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A modification of a conventional technique for the synthesis of hydrazones of racemic carbonyls: prevention of spontaneous chiral inversion

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Conventionally hydrazones and other derivatives of carbonyls are synthesized under acidic conditions when spontaneous chiral inversion is a common problem if the carbonyl compound is chiral. A new method has been developed involving solid phase microwave-assisted conditions for the synthesis of 2,4-dinitrophenyl hydrazone(s) of chiral carbonyl compounds wherein there occurred no inversion of configuration. The method provided high yields (91–95%) in short reaction times (4–6 min). The method proposed clearly has synthetic advantages over current practices. The hydrazones were characterized by IR, ^{1}H NMR and CHN analysis. The hydrazones represent enantiomeric pairs tagged with a strong chromophore rather than diastereomers. The enantiomeric pairs were separated by HPLC using an α_1-acid glycoprotein column and the best resolution of all the analytes was achieved with a mobile phase containing 0.5% 2-propanol in 10 mM citrate phosphate buffer at pH 6.5. The chromatographic peaks clearly showed base line separation with comparable peak areas and thus the results confirmed that there was no spontaneous inversion of configuration during derivatization. The chromatograms corresponding to the products obtained by conventional procedure (from the racemic mixtures of analytes) showed peaks with unequal areas suggesting formation of enantiomers in unequal amounts (*i.e.*, non racemic mixtures) because of spontaneous inversion of the configuration during derivatization.

1. Introduction

It may not be an exaggeration to state that the carbonyl group is the centerpiece of organic chemistry. It is not only present itself in most of the main functional groups with multiple bond from carbon to heteroatom but it also serves as a model for reactions of all functions with π-bonds between dissimilar atoms. Besides, its modes of reaction are simple and are very versatile in terms of synthetic applications. Compounds having a primary amino group cause a nucleophilic addition at the electron deficient carbonyl carbon of aldehydes and ketones with a subsequent condensation step to replace the carbonyl oxygen by nitrogen ($\rangle C{=}O \rightarrow \rangle C{=}N + H_2O$). Notable among such reagents are phenyl-, *p*-nitrophenyl-, and 2,4-dintrophenyl-hydrazines which are used much more often and give the corresponding hydrazones with most aldehydes and ketones.[1] These make excellent derivatives as sharp melting solids (and having characteristic IR spectra), useful for characterization of the parent aldehydes or ketones.

Such derivatizations (including D-exchange and bromination) proceed *via* enol formation which is rate determining (and are generally acid catalyzed). Ketones offer a choice of enolization in two directions. In saturated molecules, the enolate from the less substituted side is favoured in base catalysed reaction while in acid the more substituted enol is preferred. It is the more resonance-stabilized enol or enolate that is always preferred.

Enolization causes inversion of configuration at the asymmetric α-carbon to carbonyl since the enol has no asymmetry. The most stable configuration at the α-carbon will result from equilibration by enolization. In addition reactions to carbonyl carbon, the asymmetry present on the carbon α- to carbonyl makes the less hindered side of the carbonyl π-orbital more accessible and may cause spontaneous configurational inversion resulting into enantiomeric mixtures (which are often not predictable).

Enantioresolution of chiral aliphatic or alicyclic aldehydes and ketones by direct approach using liquid chromatography would require the presence of a suitable chromophore for on-line detection in UV-visible region. Direct enantioresolution based on reversible diastereomeric association between solute enantiomers and chiral stationary phases (CSPs) offers the advantages of simple chromatographic runs and absence of kinetic resolution and racemization over indirect method of chiral separation.[2,3] Among the various nucleophilic reagents containing amino group 2,4-dinitrophenyl hydrazine is the one that could act as an achiral strong chromophore for carbonyl compounds for its DNP moiety.

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1.1. DNP-derivatives

Since the pioneering work of Sanger[4] on the preparation of dinitrophenyl (DNP) derivatives of amino acids their use for sequence analysis has declined in the modern times. Nevertheless, DNP moiety attracted attention for its application, as a strong chromophore, for synthesis of several chiral derivatizing reagents (CDRs) which have been used for separation and detection of diastereomers of a variety of pharmaceutically important racemic compounds.[5–7]

1.2. Enantioresolution of chiral carbonyl compounds

Literature reveals sporadic reports on enantioresolution of chiral carbonyl compounds involving application of DNP moiety or hydrazone derivative. These include indirect enantioresolution using CDRs developed from 1,5-difluoro-2,4-dinitrobenzene[8] wherein the DNP moiety serves as a chromophore for on-line detection of the corresponding diastereomers. CSP derived from (*S*)-1-(6,7-dimethyl-1-naphthyl) isobutylamine was used for resolution of cyclic and acyclic chiral ketones as their oxime 3,5-dinitrophenyl carbamates.[9] A chiral phosphorylhydrazine reagent was used to prepare hydrazone diastereomers of chiral ketones which were analyzed by ^{31}P NMR and HPLC.[10]

Some other reports include resolution of chiral cyclic ketones by direct approach using CSPs based on amylose tris(3,5-dimethylphenyl carbamates) and cellulose tris(3,5-dimethylphenyl carbamates),[11] cellulose tribenzoate,[12] and β-cyclodextrin.[13,14] Enantioresolution of Wieland–Miescher ketones, their C(5) homologue, and their C(1) dioxolane derivatives has been reported[15] using commercially available CSPs like cellulose tris-(3,5-dimethyl-phenylcarbamate), native β-cyclodextrin, and acetylated, carboxymethylated and permethylated β-cyclodextrins. Direct enantioresolution of Mannich ketones has been achieved by using aqueous copper(II) acetate and L-aspartame[16] and, cellulose and cyclodextrin derivatives[17] as chiral mobile phase additives.

Thus, it is evident that till now the scientific issue with respect to spontaneous configurational inversion of any chiral carbonyl compound (used either as an enantiomerically pure sample or a racemic mixture) undergoing derivatization with an amino group containing nucleophilic reagent has not been investigated.

1.3. Present work

Taking into account the literature, as noted above, and the references cited therein the objective of the present report has been to develop method of synthesis of derivatives (DNP-hydrazones in the present case) of certain didactic racemic carbonyl compounds (four ketones and two aldehydes **I–VI**, Fig. 1) which would proceed without spontaneous chiral inversion, and to establish 'racemic' and 'non-racemic' composition of the products obtained under newly developed method, and the products obtained by derivatization of the same racemic carbonyl compound under conventional acidic conditions. To achieve the objective, (a) solid phase microwave-assisted conditions were developed for synthesis of 2,4-dinitrophenyl hydrazone(s) (DNPHz) of certain didactic chiral carbonyl compounds, and (b) the DNPHz derivatives of racemic carbonyl compounds were then resolved by chiral HPLC into enantiomers using α_1-AGP column. The emphasis was not on developing a method for enantioseparation but it was to verify the 'racemic' and 'non-racemic' nature of the product. And the novelty of the present work lies in the above said two aspects.

Literature reports on the importance of carbonyl compounds in organic synthesis or in pharmaceutical industry or the methods of their enantioresolution have not been discussed because the focus of the present paper is to report the aspects mentioned above.

2. Experimental

2.1. Apparatus

The HPLC system of Waters (Milford, MA, USA) was used that consisted of a 515 HPLC pump, a Waters 2489 UV-vis dual wavelength detector, high pressure binary gradient pump

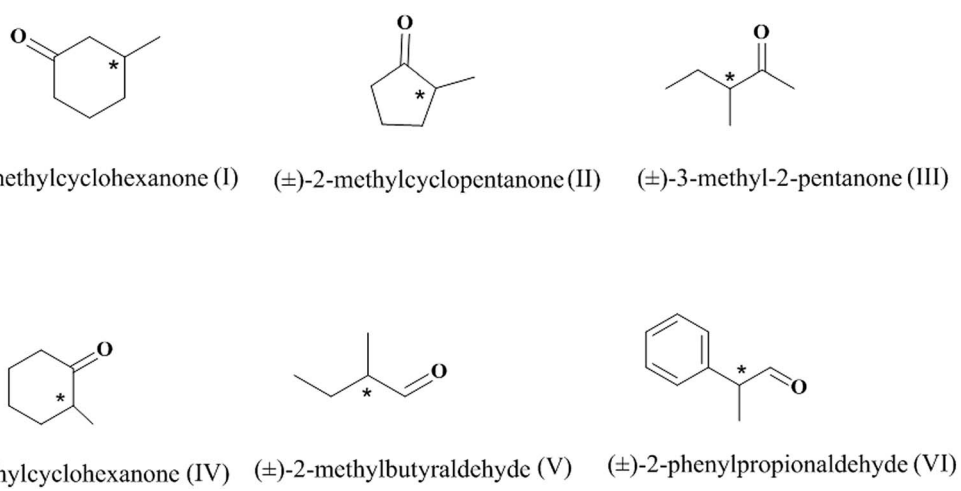

Fig. 1 Structures of chiral aldehydes and ketones.

control module II, a manual injection valve, an empower2 operating software (build number, 2154). Other equipment used were Microwave-Multiwave 3000 (800 W, Perkin-Elmer, Shelton, CT, USA), α_1-AGP (L × I.D. 10 cm × 4 mm, 5 μm particle size) column from Chromtech Merck (Darmstadt, Germany), pH meter Cyberscan 510 (Singapore), Polarimeter P-3002 (Krüss, Hamburg, Germany), FT-IR spectrometer 1600 (Boardman, OH, USA), Vario EL III elementar analyzer, and Shimadzu UV-1601 spectrophotometer (spectra were recorded in MeOH). ^{1}H NMR spectra were recorded on a Bruker 500 MHz instrument using $CDCl_3$ as the solvent.

2.2. Chemicals and reagents

(±)-2-Methylcyclopentanone; (±)-2-methylcyclohexanone; (±)-3-methylcyclohexanone, (±)-3-methyl-2-pentanone, (±)-2-methylbutyraldehyde, (±)-2-phenylpropionaldehyde and 2,4-dinitrophenyl hydrazine (2,4-DNPH) were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other analytical-grade chemicals, HPLC grade solvents such as acetonitrile (MeCN) and silica gel 60 were also from E. Merck (Mumbai, India). Double distilled water purified (18.2 MΩ cm^3) with Milli-Q system of Millipore (Bedford, MA, USA) was used throughout.

2.3. Synthesis and characterization of dinitrophenyl hydrazones (2,4-DNPHz)

Solid phase microwave-assisted approach. Representative synthesis of 2,4-DNPHz of (±)-3-methyl-2-pentanone and characterization data of all the resulting six hydrazones is given below.

*Synthesis of 2,4-DNPHz of (±)-3-methyl-2-pentanone (**3**).* 2,4-DNPH (0.019 g; 10 mmol) and (±)-3-methyl-2-pentanone (0.010 g; 10 mmol) were dissolved in MeOH (10 mL) followed by addition of silica gel (6 g) to this solution. After about 20 minutes, the solvent was evaporated and the silica gel (on which the two reactants were adsorbed) was irradiated with MW in an oven at 500 W for 4 min (with 1 min interval). The MW irradiated silica gel was then stirred in ethyl acetate (10 mL) for 10 min and then filtered. The residual silica gel was washed twice with 5 mL ethyl acetate; the combined extract was concentrated under the stream of nitrogen and was left for crystallization. The yields were in the range of 91–95%. The hydrazones obtained, as the product, under experimental conditions (i) were designated as (**1–6**). These derivatives were analysed by chiral HPLC.

Conventional acid catalyzed synthesis. 2,4-DNPH (0.019 g; 10 mmol) was dissolved in 10 mL MeOH, in a small conical flask; as a representative, solution of (±)-3-methyl-2-pentanone (0.010 g; 10 mmol) in 10 mL MeOH was added to it. Concentrated sulphuric acid was then added drop by drop with constant stirring till pH 4 was obtained. The reaction mixture was allowed to stand for 10 min. Formation of corresponding derivative as 2,4-DNPHz occurred during this time. It was filtered and recrystallized from MeOH. The yields were in the range of 78–82%. The hydrazones obtained, as the product, under experimental condition (ii) were analysed by chiral HPLC.

2.4. Characterization of the hydrazones

Melting points were determined in open ended glass capillaries and were uncorrected. The hydrazones were characterized by IR, ^{1}H NMR and CHN analysis; the data is given below.

2,4-DNPHz of (±)-3-methylcyclohexanone (1). Color: yellow; mp 103 ± 2 °C; UV (λ_{max}, 365 nm, MeOH): 365; IR (KBr): 3308 (NH), 1617 (C=N), 1590 (Ar); ^{1}H NMR: δ 9.15 (1H, s, ArH), δ 8.31–8.30 (1H, d, ArH), δ 8.00–7.98 (1H, d, ArH), δ 1.07 (3H, d), δ 2.07 (1H, m), δ 1.36 (2H, m), δ 1.98 (2H, m), δ 1.58 (2H, m), δ 11.2 (1H, s); anal. calcd for $C_{13}H_{16}N_4O_4$: C, 53.42%; H, 5.52%; N, 19.17%. Found: C, 53.22%; H, 5.30%; N, 19.08%.

2,4-DNPHz of (±)-2-methylcyclopentanone (2). Color: yellow; mp 105 ± 2 °C; UV (λ_{max}, 363 nm, MeOH); IR (KBr): 3416 (NH), 1619 (C=N), 1511 (Ar); ^{1}H NMR: δ 10.8 (1H, s), δ 9.15 (1H, s, ArH), δ 8.31 (1H, d, ArH), δ 8.01–7.99 (1H, d, ArH), δ 1.28 (3H, d), δ 2.76 (2H, m), δ 2.10 (2H, m), δ 2.24 (2H, m), δ 2.41 (1H, m); anal. calcd for $C_{12}H_{14}N_4O_7$: C, 51.80%; H, 5.07%; N, 20.13%. Found: C, 51.62%; H, 4.95%; N, 19.99%.

2,4-DNPHz of (±)-3-methyl-2-pentanone (3). Color: orange; mp 98 ± 2 °C; UV (λ_{max}, 361 nm, MeOH); IR (KBr): 3290 (NH), 1617 (C=N), 1516 (Ar); ^{1}H NMR: δ11.04 (1H, s), δ 9.13 (1H, s, ArH), δ 8.31–8.29 (1H, d, ArH), δ 7.98 (1H, d, ArH), δ 2.51 (1H, q), δ 1.98 (3H, s), δ 1.66 (2H, m), δ 1.18 (3H, d), δ 0.93 (3H, t); anal. calcd for $C_{12}H_{16}N_4O_4$: C, 51.42%; H, 5.75%; N, 19.99%. Found: C, 51.11%; H, 5.52%; N, 19.46%.

2,4-DNPHz of (±)-2-methylcyclohexanone (4). Color: yellow-brown; mp 112 ± 2 °C; UV (λ_{max}, 364 nm, MeOH); IR (KBr): 3320 (NH), 1621 (C=N), 1586 (Ar); ^{1}H NMR: δ 9.14 (1H, s, ArH), δ 8.30–8.29 (1H, d, ArH), δ 8.00–7.98 (1H, d, ArH), δ 2.00 (1H, m), δ 1.06 (3H, d), δ 1.37 (2H, m), δ 1.86 (2H, m), δ 1.24 (3H, d), δ 1.58 (2H, m), δ 11.2 (1H, s); anal. calcd for $C_{13}H_{16}N_4O_4$: C, 53.42%; H, 5.52%; N, 19.17%. Found: C, 53.2%; H, 5.23%; N, 19.01%.

2,4-DNPHz of (±)-2-methylbutyraldehyde (5). Color: yellow-brown; mp 95 ± 2 °C; UV (λ_{max}, 361 nm, MeOH); IR (KBr): 3287 (NH), 1621 (C=N), 1516 (Ar); ^{1}H NMR: δ 9.17 (1H, s, ArH), δ 8.30 (1H, d, ArH), δ 8.00–7.98 (1H, d, ArH), δ 10.9 (1H, s), δ 1.21 (6H, d), δ 1.48 (2H, m), δ 9.87 (1H, d); anal. calcd for $C_{11}H_{14}N_4O_4$: C, 49.62%; H, 5.30%; N, 21.04%. Found: C, 49.43%; H, 5.21%; N, 20.98%.

2,4-DNPHz of (±)-2-phenylpropionaldehyde (6). Color: yellow; mp 98 ± 2 °C; UV (λ_{max}, 362 nm, MeOH); IR (KBr): 3290 (NH), 1617 (C=N), 1518 (Ar); ^{1}H NMR: δ 9.30 (1H, s, ArH), δ 8.31 (1H, d, ArH), δ 8.00 (1H, d, ArH), δ 10.8 (1H, s), δ 7.28 (2H, m), δ 7.98 (2H, m), δ 7.26 (1H, m), δ 1.24 (3H, d), δ 2.59 (1H, m), δ 10.1 (1H, m); anal. calcd for $C_{15}H_{14}N_4O_4$: C, 57.32%; H, 4.49%; N, 17.83%. Found: C, 57.17%; H, 4.32%; N, 17.44%.

Stock solutions. (i) Solutions of 2,4-DNPHz of each of the six carbonyl compounds were prepared in 2-propanol at a concentration of 10 mM and then diluted to a final concentration of 0.1 mM.

(ii) Citrate phosphate buffer was prepared using 0.1 M solution of citric acid and 0.2 M solution of dibasic sodium phosphate.[18]

Chiral HPLC of reaction products. The composition of mobile phase for achieving enantioresolution was optimized by using binary mobile phase system consisting of citrate

phosphate buffer (in the concentration range 5–25 mM, and pH 3.5–6.5) and 2-propanol (in the range 0.5% to 3.0%) or MeCN (in the range 1 to 5%) at a flow rate of 1 mL min^{-1}. Mobile phase was filtered through a 0.45 μm filter and degassed by sonication and passing nitrogen before use. 20 μL of the sample was injected onto the column. Detection was at 365 nm.

3. Results and discussion

The reaction of racemic carbonyl compounds (**I–VI**) with the reagent (2,4-DNPH) does not lead to formation of diastereomers as the reagent is achiral. There occurs '*tagging*' of enantiomers with a strong chromophore in the form of DNP moiety of 2,4-DNPH. The scheme showing synthesis of derivatives is given in Fig. 2. The hydrazones obtained, as the product, under experimental condition (i) were designated as (**1–6**) and are also shown in Fig. 2.

The reaction under condition (ii) requires nearly pH 4 for maximum rate while basic or highly acidic conditions lower the rate. In more strongly acid solution (pH < 3.5) the unshared pair of electrons (the nucleophilic site) of N is protonated and is no more a nucleophile.[19] It was interesting to observe that addition of sulphuric acid (till pH 4 is obtained) to the mixture of 2,4-DNPH and the carbonyl compound resulted into higher yield of the product hydrazone in comparison to an approach in which sulphuric acid was added at first to the solution of 2,4-DNPH followed by addition of the solution of carbonyl compound.[20]

The problem or the question of spontaneous configurational inversion in each of the enantiomers (present in the racemic mixture of the carbonyls) cannot be overruled when the synthesis of hydrazones was taking place in acidic liquid medium (ii). As a result the ratio of the two enantiomers is expected to get disturbed resulting into possibly a non-racemic mixture of hydrazones of the chiral carbonyls under study. Configurational inversion would occur only when the asymmetric carbon (α- to carbonyl function) is involved in the formation of enol. The possible mechanism for enol formation and configurational inversion at the asymmetric α-carbon to carbonyl and the formation of hydrazone in the subsequent condensation step (replacing the carbonyl oxygen by nitrogen) is shown in Fig. 3.

The characterization data based on IR, ^{1}H NMR and CHN analysis does not differentiate in the enantiomeric ratio of the products obtained from the two approaches, as the products are structurally and chemically the same. In order to investigate the issue of spontaneous configurational inversion chiral HPLC of the products obtained under conditions (i) and (ii) was performed; racemic and non-racemic nature of products (enantiomeric composition) was a decisive factor.

3.1. Determination of racemic and non-racemic nature of products by chiral HPLC

HPLC analysis of the products obtained by approach (ii) showed peaks with unequal areas and this observation led to inference that the product was non-racemic though the reactant carbonyl compound was racemic in nature. Since the products are formed *via* enol and the enol (formed in rate determining step involving asymmetric carbon α- to carbonyl group) is a planar moiety it receives H from either side (side 'a' or side 'b' shown in Fig. 3, depicting mechanism) configurational inversion occurs and the product hydrazone is a non racemic mixture. It was, therefore, contended that spontaneous inversion of configuration was taking place during derivatization under acidic conditions. A representative chromatogram with unequal areas corresponding to the products of the analyte (**III**) is shown in Fig. 4.

It was further confirmed by the observation that the products corresponding to analyte (**I**), *i.e.*, (±)-3-methylcyclohexanone did not show peaks with unequal areas. It was because in the molecule (**I**, Fig. 1) the carbon α- to carbonyl is not asymmetric and it is not involved in the formation of enol.

On the other hand, the chromatograms corresponding to the products obtained under solid state microwave-assisted conditions, approach (i), clearly showed base line separation with comparable peak areas (as provided by the system software). Sections of chromatograms showing baseline resolution of all the six enantiomeric pairs of hydrazones, corresponding to approach (i), are shown in Fig. 5; a full chromatogram as

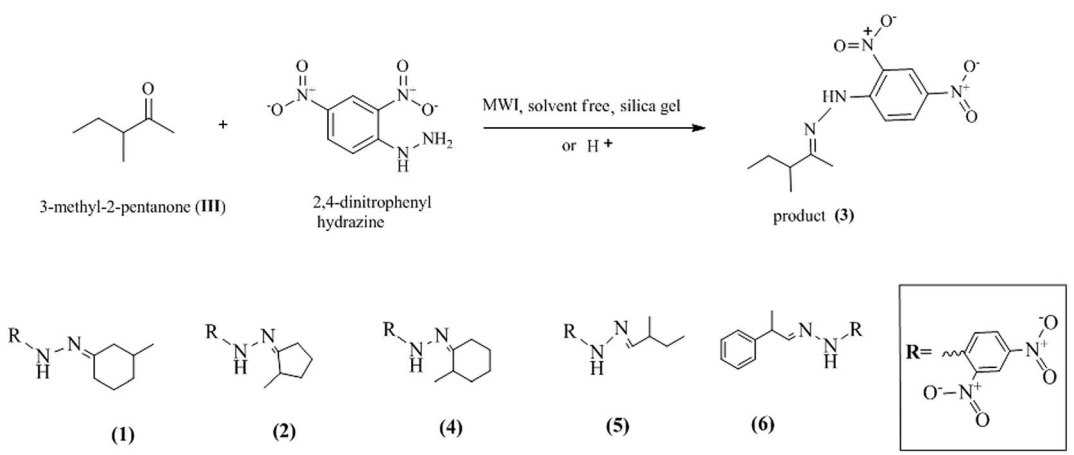

Fig. 2 Scheme showing synthesis of hydrazones.

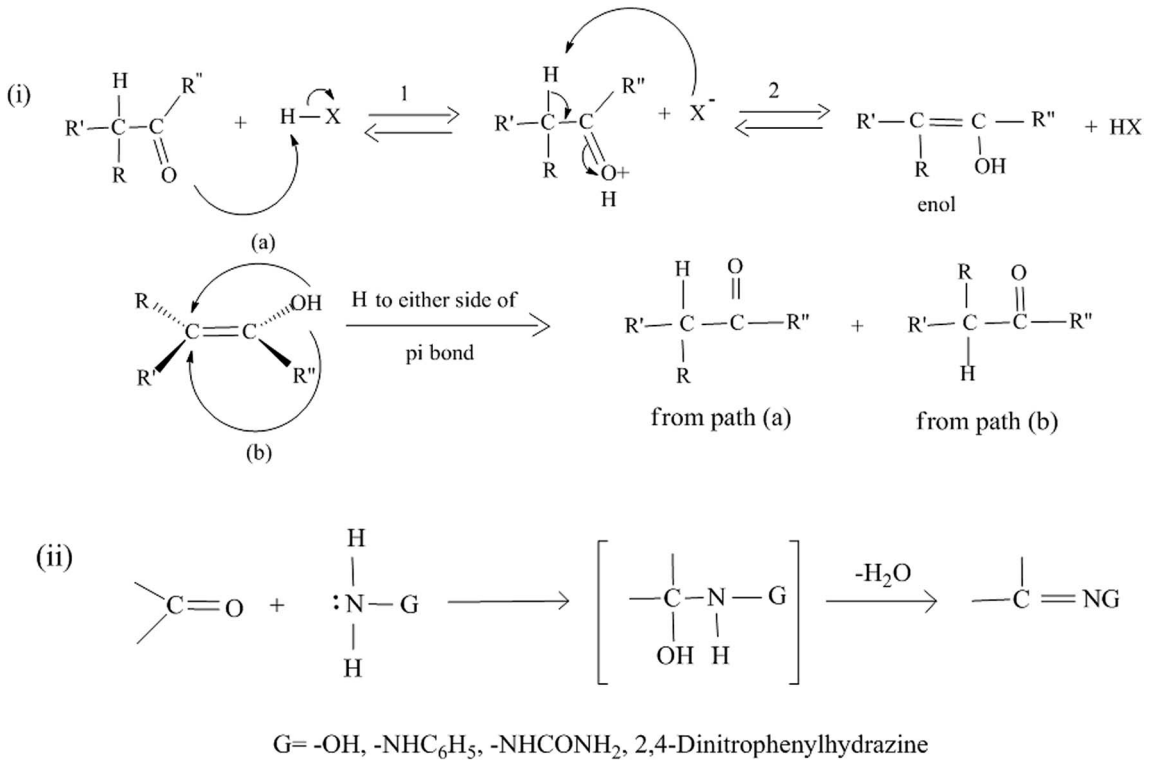

Fig. 3 The possible mechanism for the formation of hydrazones *via* enol formation.

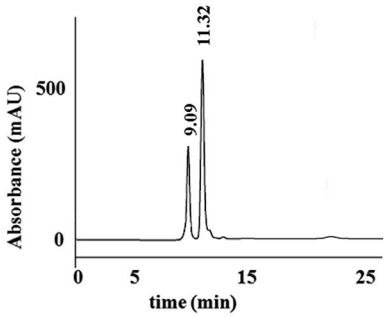

Fig. 4 Full chromatogram (as representative) showing resolution of product (**3**) obtained by approach (ii). The peak areas were 386 and 691 mAU at retention time 9.09 and 11.32 min, respectively. Peaks with unequal areas indicate non-racemic nature.

a representative is given as Fig. 6. In approach (i) silica gel allowed convenient workup. It served as a very efficient adsorbent with a large surface area for homogeneous heating and thus facilitated faster reactions with short reaction times and higher yields. The catalytic amount of acid could probably have been provided by the silica gel (having adsorbed water) and the MWI triggered reaction being very fast provided no opportunity for spontaneous chiral inversion during derivatization.

It can thus be concluded that each of the products (**1–6**) was a racemic mixture of hydrazones (*i.e.*, the tagged enantiomers) of the corresponding racemic carbonyl compound. Thus, the chiral HPLC results clearly verified that there was no configurational inversion of the chiral carbonyl compounds when they

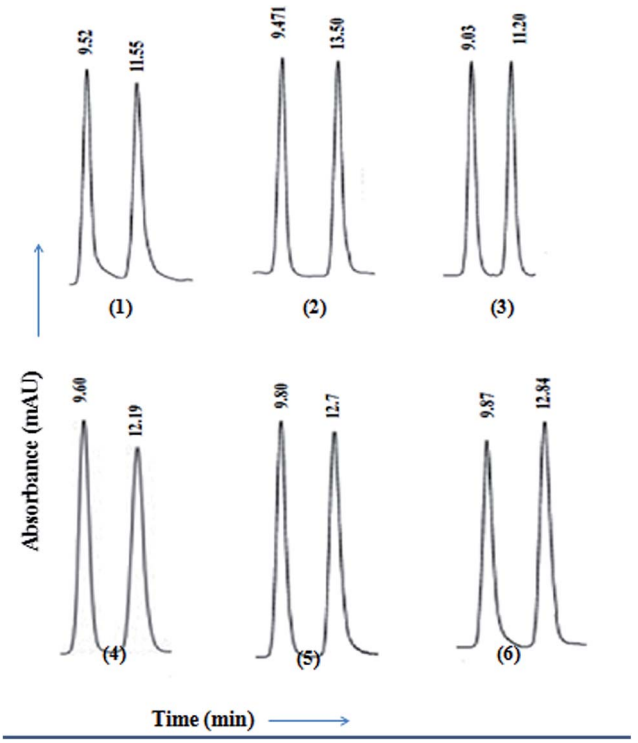

Fig. 5 Sections of chromatograms showing resolution of enantiomeric pairs of six 2,4-DNPHz (retention times are in minutes). Column, α_1-AGP (L × I.D, 10 cm × 4 mm, 5 μm particle size); mobile phase, 0.5% 2-propanol in 10 mM citrate phosphate buffer at pH 6.5; flow rate, 1.0 mL min^{-1}; detection, 365 nm.

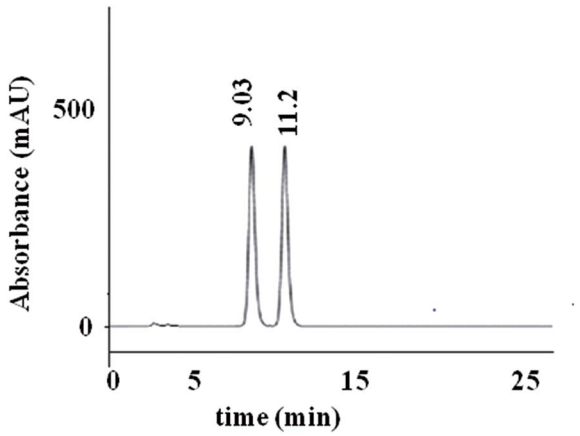

Fig. 6 Full chromatogram (as representative) showing resolution of product (**3**) obtained by approach (i). The peak areas were 402 and 403 mA U at retention time 9.03 and 11.2 min, respectively. Peaks with equal areas indicate racemic nature.

were derivatized with 2,4-DNPH under the solid state conditions using MWI. The DNP moiety of 2,4-dinitrophenyl hydrazine serves as a strong chromophore and a suitable substrate for *inclusion phenomenon* with the chiral material of the α_1-acid glycoprotein (α_1-AGP) column for enantiomeric resolution.

Chromatographic separation data for resolution of the six pairs of enantiomers (**1–6**) in the form of 2,4-DNPHz is given in Table 1. Table 1 shows the values of enantioselectivity, resolution and retention time (in terms of α, R_s and k_1) obtained by using mobile phase, 0.5% 2-propanol in citrate phosphate buffer (10 mM, pH 6.5). Varying buffer concentration above or below 10 mM (pH 6.5) resulted in decrease in enantioselectivity (Fig. 7). The enantiomers were not resolved using only the citrate phosphate buffer (10 mM, pH 6.5) as the mobile phase; a base line resolution of all the six analytes was observed after addition of 2-propanol to it at a level of 0.5%. A further increment (by a value of 0.5% at a time) in the concentration of 2-propanol up to 3% caused a decrease in resolution. 2-Propanol was found to be a better organic modifier in comparison to MeCN as lower enantioselectivity and resolution (α and R_s) and higher retention time were obtained by using MeCN in the mobile phase. Increment in the pH of mobile phase (by a value of 0.5 at a time in the range of 3.5 to 6.5) resulted in increase of α, R_s and k_1 for all the six pairs of enantiomeric hydrazones; thus finally pH 6.5 was found to be the best (Fig. 8).

3.2. Separation mechanism

Over the pH range 3.5 to 6.5, AGP bears a net negative charge. Electrostatic interactions along with hydrogen bonding play

Table 1 Chromatographic data for direct resolution of six pairs of enantiomers in the form of 2,4-DNPHz of racemic aldehydes and ketones[a]

2,4-DNPHz of racemic aldehydes and ketones																	
(**1**)			(**2**)			(**3**)			(**4**)			(**5**)			(**6**)		
k_1	α	R_s	k_1	α	R_s	k_1	α	R_s	k_1	α	R_s	k_1	α	R_s	k_1	α	R_s
5.60	1.42	4.31	5.56	1.56	5.82	5.45	1.44	4.43	5.66	1.46	4.84	5.80	1.48	5.10	5.84	1.49	5.21

[a] Chromatographic conditions: column, α_1-AGP (L × I.D. 10 cm × 4 mm, 5 μm particle size), mobile phase, 0.5% 2-propanol in citrate phosphate buffer (10 mM, pH 6.5); flow rate, 1.0 mL min^{-1}; detection at 365 nm; k_1, retention factor of first eluting enantiomer; α, separation factor; R_s, resolution. (**1–6**) represent 2,4-DNPHz of chiral aldehydes and ketones as mentioned in experimental (Section-2.3). The data presented in the table are the mean values of three independent experiments. 2,4-DNPHz synthesized by approach (i).

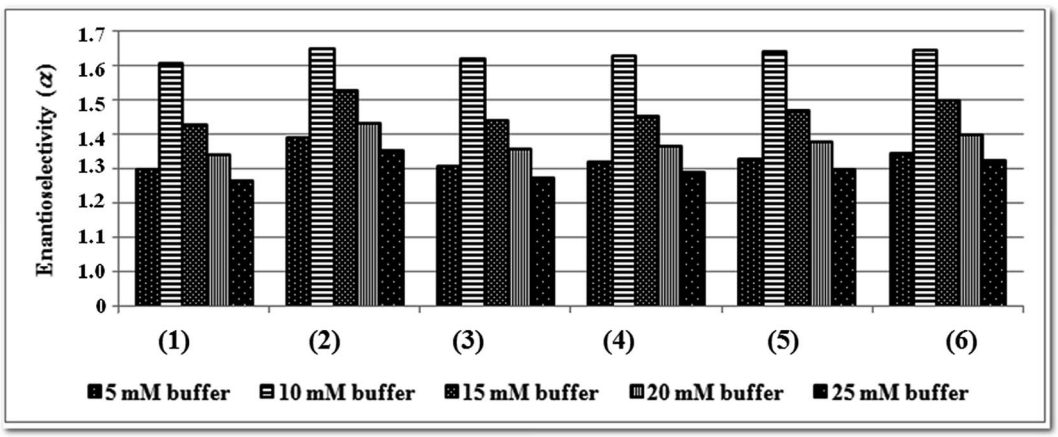

Fig. 7 Effect of buffer concentration (in the range 5–25 mM) in mobile phase consisting 0.5% 2-propanol at pH 6.5, on enantioselectivity.

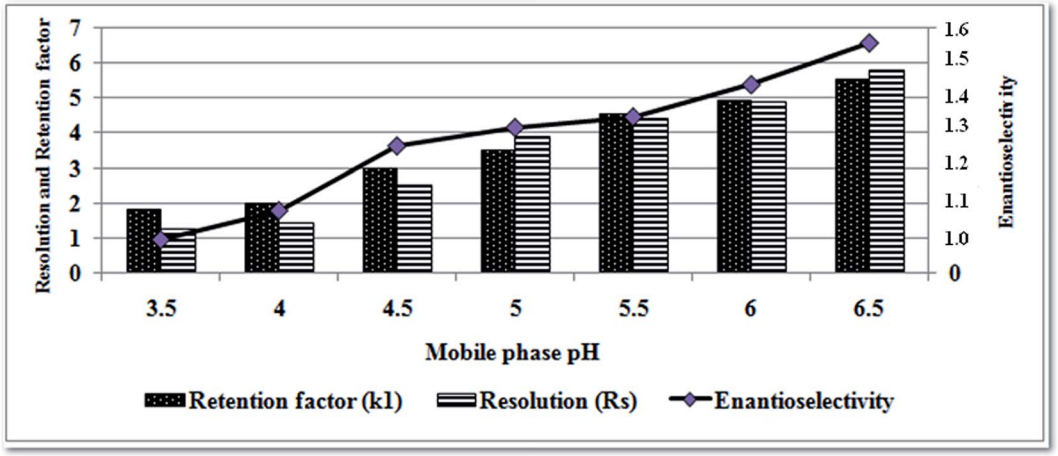

Fig. 8 Effect of pH of mobile phase (in the range 3.5–6.5) on enantioselectivity, resolution and retention times (in terms of α, R_s and k_1, respectively) for product (**2**) using mobile phase 0.5% 2-propanol in 10 mM citrate phosphate buffer at pH 6.5.

important role in the chiral discrimination on an AGP column.[21] Effect of change of pH on enantioresolution of the analytes using AGP column (as noted above) can be attributed to the involvement of coulombic interactions between the analytes and the immobilized protein as the overall charge of the protein and potential conformational changes are pH dependent.[22,23] Lowering of pH from 6.5 to 3.5 caused a decrease in the net negative charge of the protein that resulted in a reduced electrostatic attraction of cationic dinitrophenyl hydrazones with the immobilized protein resulting in decrease of retention time, enantioselectivity and resolution (Fig. 8).

AGP is also able to bind a variety of hydrophobic compounds due to interactions with an apolar cavity formed by the folding of the secondary structure of AGP.[24] DNP moiety serves as a strong chromophore and is also a suitable substrate for inclusion phenomenon with the chiral material of the AGP column for enantiomeric resolution. The baseline resolution achieved in presence of 2-propanol at a concentration of 0.5% can be attributed to the reversible changes in the secondary structure of immobilized protein; further increment of 2-propanol makes the mobile phase less polar and may cause reduction of hydrophobic interactions between the enantiomers and protein-based CSP followed by lowering of retention times and enantioselectivity.[25] In conclusion, hydrogen bonding, inclusion phenomenon and ionic interactions and/or reversible changes in the protein conformation are held responsible as the main factors for enantiomeric separation of the said dinitrophenyl hydrazones on AGP column.

4. Conclusion

The paper presents an efficient methodology for synthesis of 2,4-DNPHz of racemic (or enantiomerically pure) carbonyls under solid phase MWI conditions without spontaneous inversion of configuration. The method provided high yields (91–95%) in short reaction time (4–6 min). The method is successful in introducing a chromophore for on-line detection. The experimental results confirmed that there was no configurational inversion of any of the chiral carbonyl compounds. The study is an important step not only for derivatization of enantiomeric carbonyls without spontaneous inversion of configuration (during synthesis) but also for direct enantioresolution of several chiral carbonyl compounds *via* introducing an achiral chromophore.

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References

1 J. Buckingham, *Q. Rev., Chem. Soc.*, 1969, **23**, 37.
2 R. Bhushan and R. Kumar, *J. Chromatogr. A*, 2009, **1216**, 7941.
3 M. Laemmerhofer, *J. Chromatogr. A*, 2010, **1217**, 814.
4 F. Sanger, *Biochem. J.*, 1945, **39**, 507.
5 P. Marfey, *Carlsberg Res. Commun.*, 1984, **49**, 591.
6 R. Bhushan and H. Brückner, *Amino Acids*, 2004, **27**, 231.
7 R. Bhushan and H. Brückner, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2011, **879**, 3148.
8 R. Bhushan and V. Kumar, *J. Chromatogr. A*, 2008, **1190**, 86.
9 M. H. Hyun, Y. W. Park and I. K. Baik, *Tetrahedron Lett.*, 1988, **29**, 4735.
10 E. V. Dehmlow and C. Sauerbier, *Z. Naturforschung, B: Chem. Sci.*, 1989, **44**, 240.
11 D. V. Johnson and I. W. Wainer, *Chirality*, 1996, **8**, 551.
12 K. Oguni, H. Oda and A. Ichida, *J. Chromatogr. A*, 1995, **694**, 91.
13 Y. H. Gong, G. P. Xue, J. S. Bradshaw, M. L. Lee and H. K. Lee, *J. Heterocycl. Chem.*, 2001, **38**, 1317.
14 D. W. Armstrong, Y. I. Han and S. M. Han, *Anal. Chim. Acta*, 1988, **208**, 275.

15 F. Leonelli, B. Garofalo, L. M. Migneco, R. Marini Bettolo, F. Colais and M. Sinibaldi, *J. Liq. Chromatogr. Relat. Technol.*, 2003, **26**, 409.
16 Y. Bi, J. Yang, X. Lu, T. Shao, J. Dong and F. Li, *J. Sep. Sci.*, 2007, **30**, 1839.
17 N. Grobuschek, L. Sriphong, M. G. Schmid, T. Lorand, H. Y. Aboul-Enein and G. Gübitz, *J. Biochem. Biophys. Methods*, 2002, **53**, 25.
18 *Methods Enzymol., Guide to Protein Purification*, ed. M. P. Deutscher, Academic Press, San Diego, 1990, vol. 182, p. 32.
19 H. Meislich, H. Nechamkin and J. Sharefkin, *Theory and Problems of Organic Chemistry*, McGraw Hill International Book company, Singapore, 1983, p. 259.
20 A. I. Vogel, *Text Book of Practical Organic Chemistry*, ELBS and Longman group limited London, 1975, vol. 3, p. 721.
21 J. Hermansson and A. Grahn, *J. Chromatogr. A*, 1995, **694**, 57.
22 S. Song, L. Zhou, R. Thompson, M. Yang, D. Ellison and J. M. Wyvratt, *J. Chromatogr. A*, 2002, **959**, 299.
23 G. Massolini, E. de Lorenzi, E. Calleri, E. Tabolotti and G. Caccialanza, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2000, **738**, 343.
24 A. Rojo-Dominguez and A. Hernandez-Arana, Protein Seq, *Data Anal.*, 1993, **5**, 349.
25 J. Hermansson, *Trends Anal. Chem.*, 1989, **8**, 251.