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TETRAHEDRON: *ASYMMETRY*

Synthesis of (*S*)- and (*R*)-enantiomers of *p*-(4-hydroxybenzoyl)phenylalanine, useful photoaffinity labeling probes for peptide–protein binding sites

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Abstract

A high yield, multigram synthesis of both enantiomers of *p*-(4-hydroxybenzoyl)phenylalanine, cross-linking probes for peptide–protein binding interactions, is reported. The synthesis was accomplished using Schöllkopf chiral auxiliary reagents.

1. Introduction

Biologically active ligands containing a photoreactive and a radiolabeling moiety have proved to be highly valuable probes for many ligand-receptor interactions.[1] These photoaffinity labeling agents, in addition to having one or more radioactive atoms, possess a photosensitive organic azide, diazo ester, or diazirine moiety. On exposure of the ligand-receptor complex to light, the ligand component generates highly reactive carbene or nitrene intermediates that, in turn, can irreversibly alkylate the receptor. This allows an investigator to elucidate the primary structure of the receptor binding sites. A major drawback of these highly reactive probes is their non-specific attack on water molecules and other non-target macromolecules. Following a discovery of photoactive benzophenones,[2] which on photolysis give rise to a much milder reactive intermediate carbonyl triplet diradical, Kauer et al. reported the synthesis of a novel stable benzophenone, 4-benzoyl-L-phenylalanine (BPA).[3a] Though BPA can be obtained in tritiated form,[3b] it was not subject to radioiodination and Edman-sequencing. Recently, Wilson[4] et al. described the synthesis and use of (±)-*p*-(4-hydroxybenzoyl)phenylalanine (*rac*)-HBPA (**1**, Scheme 1), a racemic analog of BPA, which not only has the gentle attributes of BPA but also is amenable to radioiodination. The incorporation of the *rac*-HBPA into a peptide nevertheless necessitates the inconvenient separation and stereocharacterization of the two diastereomers of the

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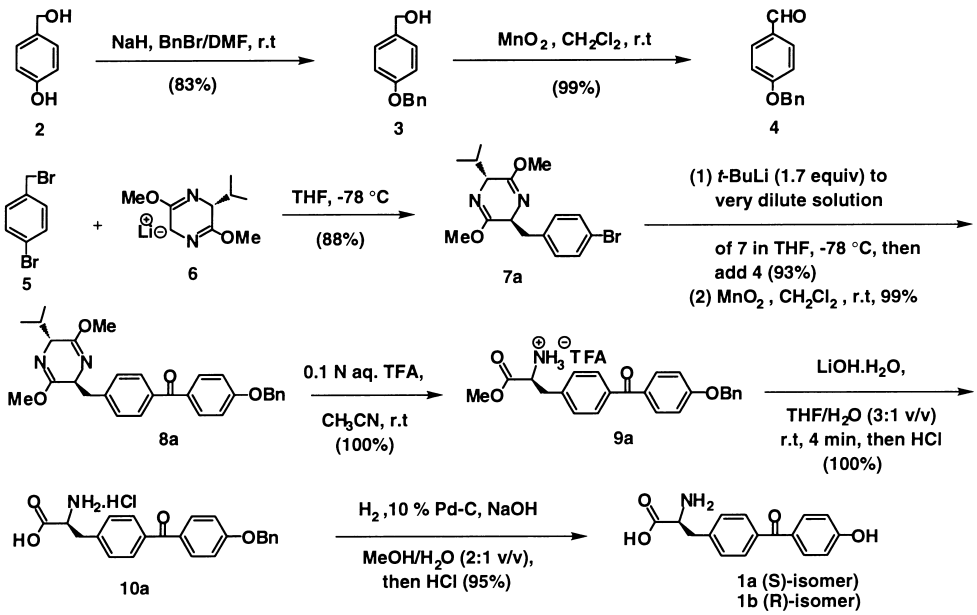
synthetic peptide ligands. As an ongoing part of our studies for exploring the structure of the binding pocket of the neurotensin receptor, we required incorporation of enantiomerically pure (*S*)-HBPA into several novel neurotensin peptide ligands. Reported herein is a simple, high yield synthesis of pure *S*- and *R*-enantiomers of HBPA for general use as a combined photophore and radiolabel for probing peptide–protein interactions.



Scheme 1.

2. Results and discussion

The entire synthesis of the (*S*)- and (*R*)-HBPA was carried out in a straightforward manner using the commercially available enantiomeric bislactim ethers **6**[5] (Scheme 1). Thus, for the synthesis of (*S*)-HBPA, *p*-bromobenzyl bromide (**5**) was coupled to the carbanion derived from the commercially available (*R*)-Schöllkopf reagent, (2*R*)-2-isopropyl-3,6-dimethoxy-2,5-dihydropyrazine (**6**), to give (*S*,*R*)-bislactim ether **7a**,[6] which was obtained as a single diastereomer (100% de) as indicated by the NMR and TLC analyses of the crude isolate after work-up. Halogen–metal exchange of **7a** at −78°C followed by the addition of *p*-benzyloxybenzaldehyde (PBB, obtained in 82% overall yield by (i) selective benzylation of 4-hydroxybenzyl alcohol (**2**) to give benzyl alcohol **3**, and (ii) MnO_2 oxidation of **3**) gave a mixture of diastereomeric benzhydryl alcohols, which, after purification, could be readily oxidized to the benzophenone **8a**. Mild acid hydrolysis of **8a** to the aminoester **9a** was followed by a very rapid saponification in commercial THF with a 1 molar excess of LiOH in H_2O/THF to give *O*-benzylated amino acid **10a**. The remarkably efficient saponification of the ester **9a** may have been due to trace amounts of H_2O_2, a possible contaminant in commercial, stabilized THF. This may be a useful solvent to use for minimizing risk of racemization in sensitive cases. The catalytic hydrogenolysis had to be conducted in 1 molar excess of NaOH to avoid concomitant overreduction of the ketone function which was a major problem with our use of several acidic or neutral solvent media. Thus, starting from *p*-benzyloxybenzaldehyde (**4**) and *p*-bromobenzyl bromide (**5**), pure (*S*)-HBPA (**1a**)[6] ($[\alpha]_D^{25}=-5.5$ (*c*=2.0 mg/ml, MeOH:H_2O=3:1 by volume) was obtained in an overall yield of 73%. Repetition of the above

procedure using (*S*)-bislactim ether reagent (**6b**) in the key Schöllkopf coupling furnished pure (*R*)-HBPA (**1b**)[6] $[\alpha]_D^{25}$=+5.1 (*c*=4.6 mg/ml, MeOH:H_2O=3:1 by volume) in a similar yield. These protocols furnished multigram lots, of both the enantiomers in a matter of days.

In summary, a highly practical synthesis of pure (L)- and (D)-HBPA (**1a**,**b**) is reported. Using this method, it is possible to quickly make multigram quantities of these valuable photoaffinity probes. The salient features of the synthesis are: (i) conciseness and high overall yield (73%) for both the enantiomers of **1**; (ii) essentially 100% diastereoselectivity in the key Schöllkopf coupling; (iii) crucial requirement of a base during the debenzylation of the compounds **10** to avoid overreduction; and (iv) extremely fast saponification of chiral aminoesters **9** using commercial, unpurified THF which might constitute a valuable procedure in highly racemization-prone cases.

3. Experimental section

3.1. General methods

Melting points were taken with a GallenKamp instrument and are uncorrected. The column chromatographic separations were performed with J. T. Baker Silica gel (230–400 mesh). THF and diethyl ether were distilled from sodium/benzophenone ketyl. Anhydrous DMF was purchased from Aldrich Chemicals. Methylene chloride was distilled over P_2O_5. Acetonitrile was reagent grade from E. M. SCIENCE and was used without further purification. Ethyl acetate and hexane were distilled prior to use. The purity of all reported compounds was shown to be >95% by TLC and high field ^{1}H NMR and ^{13}C NMR (300 and 75 MHz Brücker instrument). Optical rotations were taken with a 241-Perkin–Elmer polarimeter (Na lamp). IR spectra were measured with a 2020 GALAXY Series FT-IR instrument, from Mattson Instruments. Mass spectral analyses were performed at the Mayo Clinic Rochester Mass Spectroscopy Facility, Rochester, Minnesota. The high resolution MS and elemental analysis was performed at the Department of Chemistry, University of Florida.

3.2. [4-(Benzyloxy)phenyl]methanol (3)

Sodium hydride (2.93 g, 73 mmol) was added in small portions to a solution of 4-hydroxybenzyl alcohol (10 g, 80.5 mmol) in DMF (220 ml) at 0°C. The mixture was stirred for 1 h at RT before benzyl bromide (8.68 ml, 73 mmol) was added dropwise. The reaction mixture was stirred for 6 h, then DMF was removed by distillation under vacuum. The residue was taken up in methylene chloride (200 ml) and washed once with water. The organic layer was dried and concentrated in vacuum. The residue was purified by chomatography on silica gel (ethyl acetate:hexane=1:2 by volume, R_f=0.41) to give 12.86 g (83%) of alcohol **3** as a white solid: mp 85–86°C. ^{1}H NMR ($CDCl_3$) δ 7.40–7.20 (m, 7H), 6.92 (d, *J*=6.5 Hz, 2H), 5.02 (s, 2H), 4.55 (d, *J*=5.0 Hz, 2H), 1.64 (t, OH); ^{13}C NMR ($CDCl_3$) δ 138.9, 133.4, 128.8, 128.5, 127.9, 127.4, 115.0, 114.9, 70.0, 65.0; IR (neat, cm^{-1}) 3396, 2930.

3.3. 4-(Benzyloxy)benzaldehyde (4)

Manganese dioxide (41 g, 471.6 mmol) is added in one portion to a solution of alcohol (**3**) (11.17 g, 52 mmol) in methylene chloride (170 ml). The mixture was stirred at RT for 15 h, then filtered through Celite and concentrated to produce aldehyde **4** (11 g, 99%) as a white powder: mp 70–71°C; R_f=0.79 (ethyl acetate:hexane=1:2 by volume); ^{1}H NMR ($CDCl_3$) δ 9.88 (s, 1H), 7.83 (d, *J*=8.9 Hz, 2H), 7.39

(m, 5H), 7.08 (d, *J*=8.7 Hz, 2H), 5.15 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 190.4, 163.4, 135.7, 129.8, 128.4, 128.0, 127.2, 114.8, 69.8; IR (neat, cm^{-1}) 2744, 1684, 1601; MS (ESI) 213 (M^++1).

*3.4. (2S,5R)-2-(4-Bromobenzyl)-5-isopropyl-3,6-dimethoxy-2,5-dihydropyrazine (**7a**)*

To a solution of (2*R*)-2-isopropyl-3,6-dimethoxy-2,5-dihydropyrazine (7.96 g, 43.2 mmol) in THF (180 ml), is added n-BuLi (33.2 ml, 1.5 M in hexane) at −78°C dropwise. After stirring for 15 min, a solution of the bromide **5** (11.916 g, 47.6 mmol) in THF (20 ml) is added dropwise. The resulting mixture is stirred for 2 h at −78°C. After removal of THF in vacuo, the residue is diluted with methylene chloride (120 ml). The organic layer is washed with 5% sulfuric acid, water, and brine, then dried and concentrated in vacuo. Purification by chomatography on silica gel (ethyl acetate:hexane=1:7 by volume, R_f=0.5) afforded compound **7a** as a single diastereomer (100% de, 13.42 g, 88%) as a white solid: $[\alpha]_D^{25}$=+49.1 (*c*=6.8 mg/ml, CH_2Cl_2); mp 54–55°C; ^{1}H NMR ($CDCl_3$) δ 7.33 (d, *J*=8.3 Hz, 2H), 6.97 (d, *J*=8.3 Hz, 2H), 4.3 (dd, *J*=8.4, 4.7 Hz, 1H), 3.7 (s, 3H), 3.66 (s, 3H), 3.39 (t, 1H), 3.04 (d, *J*=4.8 Hz, 2H), 2.16 (m, 1H), 0.96 (d, *J*=6.8 Hz, 3H), 0.61 (d, *J*=6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 163.6, 162.2, 136.2, 131.4, 130.6, 120.1, 60.1, 56.1, 52.0, 51.8, 39.1, 31.0, 18.8, 16.2; IR (neat, cm^{-1}) 2961, 2870, 1693, 1437, 1238, 1014; MS (ESI) 353 (M^++1) and 355 (M^++1).

The enantiomer of **7a**, i.e., (2*R*,5*S*)-2-(4-bromobenzyl)-5-isopropyl-3,6-dimethoxy-2,5-dihydropyrazine (**7b**) was made in a similar manner using (2*S*)-2-isopropyl-3,6-dimethoxy-2,5-dihydropyrazine (**7b**) as the chiral auxiliary: $[\alpha]_D^{25}$=−50.3 (*c*=6.7 mg/ml, CH_2Cl_2).

3.5. [4-(Benzoyloxy)phenyl](4-{[(2S,5R)-5-isopropyl-3,6-dimethoxy-2,5-dihydro-2-pyrazinyl]methyl}-phenyl)methanols

(Epimeric at the alcholic carbon). *t*-Butyllithium (28 mmol, 1.7 M in pentane) was added dropwise at −78°C to a solution of the bromide **7a** (5.82 g, 16.47 mmol) in distilled THF (350 ml). Five minutes later, a solution of the aldehyde **4** (4.2 g, 19.8 mmol) in distilled THF (60 ml) was slowly cannulated into the reaction mixture. The mixture was stirred for an additional 2 h at the same temperature. A saturated ammonium chloride solution was added and the mixture was warmed to RT. The volatiles were removed in vacuo and the residue is taken up in ethyl acetate and washed once with water. The aqueous layer was extracted twice with ethyl acetate. The combined organic extracts were dried, concentrated, and the residue was purified by chromatography on silica gel to furnish the desired diastereomeric alcohols (7.468 g, 93%) as a colorless oil: $[\alpha]_D^{25}$=+34.7 (*c*=11.8 mg/ml, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.44–7.28 (m, 5H), 7.23 (m, 4H), 7.06 (d, *J*=8.1 Hz, 2H), 6.92 (dt, *J*=2.9 Hz, *J*=8.7, 2.9 Hz, 2H), 5.75 (d, *J*=2.9 Hz, 1H), 5.03 (s, 2H), 4.29 (dd, *J*=8.47, 4.8 Hz, 1H), 3.70 (d, *J*=0.9 Hz, 3H), 3.68 (d, *J*=2.1 Hz, 3H), 3.26 (dd, *J*=6.2, 3.2 Hz, 1H), 3.06 (d, *J*=4.8 Hz, 2H), 2.23 (d, *J*=3.7 Hz, OH), 2.12 (m, 1H), 0.93 (dd, *J*=6.9, 2.3 Hz, 3H), 0.60 (d, *J*=6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 171.1, 163.9, 162.4, 158.2, 141.9, 138.6, 130.0, 128.5, 127.9, 127.4, 125.9, 114.7, 75.5, 70.0, 60.2, 56.5, 52.3, 52.1, 39.6, 31.1, 21.0, 19.0, 16.4, 14.2; IR (neat, cm^{-1}) 3368, 2961, 2870, 1695, 1240, 1016, 736; MS (ESI) 487 (M^++1).

Following the above procedure, the diastereomeric mixture of alcohols of opposite configuration was obtained in a similar yield by coupling **7b** with the aldehyde **4**. $[\alpha]_D^{25}$=−33.4 (*c*=13.0 mg/ml, CH_2Cl_2).

3.6. [4-(Benzoyloxy)phenyl](4-{[(2S,5R)-5-isopropyl-3,6-dimethoxy-2,5-dihydro-2-pyrazinyl]methyl}-phenyl)methanone (**8a**)

Manganese dioxide (26.66 g, 307.15 mmol) was added in one portion at 0°C to a solution of the benzylated alcohol (7.468 g, 15.35 mmol) in methylene chloride (250 ml). The mixture was stirred overnight at RT, then filtered though Celite and concentrated in vacuo to yield 7.43 g (99%) of ketone **8a** as white crystals: R_f=0.56 (ethyl acetate:hexane=1:4 by volume); $[\alpha]_D^{25}$=+31.8 (*c*=4.2 mg/ml, CH_2Cl_2); mp 100–101°C; ^{1}H NMR ($CDCl_3$) δ 7.79 (d, *J*=6.8 Hz, 2H), 7.63 (d, *J*=8.2 Hz, 2H), 7.46–7.34 (m, 5H), 7.22 (d, *J*=8.2 Hz, 2H), 7.03 (d, *J*=6.8 Hz, 2H), 5.14 (s, 2H), 4.36 (dd, *J*=8.8, 5.0 Hz, 1H), 3.72 (s, 3H), 3.67 (s, 3H), 3.42 (t, *J*=3.5 Hz, 1H), 3.17 (d, *J*=5.4 Hz, 2H), 2.17 (m, 1H), 0.96 (d, *J*=6.9 Hz, 3H), 0.62 (d, *J*=6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 195.3, 163.9, 162.1, 142.2, 136.2, 136.0, 132.3, 130.5, 129.7, 129.4, 128.6, 128.1, 127.4, 114.2, 70.1, 60.3, 56.3, 52.3, 52.1, 39.9, 31.3, 18.9, 16.4; IR (neat, cm^{-1}) 2943, 1695, 1601, 1242, 1014; MS (ESI) 485 (M^++1).

Following the above procedure, a ketone of the opposite configuration, **8b**, was obtained in a similar yield. $[\alpha]_D^{25}$=−29.4 (*c*=4.7 mg/ml, CH_2Cl_2).

3.7. Methyl (2S)-2-amino-3-{4-[4-(benzyloxy)benzoyl]phenyl}propanoate (**9a**)

Trifluoroacetic acid (TFA, 45.9 mmol, 0.1 N in water, 3 equiv.) is added to a solution of the compound **8a** (7.43 g, 15.35 mmol) in a mixture of methylene chloride (30 ml) and acetonitrile (300 ml). The resulting mixture was stirred at RT for 3 h. The acetonitrile and TFA were removed in vacuo to produce a suspension of the methyl ester **9a** and (D)-valine methyl ester as TFA salts in water. This suspension, without neutralization with base, was then extracted 5 times with methylene chloride. The combined organic phase was dried and concentrated in vacuo to give 5.91 g (99%) of the amino ester **9a** as a white powder. This procedure effectively eliminated the TFA salt of (D)-valine methyl ester which remained in the aqueous phase: R_f (free amine)=0.32 (ethyl acetate); $[\alpha]_D^{25}$ (free amine)=+5.0 (*c*=10.2 mg/ml, MeOH); mp 102–103°C; ^{1}H NMR ($CDCl_3$, free amine) δ 7.81 (d, *J*=8.6 Hz, 2H), 7.72 (d, *J*=8.1 Hz, 2H), 7.46–7.29 (m, 5H), 7.03 (d, *J*=8.9 Hz, 2H), 5.12 (s, 2H), 3.78 (dd, *J*=7.8, 5.2 Hz, 1H), 3.73 (s, 3H), 3.15 (dd, *J*=13.5, 5.2 Hz, 1H), 2.95 (dd, *J*=13.5, 7.9 Hz, 1H), 1.54 (br, NH_2); ^{13}C NMR ($CDCl_3$, free amine) δ 195.0, 175.2, 162.3, 136.7, 136.0, 132.4, 130.4, 130.1, 129.1, 128.6, 128.0, 127.4, 114.4, 70.13, 55.6, 52.1, 40.9; IR (free amine, neat, cm^{-1}) 1738, 1649, 1599, 1251; MS (ESI) 390 (M^++1).

Following the above procedure, methyl ester of the opposite configuration, **9b**, was obtained in a similar yield by coupling **7b** with the aldehyde **4**.

3.8. (2S)-2-Amino-3{4-[4-(benzyloxy)benzoyl]phenyl}propanoic acid (**10a**)

Methyl (2*S*)-2-amino-3-{4-[4-(benzyloxy)benzoyl]phenyl}propanoate (**9a**) (0.83 g, 1.65 mmol) was dissolved in 60 ml of THF. An aqueous solution of $LiOH\cdot H_2O$ (189 mg, 4.95 mmol) in 30 ml of water was added at RT and the mixture stirred for 4–5 min. Following neutralization of the mixture with 6 N HCl to pH 3, the THF was evaporated in vacuo. A white precipitate of the amino acid–HCl salt appeared, on concentration, that was cooled to 0°C for 1 h, then filtered, washed with chilled water (×2) and ether (×3), and dried under high vacuum over P_2O_5 overnight (yield=674 mg, 99.6%): ^{1}H NMR (DMSO-d_6) δ 7.74 (d, *J*=8.7 Hz, 2H), 7.62 (d, *J*=7.8 Hz, 2H), 7.49–7.35 (m, 7H), 7.17 (d, *J*=8.7 Hz, 2H), 5.21 (s, 2H), 3.54 (dd, *J*=8.0, 5.0 Hz, 1H), 3.24 (dd, *J*=14.0, 4.23 Hz, 1H), 2.99 (dd, *J*=14.0, 8.0 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 194.2, 168.2, 162.0, 141.9, 139.2, 136.4, 136.1, 132.1, 129.4, 128.5, 128.1, 127.9, 124.9, 114.7, 69.6, 34.4, 30.4; IR (NaCl plate, cm^{-1}) 3431, 1741, 1599, 1249; MS (ESI) 376 (M^++1).

3.9. (S)-p-(4-Hydroxybenzoyl)phenylalanine (**1a**)

The benzyl group was removed by hydrogenalizing protected amino acid **10a** (608 mg, 1.48 mmol suspended in 25 ml of water) in the presence of NaOH (118 mg, 2.95 mmol) and 10% Pd–C (152 mg) at RT and atmospheric pressure for 1.5 h in the dark. After filtration through Celite, the aqueous solution was acidified to pH 3 with 6 N HCl. Charcoal (0.5 g) was added and the mixture was heated to 60°C for 5 min. Filtration and lyophilization gave a slightly off-colored powder which was quickly washed once with chilled water and dried over P_2O_5 overnight (yield=455 mg, 95%). This product was suitable for conversion to the fluorenylmethoxycarbonyl (Fmoc) group for the solid phase synthesis of peptides. An analytical sample of **1a** (TFA salt) was obtained by HPLC purification (RT=21.5 min on a Vydac column, 22 mm×250 mm, C-8, 10 μ particle size; gradient elution with 5% B to 100% B in 50 min at 8.0 ml flow rate; UV detection at λ=254 nm): $[\alpha]_D^{25}=-5.5$ (*c*=2.0 mg/ml, MeOH:H_2O=3:1 by volume); ^{1}H NMR (as TFA salt, D_2O) 7.72 (d, *J*=8.7 Hz, 2H), 7.69 (d, *J*=8.1 Hz, 2H), 7.42 (d, *J*=8.1 Hz, 2H), 6.93 (d, *J*=8.7 Hz, 2H), 4.27 (t, *J*=7.2 Hz, 1H), 3.39 (dd, *J*=14.3, 5.73 Hz, 1H), 3.73 (s, 3H), 3.26 (dd, *J*=14.5, 7.7 Hz, 1H); ^{13}C NMR (H_2O, as disodium salt, pH 10) δ 201.3, 186.7, 177.6, 145.0, 139.4, 137.3, 132.1, 131.6, 124.5, 121.5, 59.8, 43.3; IR (NaCl plate, cm^{-1}) 3431, 3317, 1645, 1602, 1249; MS (ESI) 286 (M^++1). HRMS *m/z* calcd for $C_{16}H_{16}NO_4$ (M^++1) 286.1079, found 286.1079. Anal. calcd for $C_{16}H_{17}NO_5$ (**1a**, as monohydrate): C=63.36; H=5.65; N=4.62. Found: C=63.88; H=5.53; N=4.68.

3.10. (R)-p-(4-Hydroxybenzoyl)phenylalanine (**1b**)

Compound **1b** was obtained by hydrogenolysis of **10b**. The spectral data of **1b** were identical to **1a**: $[\alpha]_D^{25}=+5.1$ (*c*=4.6 mg/ml, CH_2Cl_2). Anal. calcd for $C_{16}H_{17}NO_5$ (**1a**, as monohydrate): C=63.36; H=5.65; N=4.62. Found: C=63.60; H=5.58; N=4.67.

Acknowledgements

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