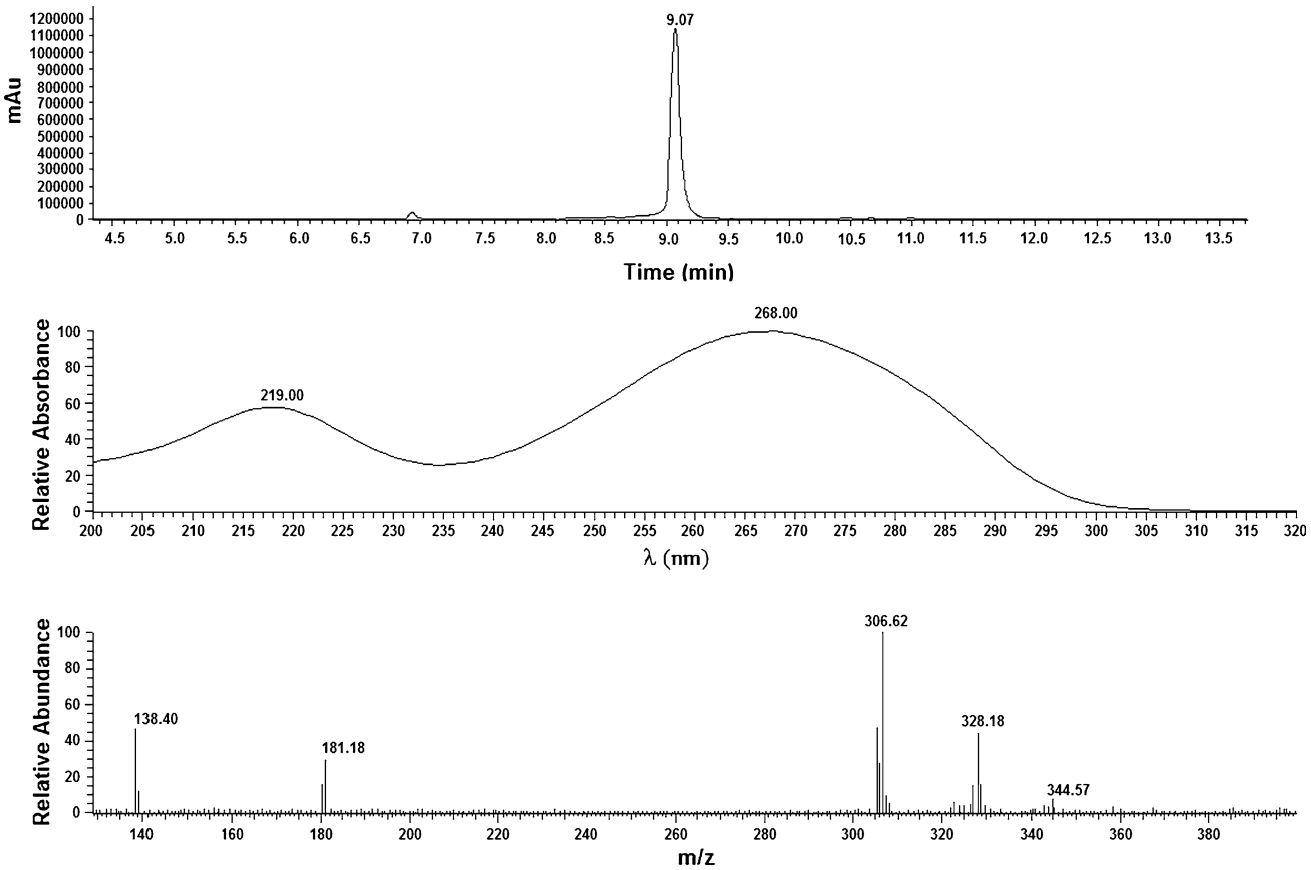


Fig. 2 LC ESI MS profile (positive ions) for T_2CO. Analytical HPLC profile was obtained with a C18 column (Phenomenex Jupiter C18 300 Å, 5 µm, 4.6 × 150 mm) using a flow rate of 0.8 mL/min and a linear gradient from 2% (for 5 min) to 30% B in A over 10 min: $t_R = 9.1$ min (A = 0.1% TFA in water; B = 0.1% TFA in acetonitrile)

to fill the asymmetric unit). Cell parameters were determined from 165 reflections in the range $3.25° \leq \theta \leq 21.22$. Semi-empirical absorbtion corrections (multi-scan SADABS by Sheldrick (1996)) were applied. The structure was solved by direct methods (SIR 97 package by Altomare et al. (1999)) and refined by the full matrix least-squares method (SHELXL program of SHELX97 package by Sheldrick (1997)) on F2 against all independent measured reflections, using anisotropic thermal parameters for all non-hydrogen atoms. All H atoms were positioned geometrically, except for the N–H and the water crystallization that were located in a difference Fourier map due to the influence on their position of possible hydrogen bonds. The coordinates of these H atoms were refined, with an isotropic displacement parameter 214 refined parameters, R1 = 0.0529; wR2 = 0.1331 [on reflections with $I > 2\sigma(I)$] and R1 = 0.1300, wR2 = 0.1807 on all reflections. Maximum and minimum residual electron density ($e \cdot$ Å^{-3}): +0.169 and −0.209. Crystal data and details of the data collection are reported in Table 1. Crystallographic data for the structural analysis have been deposited with the Cambridge Crystallographic Data Centre, CCDC No. 821514. Copies of this information may be obtained free of charge from the Director, CCDC, 12, Union Road, Cambridge CB2 1EZ (Fax +44-1223-336033) or e-mail deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk.

Results and discussion

Synthesis, purification and characterization of 1,3-bis(1-thyminyl)-2-propanone (T_2CO)

T_2CO was synthesized following a procedure similar to the preparation of 1,3-diaryl-2-propanone derivatives by a modified Dakin–West reaction (Dakin and West 1928; Curran 1995; Tran and Bickar 2006; Kawase et al. 2000) performed on 1-thyminyl acetic acid (TCH_2COOH) as schematically represented in Scheme 1.

After lyophilization the crude reaction mixture was treated with water and the precipitation of a white compound was observed in the aqueous solution. After crystallization from water and acetone the desired product was obtained with a 45% yield. Identity of T_2CO was confirmed by $^1H/^{13}C$ NMR, LC–ESI MS (Fig. 2) and X-ray characterization studies. More particularly, by examining the ESI MS spectrum (positive ions) besides the occurrence of

Table 1 Crystallographic data for $T_2CO{\cdot}H_2O$

Chemical formula	C_{13} H_{16} N_4 O_6
Crystal size (mm)	0.4 × 0.2 × 0.1
Crystal habitus, color	Prism, white
Formula weight	324.30
Temperature (K)	293
λ (Å)	0.71069
Crystal system	Orthorhombic
Space group	P_{bca}
a (Å)	15.688(1)
b (Å)	8.529(2)
c (Å)	22.225(2)
α (°)	90
β (°)	90
γ (°)	90
Volume (Å^3)	2,973.8
Z	8
D_{calcd} (g cm^{-3})	1.449
μ (mm^{-1})	0.116
F (000)	1,360
Theta range (°)	3.18, 27.56
Reflections collected	23,007
Unique observed reflections	3,414 [$R_{(int)}$ = 0.0783]
Data/parameters	3,414/214
R1[a], wR2[b][$I > 2\sigma$(I)]	0.0529, 0.1331
R1[a], wR2[b] (all data)	0.1300, 0.1807
Largest diff. peak and hole (e·Å^{-3})	0.169, −0.209

[a] $R_1 = \sum||Fo| - |Fc|/\sum|Fo|$

[b] $wR_2 = [\sum w(Fo^2 - Fc^2)^2/\sum w(Fo^2)^2]^{1/2}$

$[M+H]^+$, $[M+Na]^+$ and $[M+K]^+$ carbocations, the loss of thymine as well as thymine methylene carbonyl moieties can also be deduced by the occurrence of $[M–T]^+$ and $[M–TCH_2CO]^+$ fragments (Fig. 3).

Mechanistic considerations

A plausible mechanism for the Dakin–West reaction performed by us on 1-thyminyl acetic acid can be suggested from the comparison with other literature reports (Kawase et al. 2000) as represented in Scheme 2 and involves the formation of an 1,3-oxazolium-5-olate intermediate formed through the cyclodehydratation of the 1-thyminyl acetate activated by HATU (TCH_2COOR). To the best of our knowledge our report represents the first application of the Dakin–West reaction to the heteroaromatic acetic acids.

X-ray molecular structure

Molecular structure of T_2CO is reported in Fig. 4. The molecule crystallizes in orthorhombic system (space group P_{bca}). In the unit cell, for each molecule, a crystallization water molecule is present bound through hydrogen bond to the C=O of an amido group [O6–H6···O2; O6—H6 = 0.982(1) Å, H6–O2 = 2.094(3) Å, O6–H6–O2 = 164.07(2)°]. The molecule exhibits an *endo–endo* conformational arrangement: the two arms of the heteroaromatic rings present the same orientation with respect to the carbonyl group (Varughese and Draper 2010) and the angles between the mean planes of the propanone group and the thymine rings bound to C7 and C5 are equal to 77.5(1)° [C8–N3–C7–C6 = −70.1(3)] and 87.6(1) [C4–N2–C5–C6 = 85.6(3)], respectively. It is worth noting that NH and CO groups of different thymine rings, symmetry related to each other, are strictly bonded through a close net of hydrogen bonds.

Influence of temperature and ionic strength on the spectroscopical properties of T_2CO

Subsequently, we studied the effect of parameters such as temperature and ionic strength on the spectroscopic properties of T_2CO by means of UV absorption spectrophotometry (UV). More particularly, we recorded the UV spectrum in the 250–320 nm wavelengths range relative to a 15 μM solution of T_2CO in H_2–0.1% DMSO (pH 7.0) at different temperatures (10, 20, 30, 40, 50, 60, 70, 80 and 90°C).

As it can be seen in Fig. 5, the T_2CO solution presents an absorbance decrease around 270 nm and an increase around 298 nm as an effect of the heating.

Furthermore, the effect of the ionic strength on the spectroscopical properties of T_2CO was studied by recording the UV spectrum in the 250–320 nm wavelengths-range relative to a 38 μM solution of T_2CO in H_2O–0.2% DMSO (pH 7.0) at different NaCl concentrations (0, 20, 40, 60, 80 100, 120, 140, 160, 180, 200 mM). As can be observed in Fig. 6 the T_2CO solution presents an absorbance decrease around 270 nm as a consequence of the increase in NaCl concentration and, thus, of the ionic strength of the solution. The above-reported data suggest that high values of ionic strength, as well as the increase of the temperature, allow the molecule to explore conformations in which an interaction occurs between the π electron clouds of the two thymine rings causing the slight hypochromic effect observed.

Nucleic acid-binding studies

The ability of T_2CO to interact with nucleic acids was evaluated by UV spectroscopy using a tandem cell (Rocchi et al. 1972; Krzyzanowska et al. 1998; Fig. 7). Regarding the DNA-binding assay, from experiments conducted at

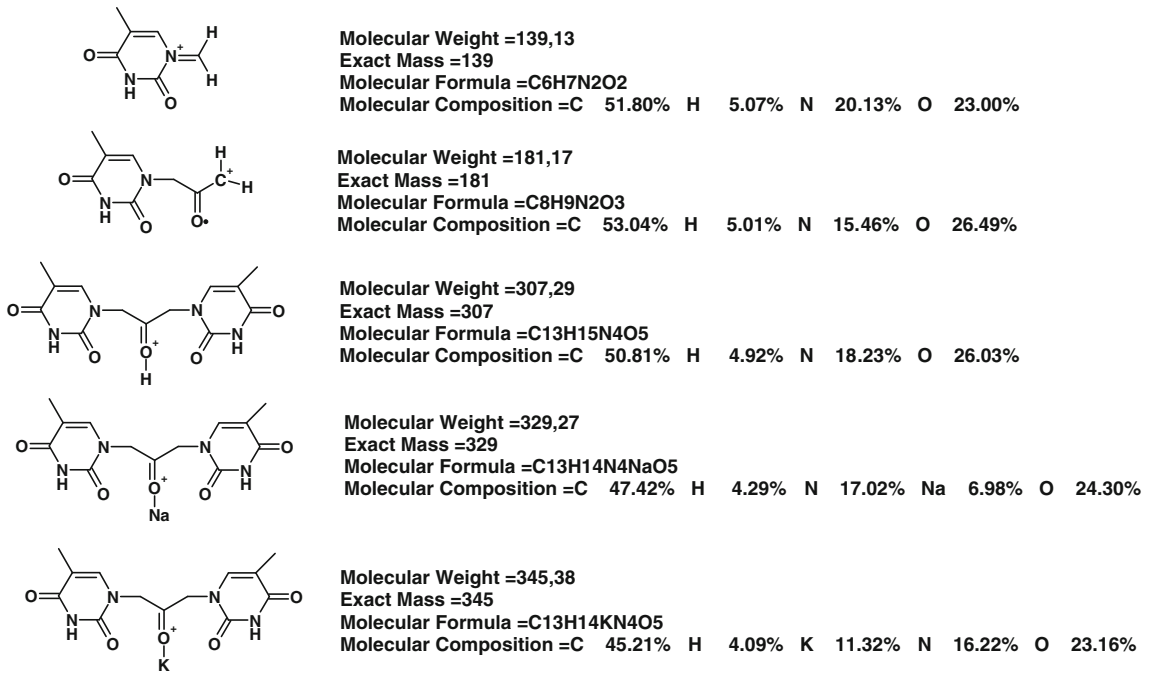
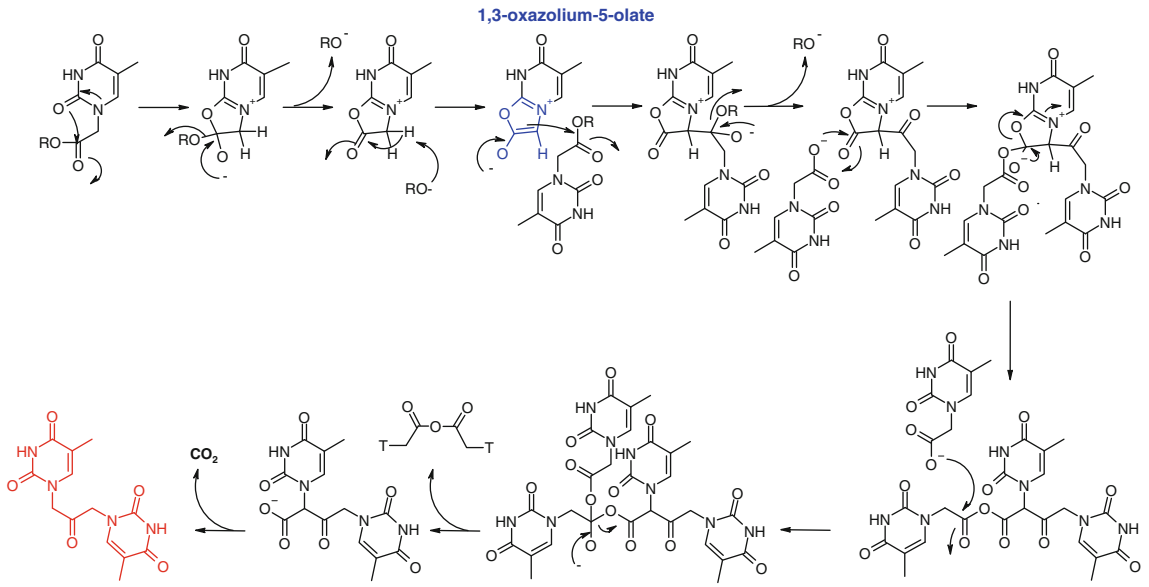


Fig. 3 Interpretation of LC–ESI MS records: representation of the carbocations and molecular fragments corresponding to the *m/z* values reported in the MS characterization of T_2CO



Scheme 2 Mechanism for the Dakin–West reaction on 1-thyminyl acetic acid for the formation of T_2CO

10°C in 10 mM phosphate buffer (pH 7.5) no ability of T_2CO to bind either double stranded (dA_{10}/dT_{10}) or single stranded (dA_{10}) DNA was detected. Indeed, no significant variation in UV profile after the mixing of the two solutions was observed in the presence of either 1:1 or 1:2 DNA:T_2CO ratios. By analogous binding experiments with RNA we did not find any interaction of T_2CO with poly-A nor poly-U single strands. On the other hand, a weak hyphochromic effect was observed after mixing a 20 μM T_2CO solution with a 20 μM (in nucleobase) poly-A/poly-U complex solution, both in 10 mM phosphate buffer (at pH 7.5) at 10°C (Fig. 7).

This finding suggests a very weak interaction of T_2CO with double-stranded RNA, a feature of interest in the field of the development of antiviral strategies based on the targeting of viral double-stranded RNA (Fei et al. 2009).

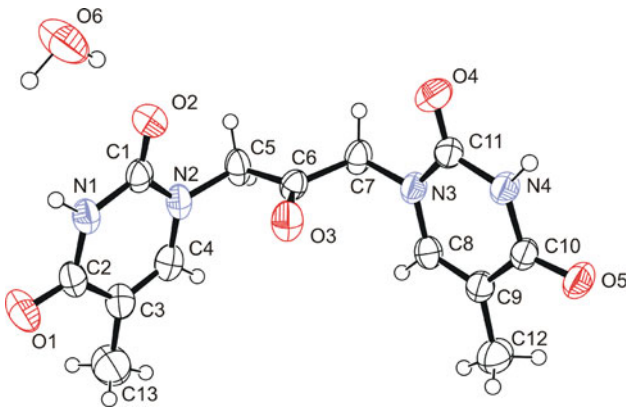


Fig. 4 ORTEP-3 view of T_2CO. Thermal ellipsoids are shown at 30% probability level. A water crystallization molecule is present in the unit cell. Selected bond distances and angles (Å, °): C6–O3 = 1.201(3), C6–C7 = 1.507(3), C5–C6 = 1.505(3), C7–N3 = 1.452(3), C5–N2 = 1.461(3), C6–C7–N3–C11 = −104.9(3), C6–C7–N3–C8 = 70.1(3), C6–C5–N2–C1 = 82.9(3), C6–C5–N2–C4 = −85.6(3)

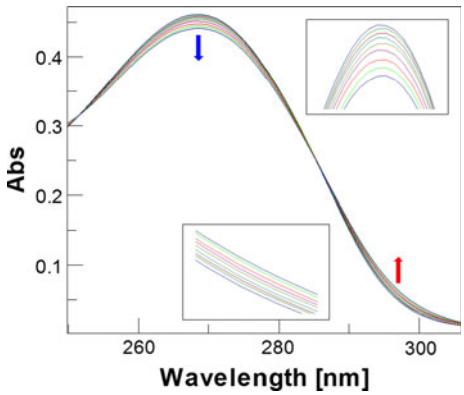


Fig. 5 Variation of the UV absorbance of a 15 μM solution of T_2CO in H_2O-0.1% DMSO as a function of temperature (10, 20, 30, 40, 50, 60, 70, 80, 90°C)

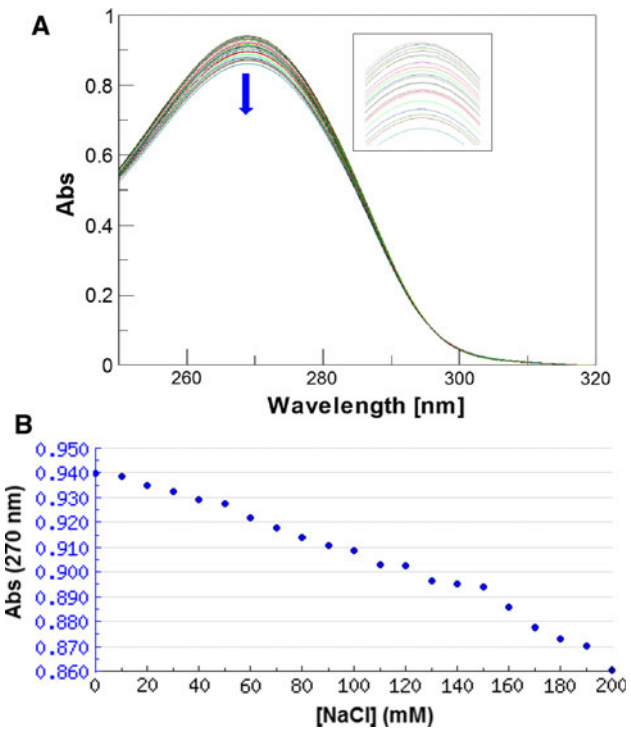


Fig. 6 Variation of the UV spectrum (**a**) and of the absorbance value recorded at 270 nm (**b**) of a 38 μM solution of T_2CO in H_2O–0.2% DMSO as a function of the NaCl concentration

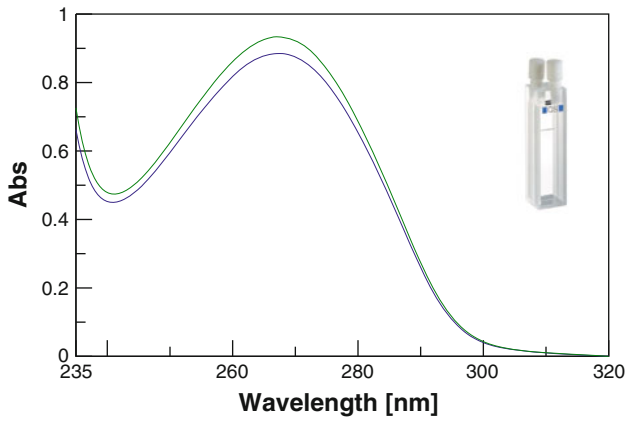


Fig. 7 Sum (*blue line*) and mix (*green line*) UV spectra recorded at 10°C in a tandem cell, relative to a poly-A/poly-U (20 μM in nucleobase) and T_2CO (20 μM) dissolved, respectively, in 1 mL of H_2O and 1 mL of H_2O–0.2% DMSO in the presence in both cases of 10 mM phosphate buffer (pH 7.5). After the mixing total volume was 2 mL (color figure online)

Interaction studies with amino acids and proteins

T_2CO was also studied for possible interactions (based on aromatic interactions, H-bonding, etc.) with molecules of biological interest different from nucleic acids such as amino acids and proteins. More particularly, by CD and UV experiments conducted on T_2CO and L-alanine or T_2CO and BSA protein no binding of these biomolecules with the heteroaromatic 1,3-disubstituted 2-propanone was detected (data not shown).

Serum stability evaluation of T_2CO

The enzymatic resistance of the thymine-containing derivative was investigated by incubating T_2CO in 92% human serum (the concentration of T_2CO was 0.3 mM) at 37°C and analyzing by RP HPLC samples withdrawn from the reaction mixture at various times (0, 1, 2, 3, 4, 5, 6 and 24 h). By analyzing the results of the stability assay (Fig. 8), it can be deduced that T_2CO is stable in human serum at 37°C for up to 24 h without degradation.

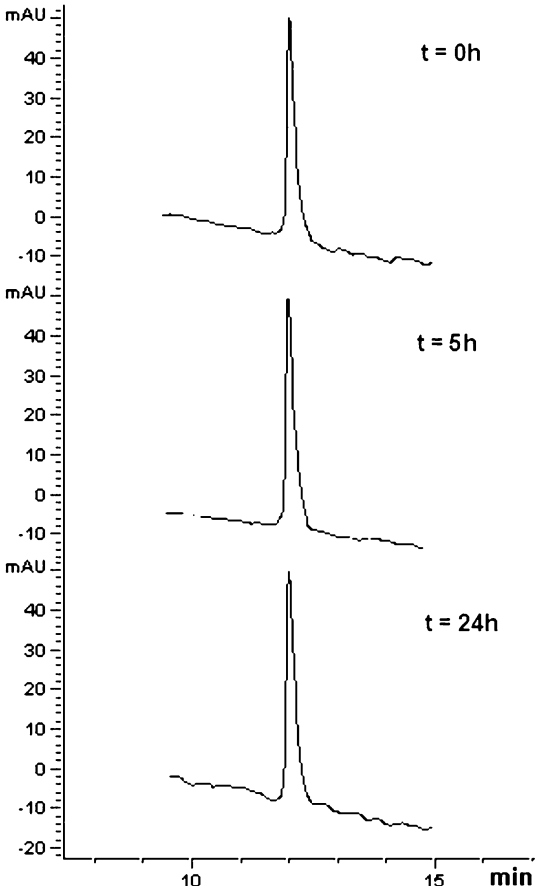


Fig. 8 RP-HPLC analysis of the serum stability assay on T_2CO relative to 0, 5 and 24 h ($T = 37°C$). RP-HPLC analytical chromatography was performed on a C_{18} column (with a flow rate of 4 mL/min) with a linear gradient from 2% (for 5 min) to 80% B in A over 20 min: $t_R = 12.1$ min (A = 0.1% TFA in water; B = 0.1% TFA in acetonitrile)

Interaction studies with Ni (II) and Cu (II) metal cations

Furthermore, we performed interaction studies with metal ions such as Ni^{2+} and Cu^{2+} using a 4 μM solution of T_2CO in H_2O–0.1% DMSO (pH 7) and two aqueous solutions of nickel (II) chloride hexahydrate and copper (II) chloride dihydrate, respectively.

As can be deduced from Fig. 9, no significant interaction of T_2CO with Ni^{2+} was evidenced, whereas a certain Cu^{2+}-binding ability was observed corresponding to the slight change in UV spectra recorded in correspondence with increasing amounts of Cu^{2+} added to the solution of 1,3-bis(1-thyminyl)-2-propanone.

Interaction studies with a nucleopeptide-based supramolecular assembly

Recently, it was evidenced by CD and laser scattering that chiral nucleopeptides are able to form large supramolecular networks held together by non-covalent interactions occurring between nucleopeptide units and able to host in their interior organic molecules, characteristics that render them molecules of clear interest for the realization of innovative drug delivery systems (Roviello et al. 2011). Prosecuting our research efforts in this field, here we present a study on the interaction of T_2CO with the above-mentioned nucleopeptide, synthesized according to literature (Roviello et al. 2011), performed in order to evaluate the potential use of the heteroaromatic compound to reinforce nucleopeptide-based supramolecular assemblies.

In particular, to a 40 μM solution of chiral nucleopeptide in 2 mL of 10 mM phosphate buffer (pH 7.5), we added increasing amounts of T_2CO and recorded CD spectra after each addition (Fig. 10). From the comparison of such spectra with the one obtained before the titration started, we found a progressive intensification of the negative band situated around 275 nm, which is characteristic of the supramolecular assembly (Roviello et al. 2011), as a consequence of the addition of the thymine-based derivative. This result suggests that the nucleopeptide-based supramolecular system, already studied in literature (Roviello et al. 2011), presents a higher level of structuration due to the influence of the T_2CO molecules. Thus, the

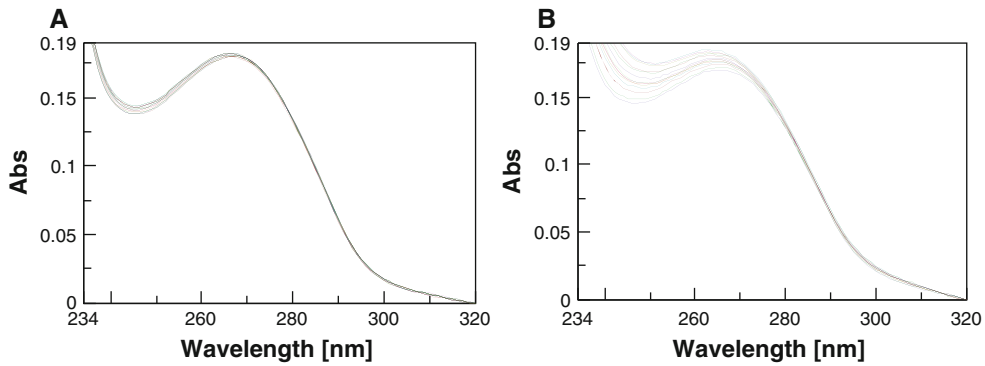


Fig. 9 Absorption spectra relative to a 4 μM solution of T_2CO in H_2O–0.1% DMSO in the presence of Ni^{2+} (A) and Cu^{2+} (B) cations in 0–40 equivalents range (at 25°C)

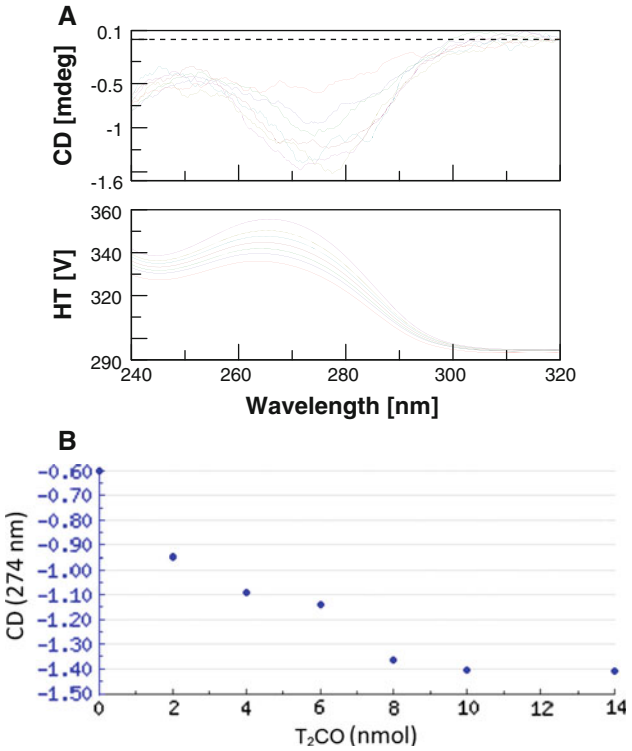


Fig. 10 **a** CD spectra recorded at 10°C in 10 mM phosphate buffer, pH 7.5, relative to a 40 μM solution of nucleopeptide (80 nmol) in the presence of 0, 2, 4, 6, 8, 10 and 14 nmol of T_2CO. **b** Variation of CD value recorded at 274 nm as a function of the T_2CO amounts (nmol) added to the nucleopeptide solution

heteroaromatic molecule herein-studied could find application in the assembly of the above-mentioned drug delivery systems thanks to its combined utilization with nucleopeptide molecules. Interestingly, the highest structuration effect observed on the nucleopeptide supramolecular network was revealed in correspondence of a 10:1 (nucleopeptide:T_2CO) ratio which corresponds to the addition of 8 nmol of thymine-based derivative to the solution containing 80 nmol of nucleopeptide. Indeed no significant intensification of the negative band centered around 275 nm was observed by adding further amounts of T_2CO.

Conclusion and perspectives

This work concerns the study of the properties of a heteroaromatic compound, which was called by us T_2CO, characterized by two thymine rings anchored to the 1 and 3 positions of a 2-propanone moiety. The spectroscopical properties of this compound, studied by UV as a function of temperature and ionic strength, suggest that the increase of temperature allows the molecule to explore conformations usually not favored, available also thank to the ion–dipole interactions occurring in correspondence of high values of ionic strength, in which the interaction between the π electron clouds of the two thymine rings caused the hypochromic effect observed in our experiments. Subsequently, preliminary nucleic acid-binding studies, performed by UV on samples containing T_2CO, evidenced a slight ability of this molecule to interact with double-stranded RNA (poly-A/poly-U), but not with single stranded nucleic acids (DNA and RNA) or with double-stranded DNA. Furthermore, in vitro studies showed that T_2CO is enzymatically stable up to 24 h in fresh human serum, whereas CD studies evidenced its ability to induce higher levels of structuration on nucleopeptide-based supramolecular networks, behavior of great interest in view of the realization of innovative drug delivery systems. In conclusion, all the interesting characteristics of the heteroaromatic compound studied in our work strongly encourage us to deepen the study of the possible applications that this molecule in conjunction with nucleobase-containing peptides can have in the field of biomedicine thanks to its molecular recognition properties. Nevertheless, future biological studies on this 2-propanone-based analogue of (−)-anaferine are also auspicable in order to evaluate its potential as anticancer and antimuscarinic drug.

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Conflict of interest The authors state that there is no conflict of interests.

References

Altomare A, Burla MC, Camalli M, Cascarano GL, Giacovazzo C, Guagliardi A, Moliterni AGG, Polidori G, Spagna R (1999) SIR97: a new tool for crystal structure determination and refinement. J Appl Cryst 32:115–119

Curran TT (1995) Implementation of the Dakin–West reaction for the preparation of an α-amino-pentafluoroethyl ketone. J Fluorine Chem 74:107–112

Dakin HD, West RA (1928) General reaction of amino acids I and II. J Biol Chem 78:91–105

Fei Z, Liu Y, Yan Z, Fan D, Alexander A, Yang JH (2009) Targeting viral dsRNA for antiviral prophylaxis. FASEB J. doi: 10.1096/fj.09-144915

Kaiser C, Audia VH, Carter JP, McPherson DW, Waid PP, Lowe VC, Noronha-Blob L (1993) Synthesis and antimuscarinic activity of some 1-cycloalkyl-1-hydroxy-1-phenyl-3-(4-substituted piperazinyl)-2-propanones and related compounds. J Med Chem 36:610–616

Kawase M, Hirabayashi M, Kumakura H, Saito S, Yamamoto K (2000) The Dakin–West reaction of *N*-alkoxycarbonyl-*N*-alkyl-α-amino acids employing trifluoroacetic anhydride. Chem Pharm Bull 48:114–119

Krzyzanowska D, Lisowski M, Kochman M (1998) UV-difference and CD spectroscopy studies on juvenile hormone binding to its carrier protein. J Pept Res 51:96–102

Rocchi R, Borin G, Marchiori F, Moroder L, Peggion E, Scoffone E, Crescenzi V, Quadrifoglio F (1972) Interaction of S-protein with S-peptide and with synthetic S-peptide analogs. A spectroscopic and calorimetric investigation. Biochemistry 11:50–57

Rother A, Bobbitt JM, Schwarting AE (1962) Structure and synthesis of the alkaloid anaferine. Chem Ind (London):654–655

Roviello GN, Musumeci D, Castiglione M, Bucci EM, Pedone C, Benedetti E (2009) Solid phase synthesis and RNA-binding studies of a serum-resistant nucleo-epsilon-peptide. J Pept Sci 15:155–160

Roviello GN, Benedetti E, Pedone C, Bucci EM (2010) Nucleobase-containing peptides: an overview of their characteristic features and applications. Amino Acids 39:45–57

Roviello GN, Musumeci D, Bucci EM, Pedone C (2011) Evidences for supramolecular organization of nucleopeptides: synthesis, spectroscopic and biological studies of a novel dithymine L-serine tetrapeptide. Mol BioSyst 7:1073–1080

Sheldrick GM (1996) SADABS, Program for empirical absorption correction. University of Göttingen, Germany

Sheldrick GM (1997) SHELX-97 University of Göttingen, Germany

Tran K, Bickar D (2006) Dakin–West synthesis of β-aryl ketones. J Org Chem 71:6640–6643

Varughese S, Draper SM (2010) Solid state conformational preferences of a flexible molecular backbone derived from acetone: dependence on electron donating/withdrawing ability of substitutions. Cryst Grow Des 10:2298–2305