New Journal of Chemistry

Supplementary Information

Synthesis, *in vitro* anti-plasmodial potency, *in silico* cum SPR binding with inhibition of *Pf*Pyridoxal synthase and rapid parasiticidal action by 3,5-Bis {(E) arylidene}-N-methyl-4-piperidones

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$ Both authors contributed equally

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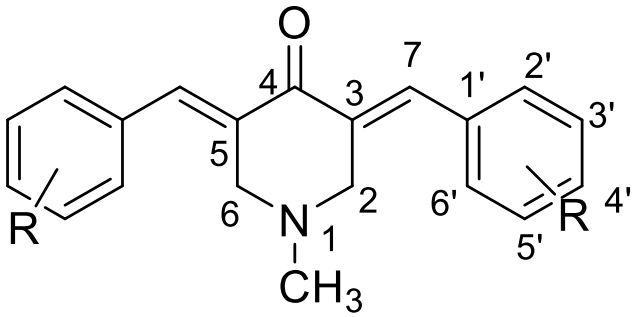

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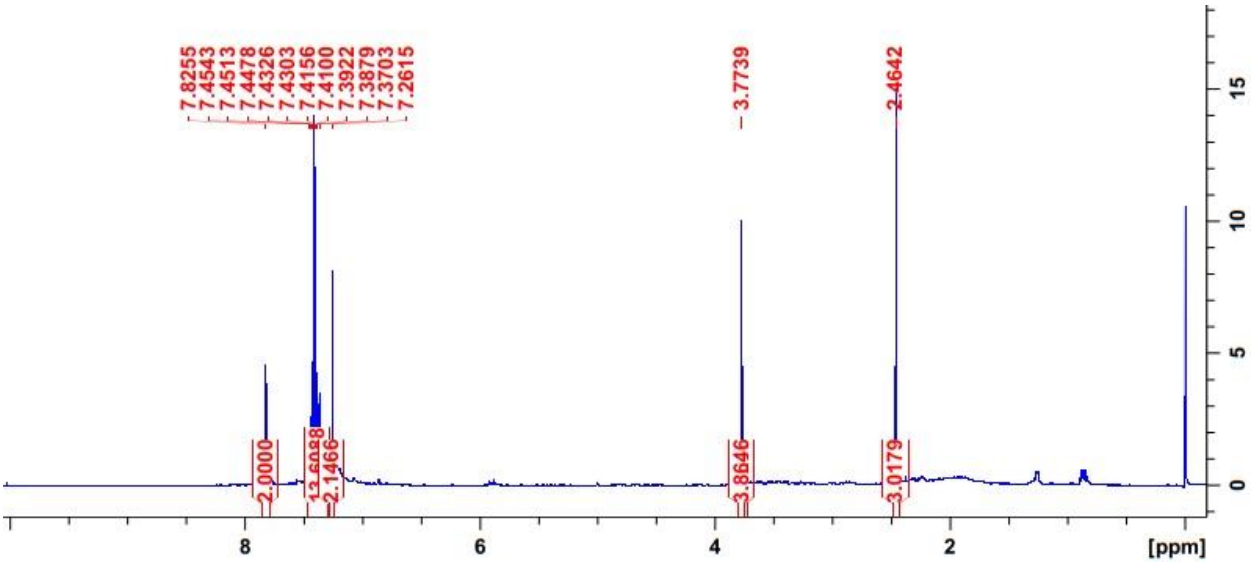
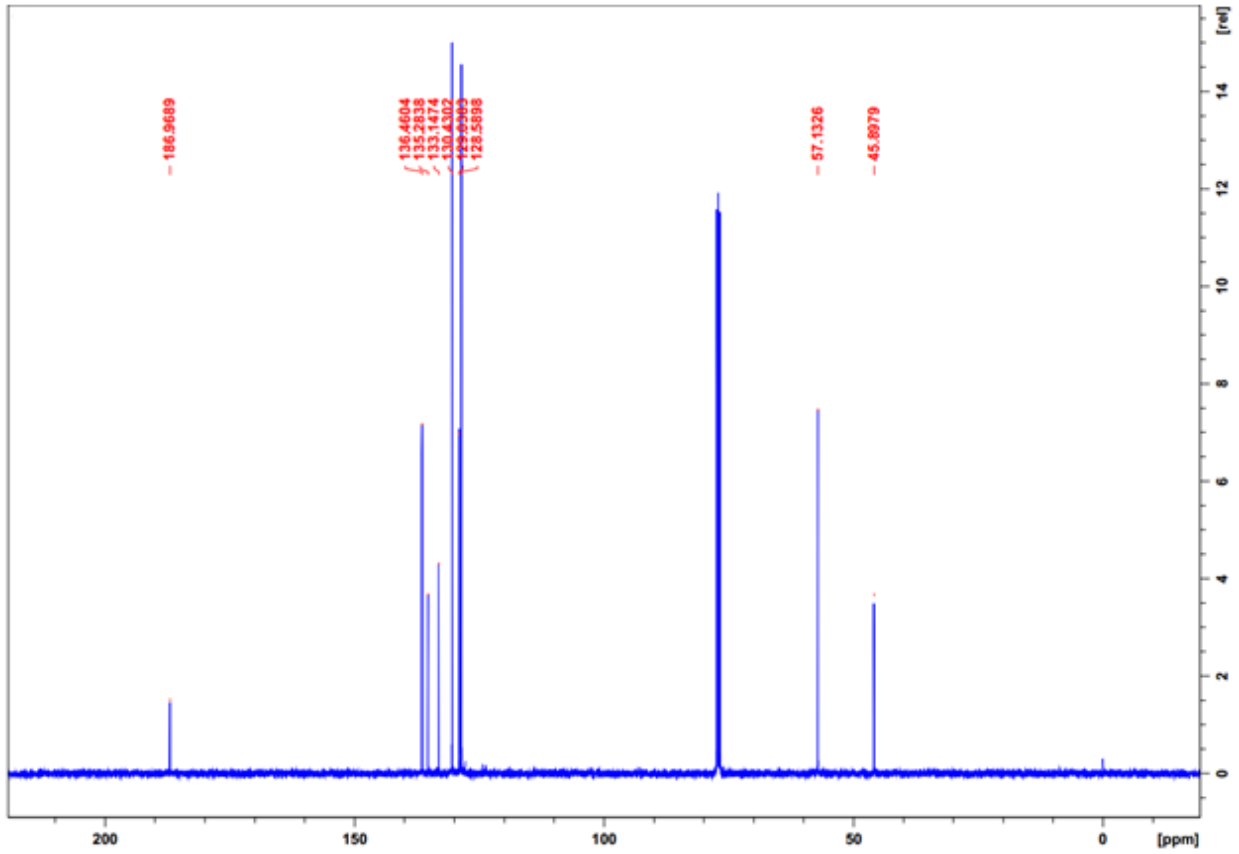
Ia) 3,5-Bis (dibenzylidene)-N-methyl-4-piperidone:

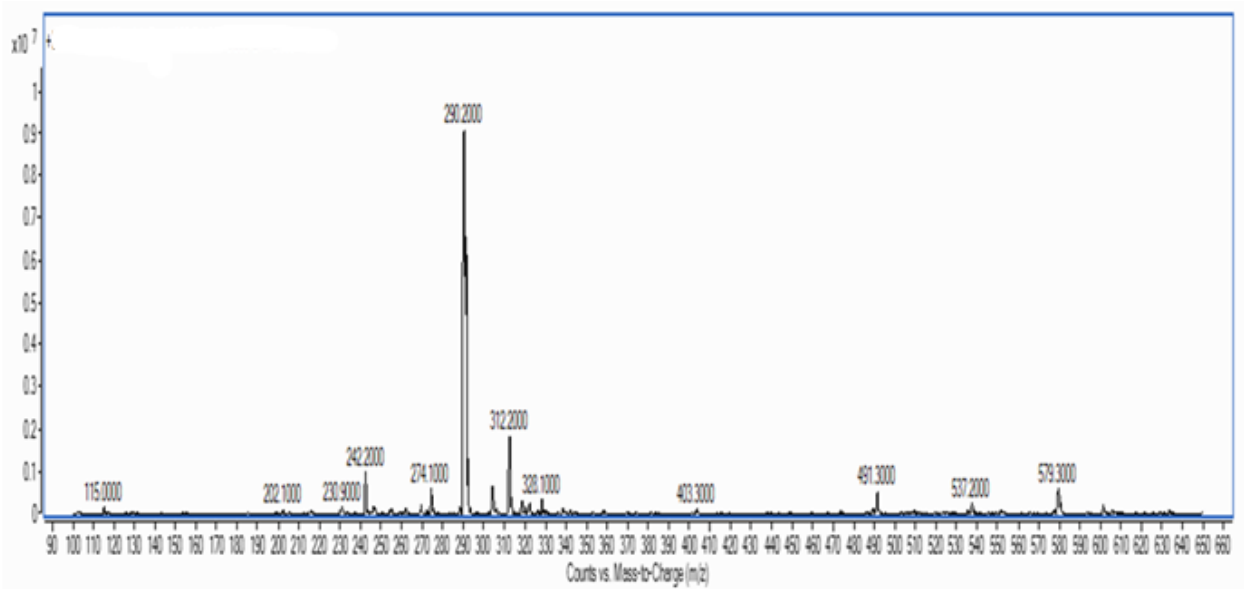
Yield: 88.9 %. Light yellow crystals. Recrystallized from pet ether and chloroform. mp: 111.5-113^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82 (H-7, 2H, s), δ 7.45 to 7.37 (Ar-H, m), δ 3.76 (H-2, H-6, 4H, s), δ 2.45, (N-CH_3, 3H, s). ^{13}C NMR (400 MHz, $CDCl_3$): δ 186.96 (C-4), δ 136.46 (C-5), δ 135.28 (C-7), δ 133.03(C-1'), δ 130.43 (C-4'), δ 129.03 (C-2$^{'}$ and 6$^{'}$), δ 128.58 (C-3$^{'}$ and C-5$^{'}$), δ 57.1 (C-6) and δ 45.89 (N-C); IR (KBr disc) (cm^{-1}): 3014 (sp^2 C-H stretch), 2880 (sp^3 C-H stretch), 1670 (C=O stretch), 1618 (C=C stretch), 1588 and 1572(Ar skeletal bands), 764 and 693 (mono substituted aromatic ring); Mass: $[M+H]^+$ = m/z 290.2000; UV (MeOH) λmax (nm): 328 and 231.



SI-Figure 1: ^{1}H NMR spectrum of 3,5-Bis (dibenzylidene)-N-methyl-4-piperidone.



SI-Figure 2: ^{13}C NMR spectrum of 3,5-Bis (dibenzylidene)-N-methyl-4-piperidone.

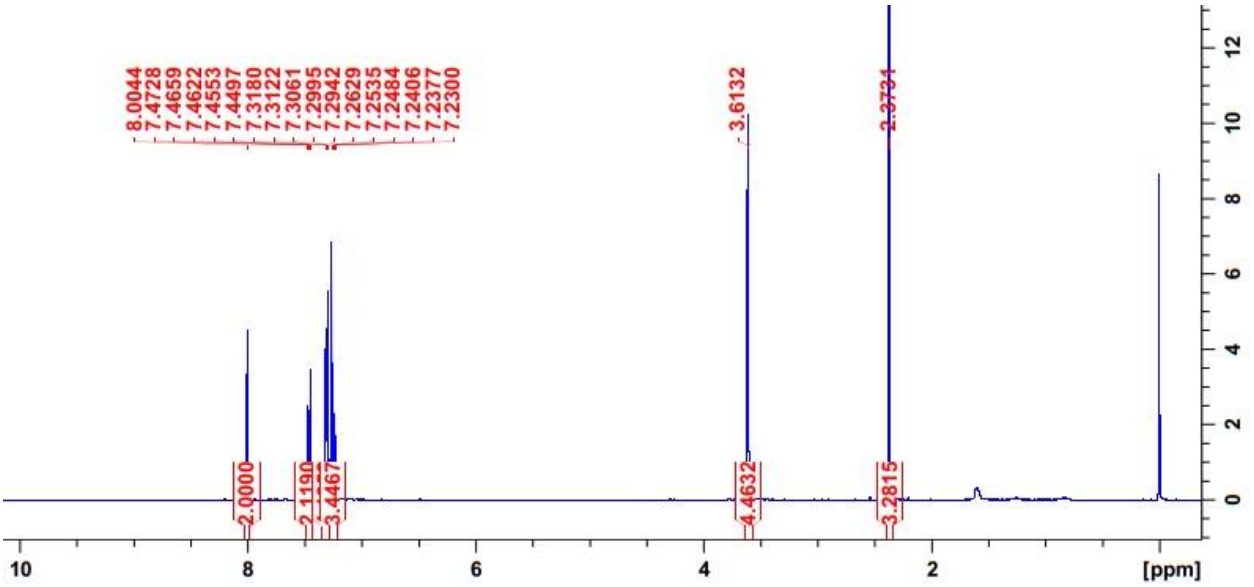


Mass: $[M+H]^+$ = m/z 290.2000

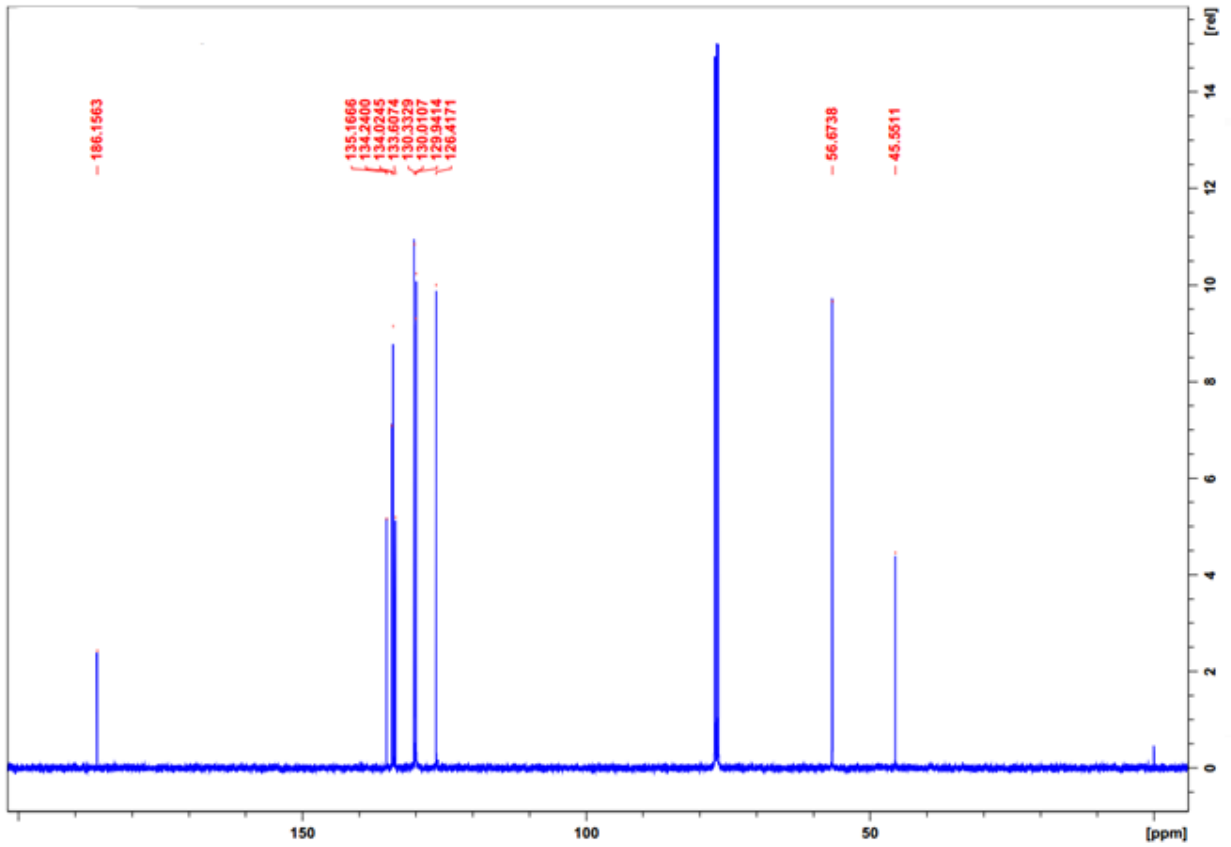
SI-Figure 3: Mass spectrum of 3,5-Bis (dibenzylidene)-N-methyl-4-piperidone.

Ib) 3,5-Bis ((E)-2-Chlorobenzylidene)-N-methyl-4-piperidone

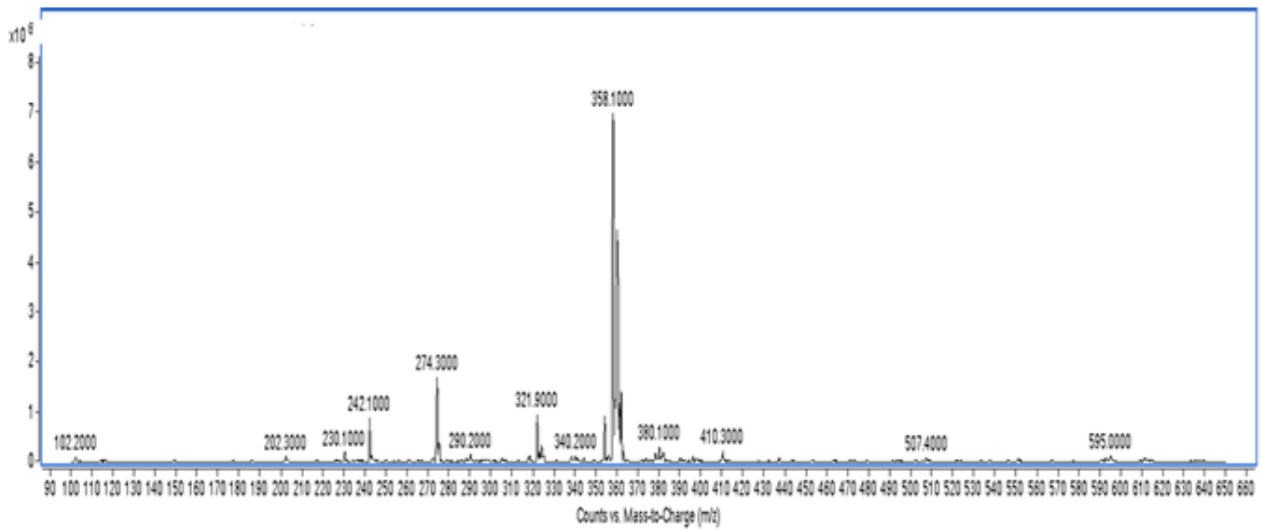
Yield: 81.4%. Light yellow crystals. Recrystallized from chloroform-methanol. mp: 145-147.8^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.00 (H-7, 2H, s), δ 7.45 (H-2', 2H, overlapping signals), δ 7.31 (H-6', 2H, overlapping signals), δ 7.29 (H-5', 2H, overlapping signals), δ 7.24 (H-4', 2H, overlapping signals), δ 3.61 (H-2, H-6, 4H, s), δ 2.37, (N-CH_3, 3H, s). ^{13}C NMR (400 MHz, $CDCl_3$): δ 186.15 (C-4), δ 135.16 (C-2'), δ 134.2 (C-5), δ 134.02 (C-7), δ 133.6 (C-1'), δ 130.33 (C-6'), δ 130.01 (C-4'), δ 129.94 (C-3'), δ 126.41 (C-5'), δ 56.67 (C-6) and δ 45.55 (N-C); IR (KBr disc) (cm^{-1}): 3067 (sp^2 C-H), 2977 (sp^3 C-H), 1675 (C=O), 1623 (C=C), 1588 and 1572 (aromatic skeletal bands), 1053 (C-Cl); Mass: $[M+H]^+$ = m/z 358.1000, $[M+2]^+$ = m/z 360, $[M+4]^+$ = m/z 362; UV (MeOH) λ_{max} (nm): 235 and 314 nm.



SI-Figure 4: ^{1}H NMR spectrum of 3,5-Bis ((E)-2-Chlorobenzylidene)-N-methyl-4-piperidone.



SI-Figure 5: ^{13}C NMR spectrum of 3,5-Bis ((E)-2-Chlorobenzylidene)-N-methyl-4-piperidone.

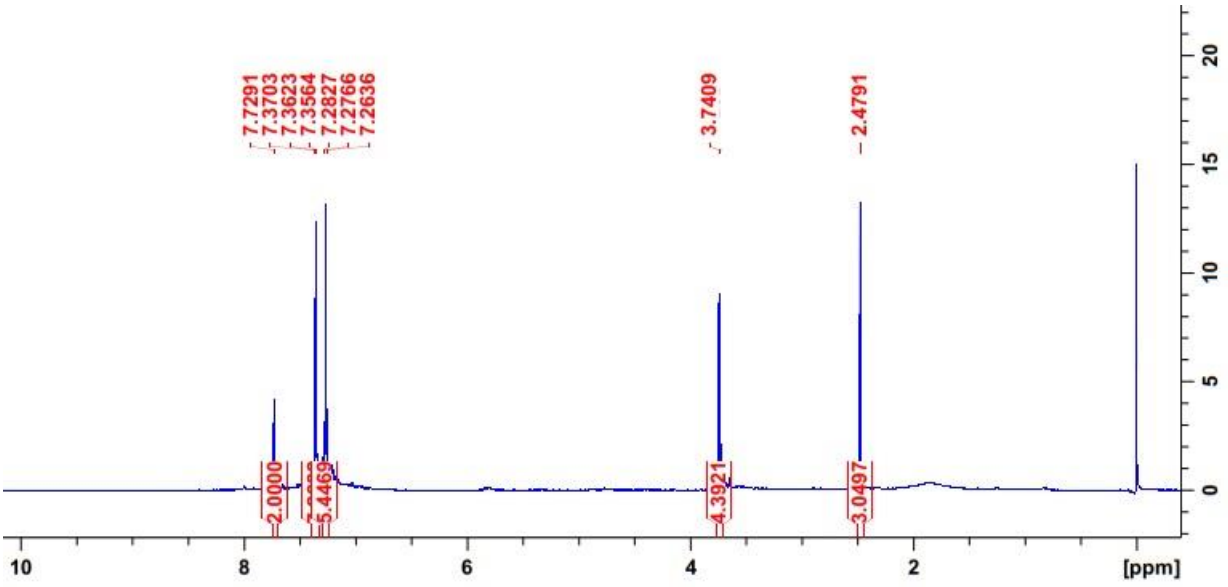


Mass: $[M+H]^+$ = m/z 358.1000

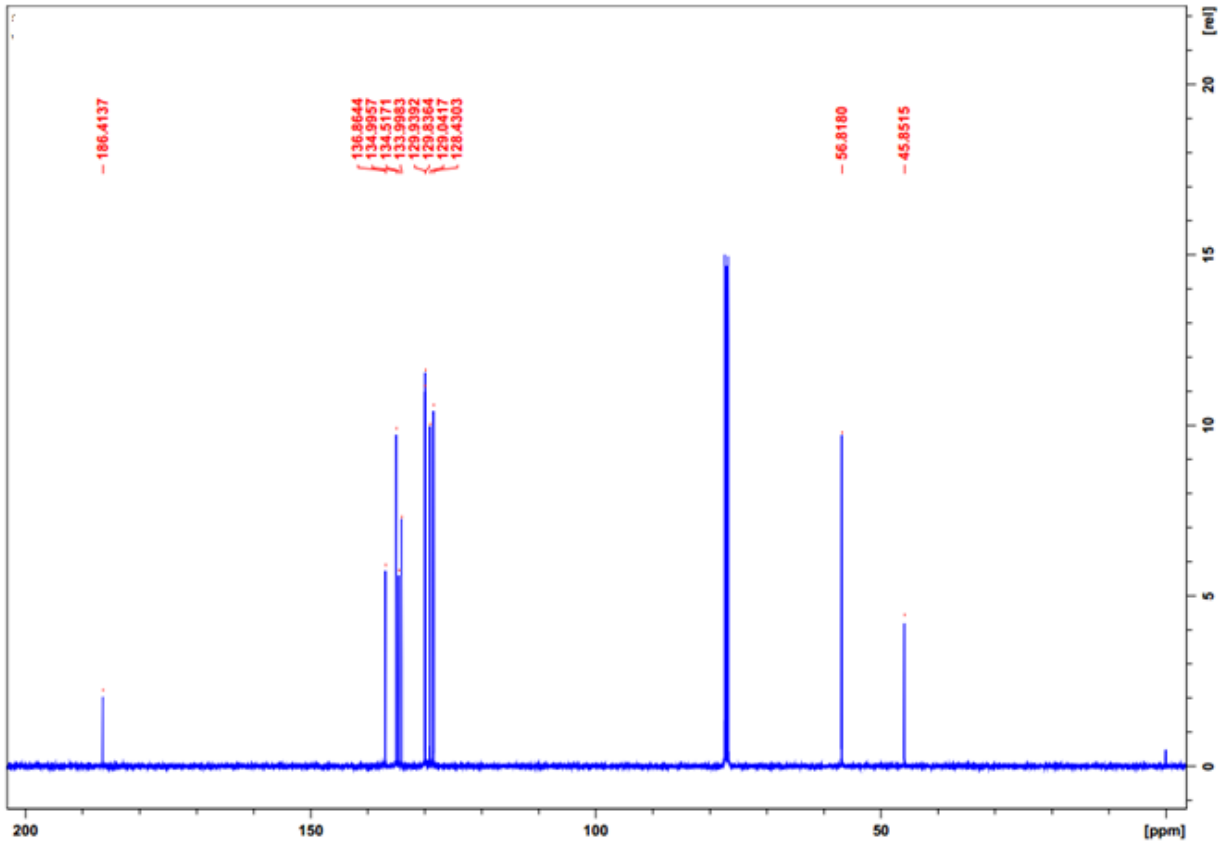
SI-Figure 6: Mass spectrum of 3,5-Bis ((E)-2-Chlorobenzylidene)-N-methyl-4-piperidone.

Ic) 3,5-Bis ((E)-3-Chlorobenzylidene)-N-methyl-4-piperidone

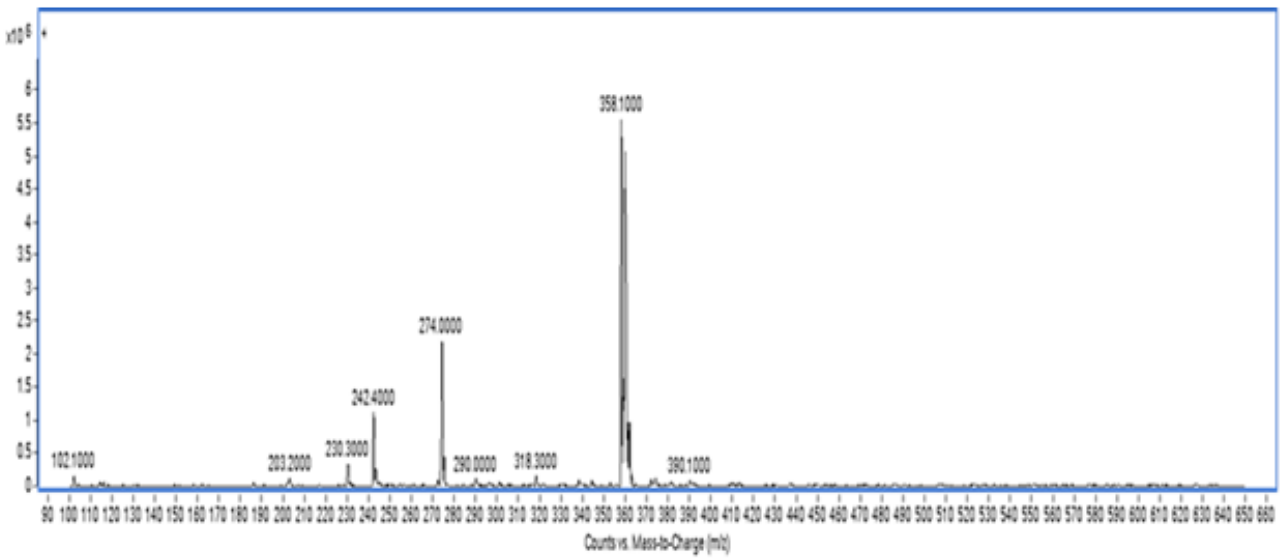
Yield: 78.6%. Yellow crystals. Recrystallized from chloroform-methanol. mp: 172.2-173.6^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72 (H-7, 2H, s), δ 7.38 (H-2', H-4', 4H, overlapping signals), δ 7.35 (H-5', 2H, overlapping signals), δ 7.27 (H-6', 2H, overlapping signals) , δ 3.73 (H-2, H-6, 4H, s), δ 2.47, (N-CH_3, 3H, s). ^{13}C NMR (400 MHz, $CDCl_3$): δ 186.41 (C-4), δ 136.86 (C-3'), δ 134.99 (C-5), δ 134.51 (C-7), δ 133.99 (C-1'), δ 129.93 (C-5'), δ 129.83 (C-2'), δ 129.04 (C-4'), δ 128.43 (C-6') δ 56.81 (C-6) and δ 45.85 (N-C); IR (KBr disc) (cm^{-1}): 3011 (sp^2 CH), 2934 (sp^3 C-H), 1669 (C=O), 1613 (C=C), 1588 and 1562 (aromatic skeletal stretch), 1096 (C-Cl), 782 (meta-substitution); Mass: $[M+H]^+$ = m/z 358.1000, $[M+2]^+$ = m/z 360, $[M+4]^+$ = m/z 362; UV (MeOH) λ_{max} (nm): 233 and 322 nm.



SI-Figure 7: ^{1}H NMR spectrum of 3,5-Bis ((E)-3-Chlorobenzylidene)-N-methyl-4-piperidone.



SI-Figure 8: ^{13}C NMR spectrum of 3,5-Bis ((E)-3-Chlorobenzylidene)-N-methyl-4-piperidone.

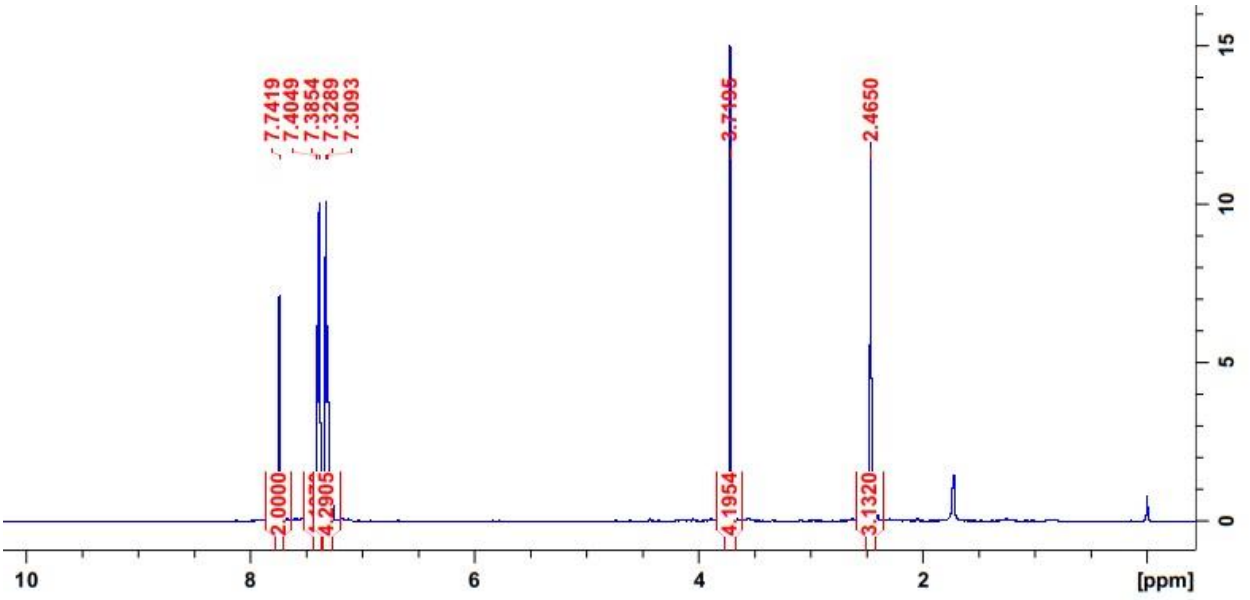


Mass: $[M+H]^+$ = m/z 358.1000

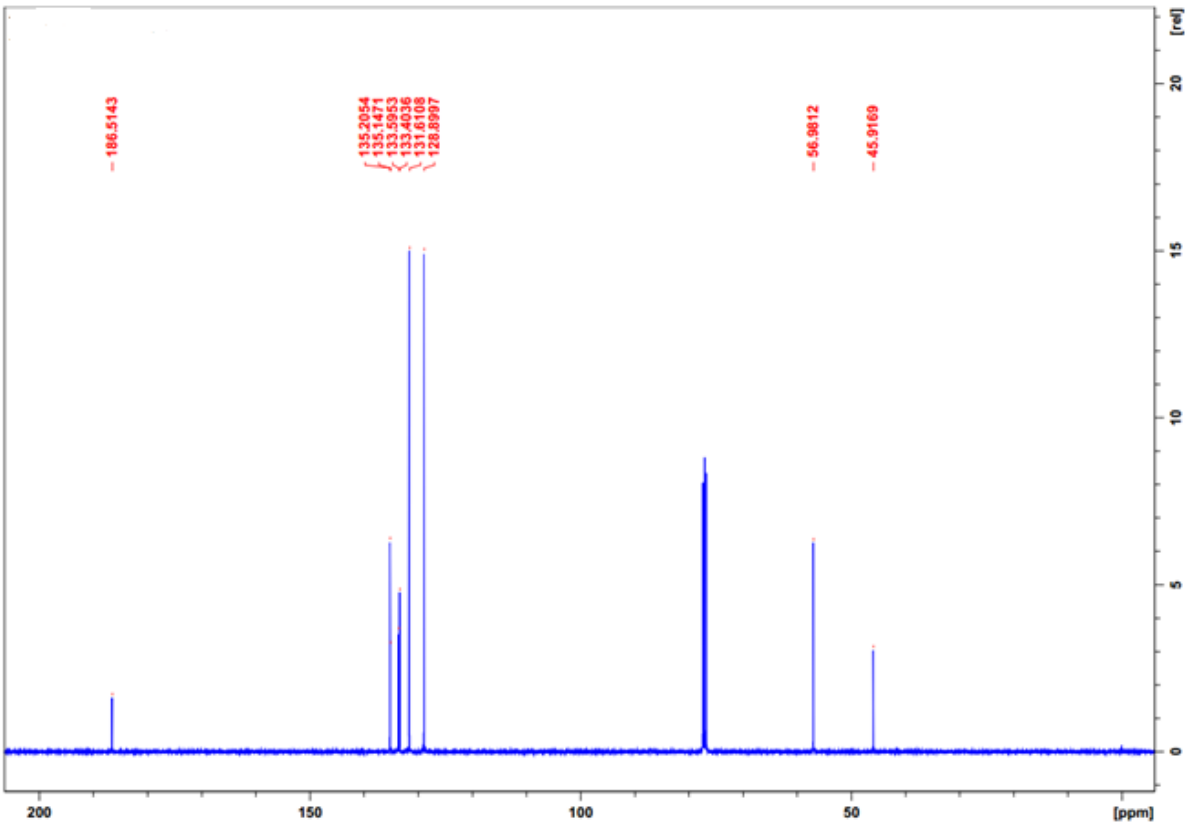
SI-Figure 9: Mass spectrum of 3,5-Bis ((E)-3-Chlorobenzylidene)-N-methyl-4-piperidone.

Id) 3,5-Bis ((E)-4-Chlorobenzylidene)-N-methyl-4-piperidone

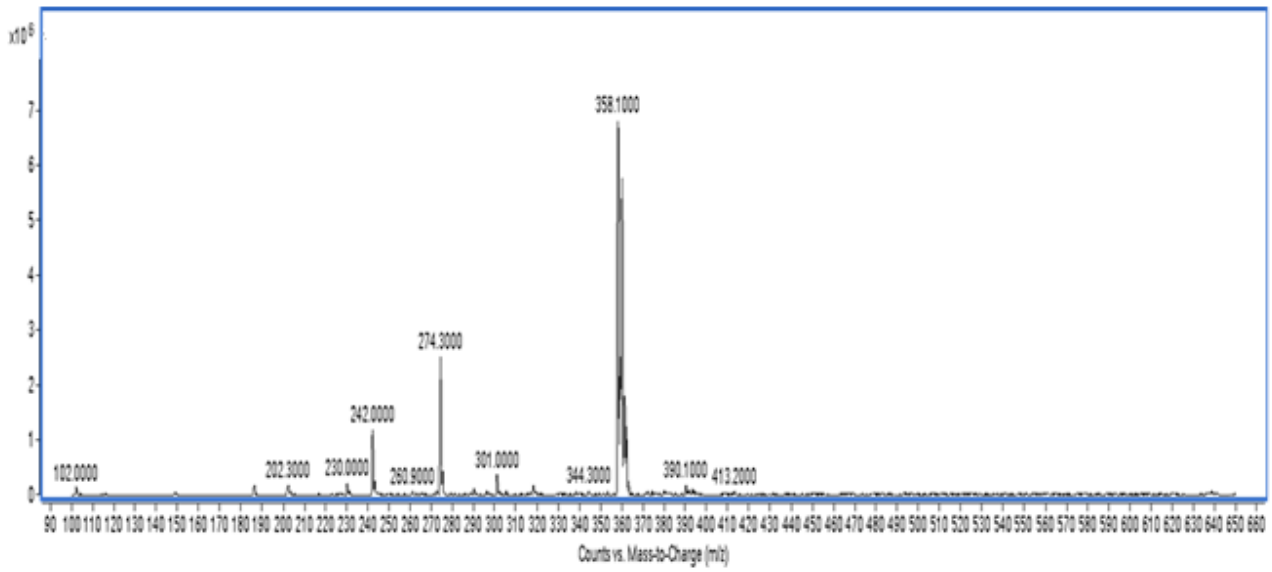
Yield: 83.9%. Light yellow crystals. Recrystallized from 1. Chloroform-methanol 2. Ethyl acetate (EtOAC). mp: 176-177.5^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.74 (H-7, 2H, s), δ 7.39 (H-2' and H-6', 4H, d, J = 7.80 Hz), δ 7.31 (H-3' and H-5', 4H, d, J = 7.80 Hz), δ 3.71 (H-2, H-6, 4H, s), δ 2.46, (N-CH_3, 3H, s). ^{13}C NMR (400 MHz, $CDCl_3$): δ 186.51 (C-4), δ 135.20 (C-4'), δ 135.1 (C-5), δ 133.5(C-7), δ 133.40 (C-1'), δ 131.61 (C-2$^{'}$ and 6$^{'}$), δ 128.89 (C-3$^{'}$ and C-5$^{'}$), δ 56.98 (C-6) and δ 45.91 (N-C); IR (KBr disc) (cm^{-1}): 3006 (sp^2 C-H), 2937 (sp^3 C-H), 1673 (C=O), 1615 (C=C), 1587 and 1546 (aromatic skeletal bands), 1097 (C-Cl); Mass: $[M+H]^+$ = m/z 358.1000, $[M+2]^+$ = m/z 360, $[M+4]^+$ = m/z 362; UV (MeOH) λ_{max} (nm): 235 and 332 nm.



SI-Figure 10: ^{1}H NMR spectrum of 3,5-Bis ((E)-4-Chlorobenzylidene)-N-methyl-4-piperidone.



SI-Figure 11: ^{13}C NMR spectrum of 3,5-Bis ((E)-4-Chlorobenzylidene)-N-methyl-4-piperidone.

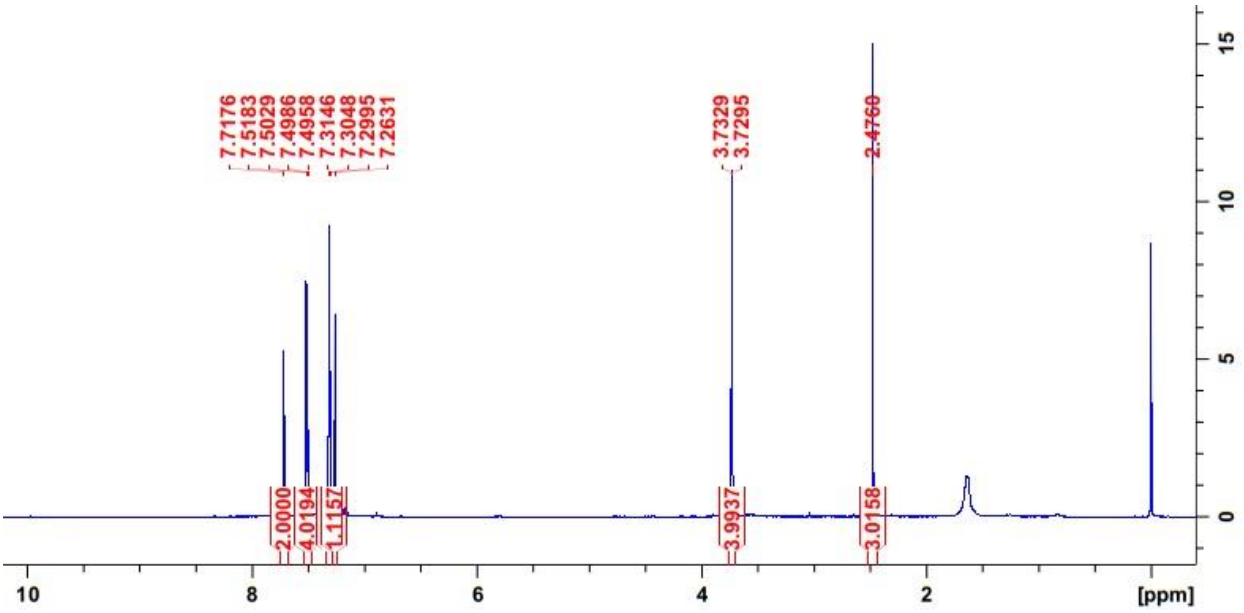


Mass: $[M+H]^+$ = m/z 358.1000

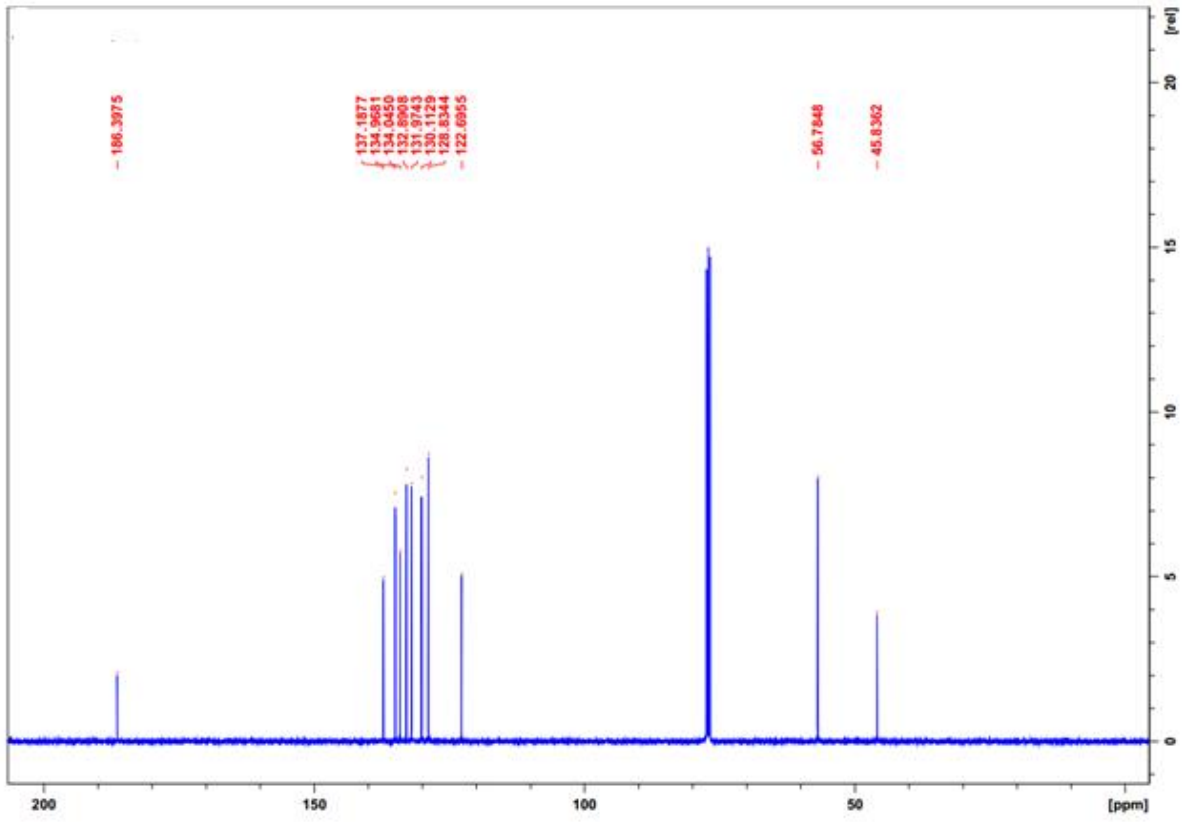
SI-Figure 12: Mass spectrum of 3,5-Bis ((E)-4-Chlorobenzylidene)-N-methyl-4-piperidone.

Ie) 3,5-Bis ((E)-3-bromobenzylidene)-N-methyl-4-piperidone

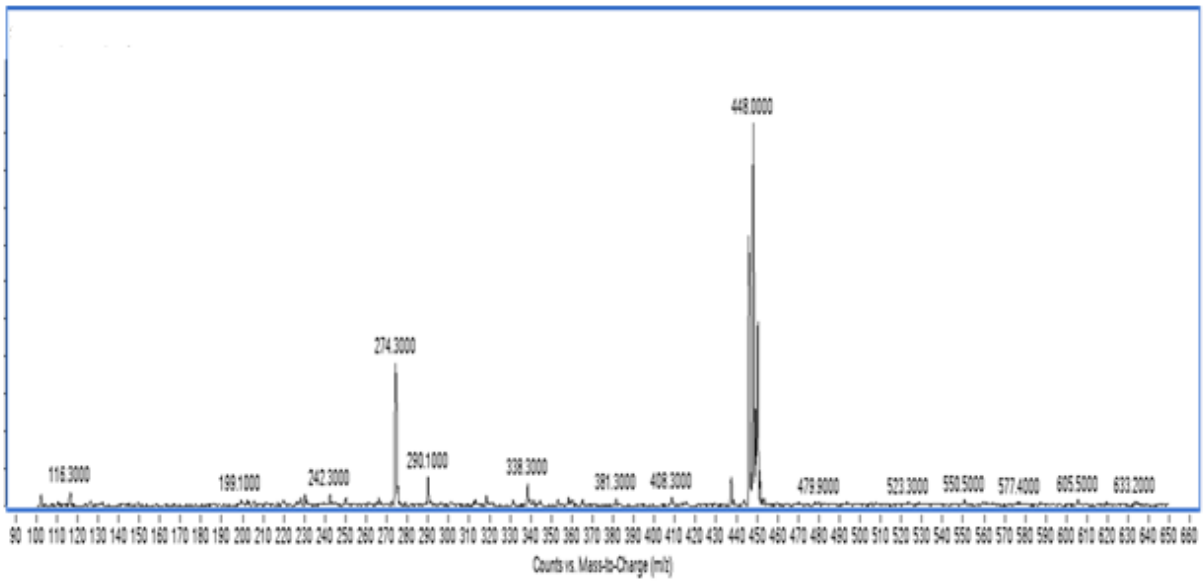
Yield: 84.3%. Yellow powder. Recrystallized from 1. Methanol-chloroform 2. Petrolium ether-acetone. mp: 123-125^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71 (H-7, 2H, s), δ 7.51-7.49 (H-2', H-4', 4H, overlapping signals), δ 7.31-7.29 (H-5', H-6', 4H, overlapping signals), δ 3.72 (H-2, H-6, 4H, s), δ 2.47, (N-CH_3, 3H, s). ^{13}C NMR (400 MHz, $CDCl_3$) : δ 186.39 (C-4), δ 137.18 (C-3'), δ 134.04 (C-2'), δ 132.89 (C-5), δ 131.97 (C-7), δ 130.11 (C-1'), δ 128.84 (C-4'), δ 128.83 (C-6'), δ 122.69 (C-3'), δ 56.78 (C-6) and δ 45.83 (N-C); IR (KBr disc) (cm^{-1}): 3070 (sp^2 C-H), 2945 (sp^3 C-H), 1670 (C=O), 1610 (C=C), 1595 and 1480 (aromatic skeletal stretch), 995 and 910 (C-H alkene out of plane bend), 845 and 870 (aromatic C-H out of plane bend); Mass: $[M+H]^+$ = m/z 448.0000. $[M+2]^+$= m/z 450, and $[M+4]^+$ = m/z 452; UV (MeOH) λ_{max} (nm): 322 nm.



SI-Figure 13: ^{1}H NMR spectrum of 3,5-Bis ((E)-3-bromobenzylidene)-N-methyl-4-piperidone.



SI-Figure 14: ^{13}C NMR spectrum of 3,5-Bis ((E)-3-bromobenzylidene)-N-methyl-4-piperidone.

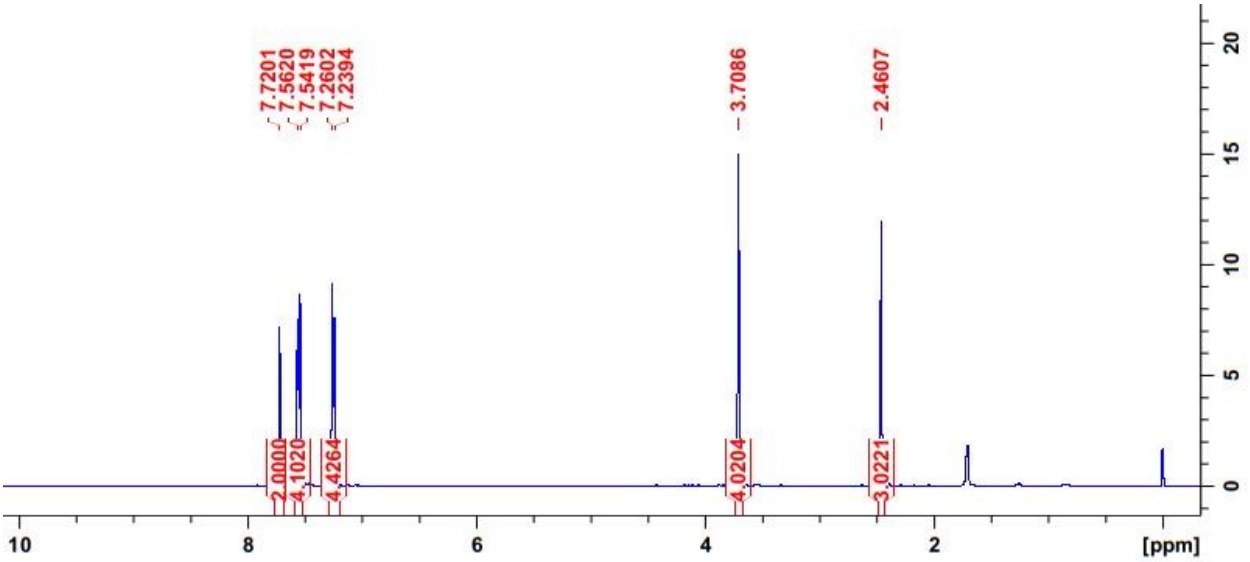


Mass: $[M+H]^+$ = m/z 448.0000

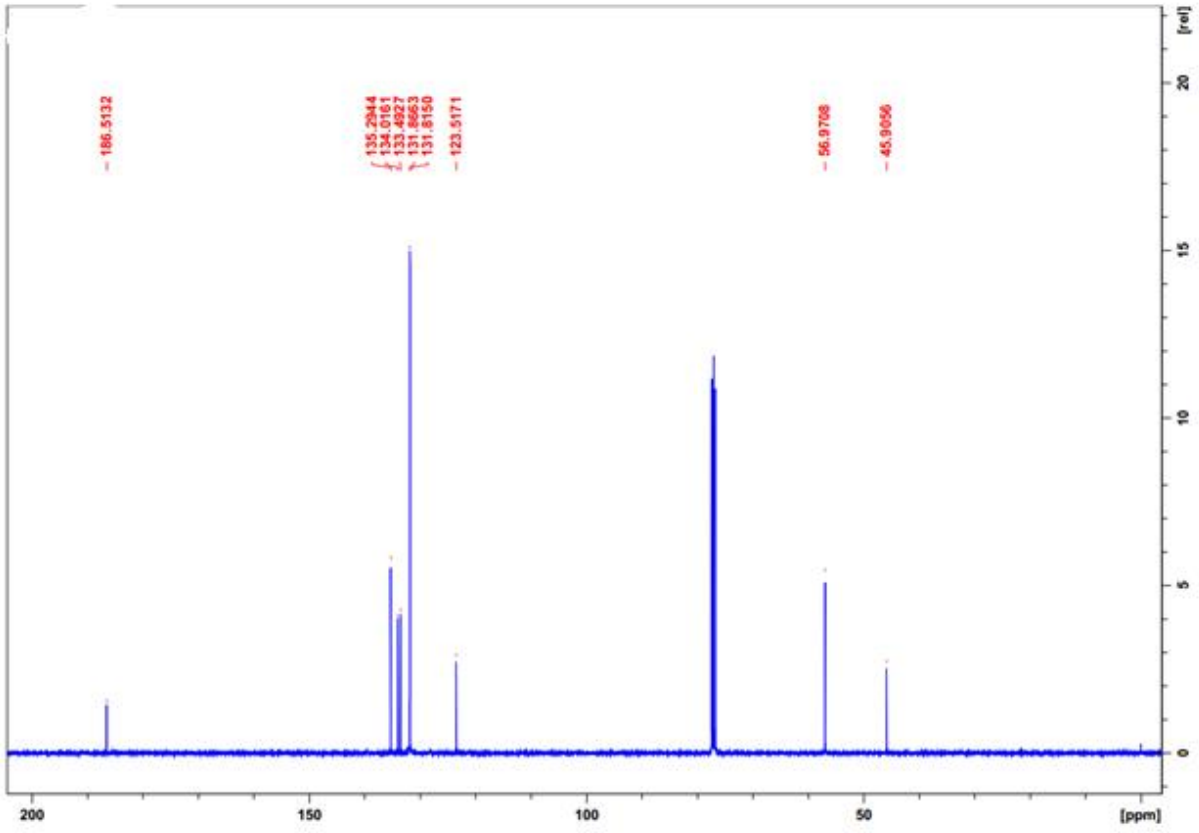
SI-Figure 15: Mass spectrum of 3,5-Bis ((E)-3-bromobenzylidene)-N-methyl-4-piperidone.

If) 3,5-Bis ((E)-4-bromobenzylidene)-N-methyl-4-piperidone

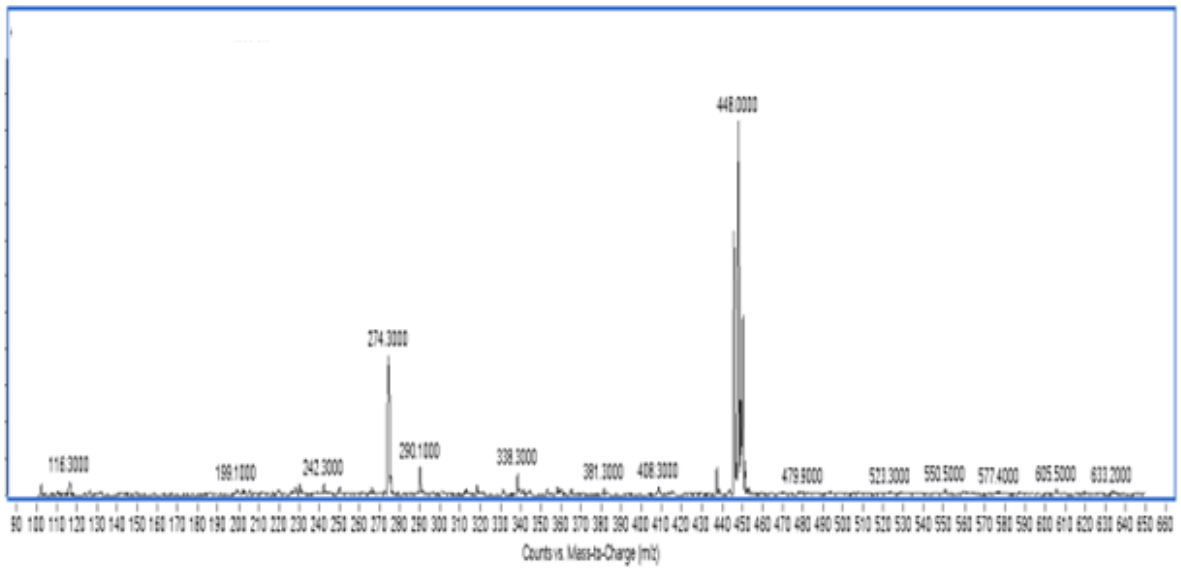
Yield: 99%. Yellow powder. Recrystallized from chloroform-ethyl acetate. mp: 183.7-186.3^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72 (H-7, 2H, s), δ 7.55 (H-3' and H-5', 4H, d, J = 8.04 Hz), δ 7.24 (H-2' and H-6', 4H, d, J = 8.04 Hz), δ 3.70 (H-2, H-6, 4H, s), δ 2.46, (N-CH_3, 3H, s). ^{13}C NMR (400 MHz, $CDCl_3$): δ 186.5 (C-4), δ 135.29 (C-5), δ 134.01 (C-7), δ 133.49 (C-1'), δ 131.86 (C-3' and C-5'), δ 131.81 (C-2' and C-6'), δ 123.51 (C-4'), δ 56.97 (C-6) and δ 45.9 (N-C); IR (KBr disc) (cm^{-1}): 3080 (sp^2 C-H), 2950 (sp^3 C-H), 1670 (C=O), 1612 (C=C), 1595 and 1500 (aromatic skeletal stretch), 1000 and 915 (C-H alkene out of plane bend), 820 and 780 (aromatic C-H out of plane bend); Mass: $[M+H]^+$ = m/z 448.0000, $[M+2]^+$ = m/z 450, and $[M+4]^+$ = m/z 452; UV (MeOH) λ_{max} (nm): 334 and 236 nm.



SI-Figure 16: ^{1}H NMR spectrum of 3,5-Bis ((E)-4-bromobenzylidene)-N-methyl-4-piperidone.



SI-Figure 17: ^{13}C NMR spectrum of 3,5-Bis ((E)-4-bromobenzylidene)-N-methyl-4-piperidone.

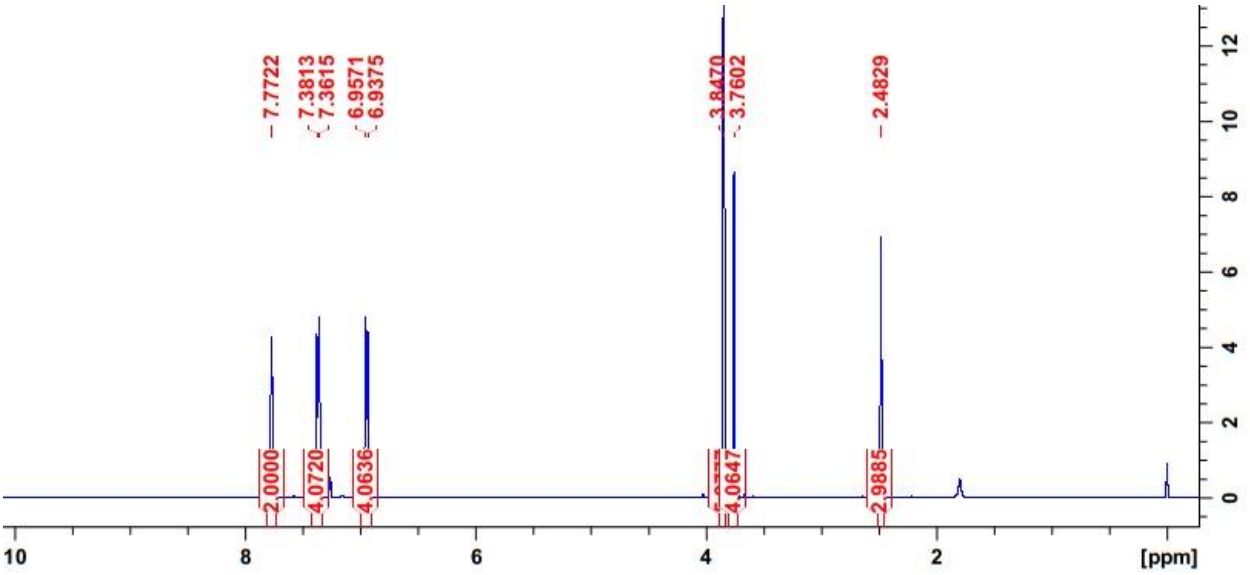


Mass: $[M+H]^+$ = m/z 448.0000

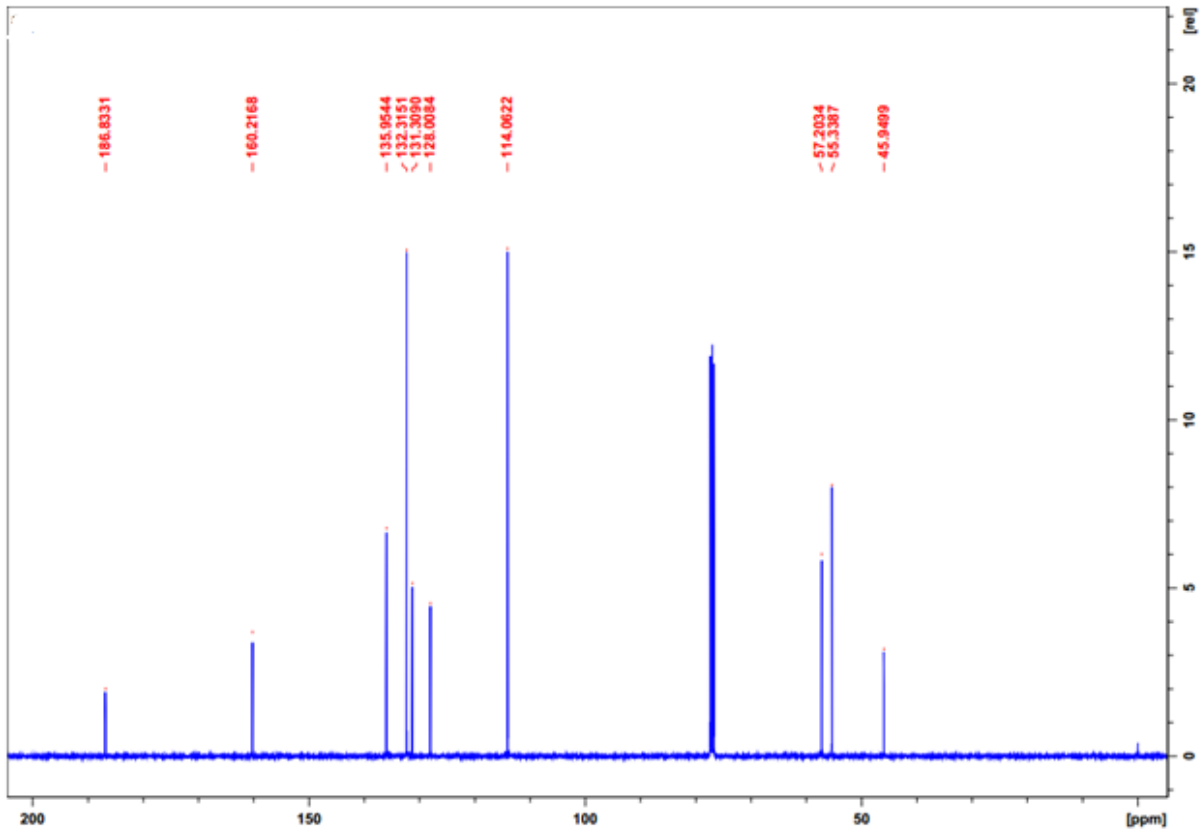
SI-Figure 18: Mass spectrum of 3,5-Bis ((E)-4-bromobenzylidene)-N-methyl-4-piperidone.

Ig) 3,5-Bis ((E)-4-methoxybenzylidene)-N-methyl-4-piperidone

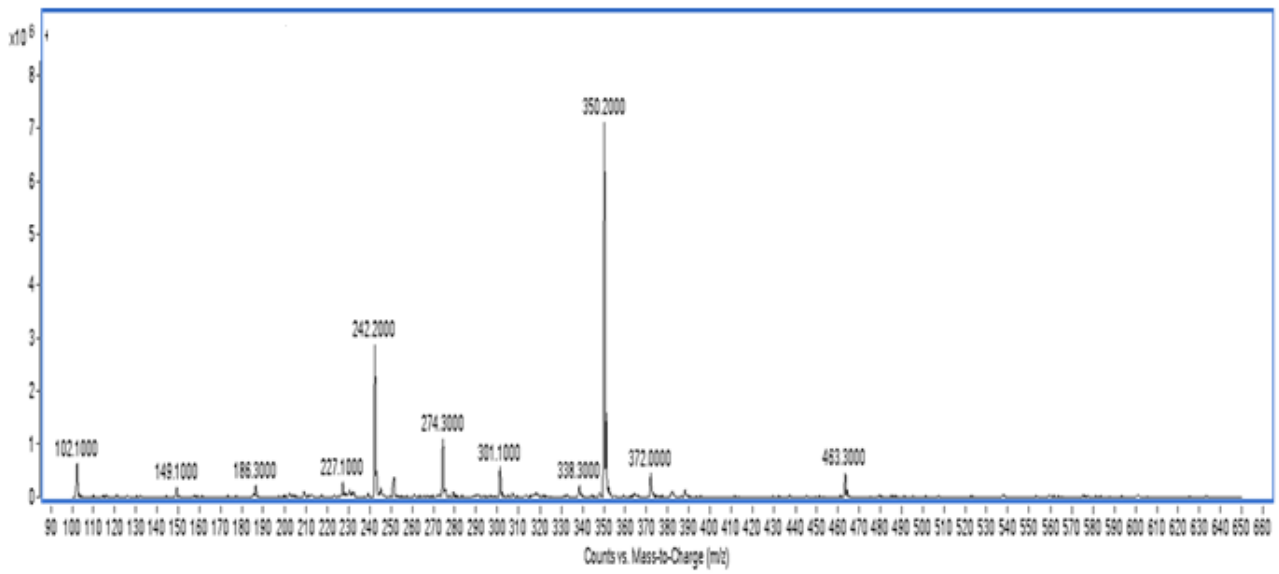
Yield: 77.8%. Yellow crystals. Recrystallized from chloroform-methanol. mp: 201-203.2^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77 (H-7, 2H, s), δ 7.37 (H-3' and H-5', 4H, d, J = 7.84 Hz), δ 6.94 (H-2' and 6', 4H, d, J = 7.84 Hz), δ 3.84 (O-CH, 3H, s), δ 3.76 (H-2, H-6, 4H, s), δ 2.48, (N-CH_3, 3H, s). ^{13}C NMR (400 MHz, $CDCl_3$): δ 186.83 (C-4), δ 160.2 (C-4'), δ 135.95 (C-5), δ 132.31 (C-2'), δ 131.30 (C-1'), δ 128.0 (C-6'), δ 114.06 (C-3'), δ 57.20 (O-CH_3), δ 55.33 (C-6) and δ 45.94 (N-C); IR (KBr disc) (cm^{-1}): 3011 (sp^2 C-H), 2929 (sp^3 C-H), 1670 (C=O), 1609 (C=C), 1163(C-O), 1580 and 1510 (aromatic skeletal bands) 830 (para substitution); Mass: $[M+H]^+$ = m/z 350.2000, $[M-108]^+$ = m/z 242.2000; UV (MeOH) λ_{max} (nm): 242 and 364 nm.



SI-Figure 19: ^{1}H NMR spectrum of 3,5-Bis ((E)-4-methoxybenzylidene)-N-methyl-4-piperidone.



SI-Figure 20: ^{13}C NMR spectrum of 3,5-Bis ((E)-4-methoxybenzylidene)-N-methyl-4-piperidone.

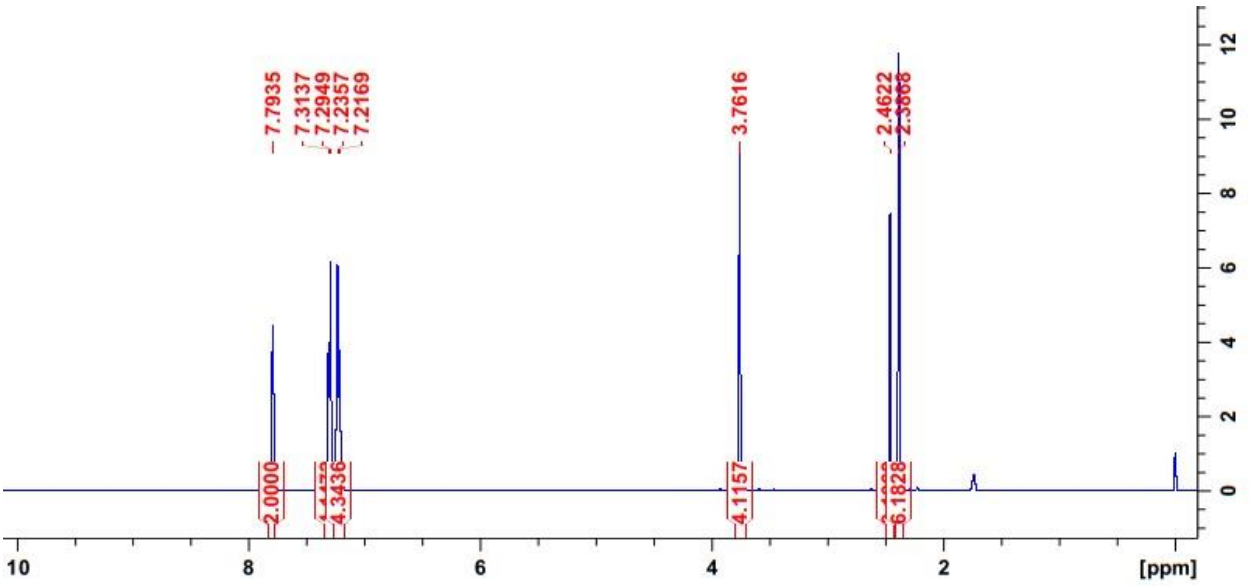


Mass: $[M+H]^+$ = m/z 350.2000

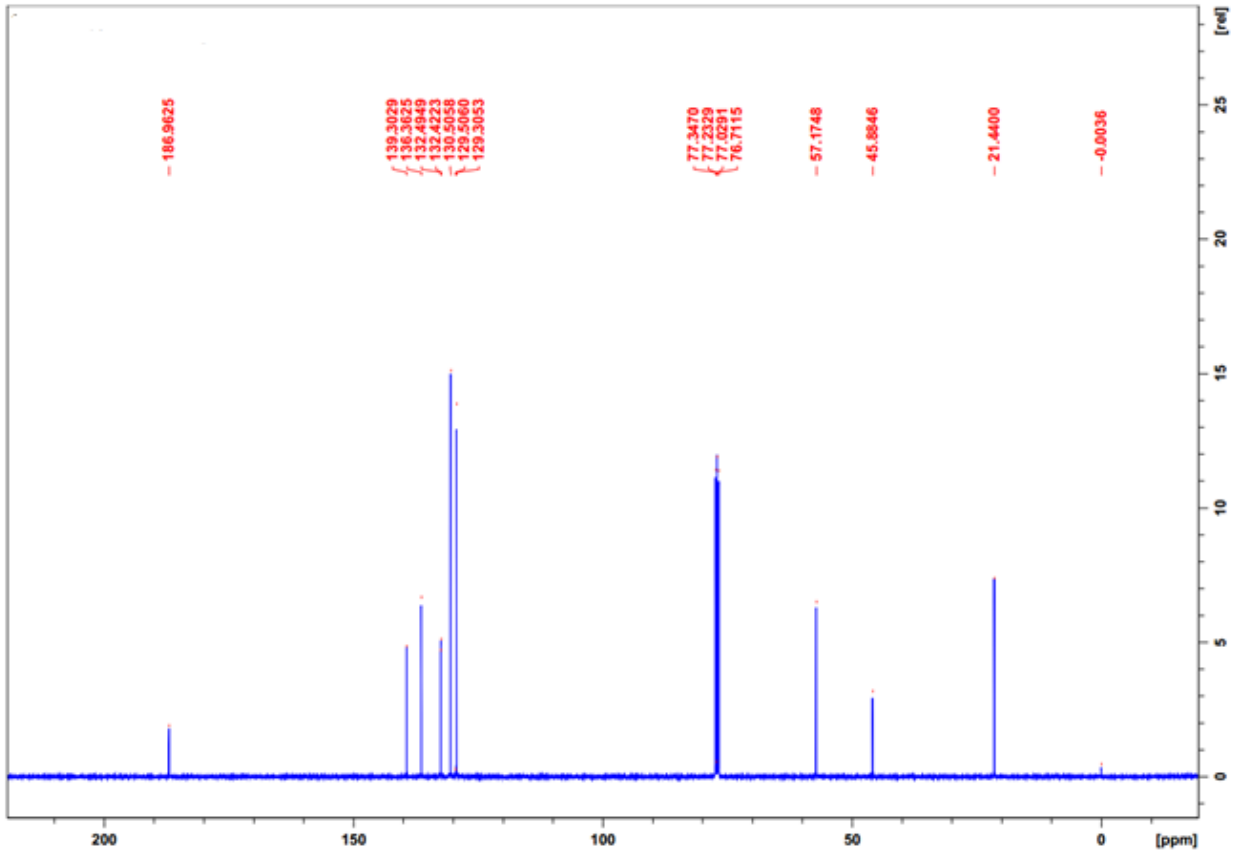
SI-Figure 21: Mass spectrum of 3,5-Bis ((E)-4-methoxybenzylidene)-N-methyl-4-piperidone.

Ih) 3,5-Bis ((E)-4-methylbenzylidene)-N-methyl-4-piperidone

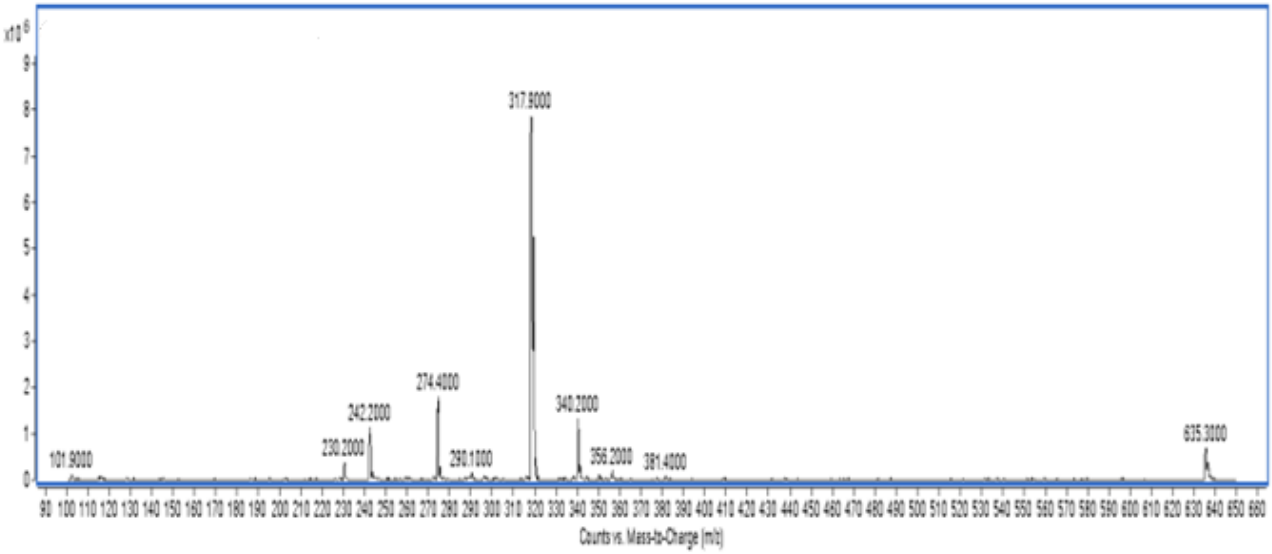
Yield: 82%. Yellow crystals were obtained. It was purified by recrystallization from methanol-chloroform. mp: 200-202^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79 (H-7, 2H, s), δ 7.29 (H-2' and H-6', 4H, d, J = 7.52 Hz), δ 7.22 (H-3' and 5', 4H, d, J = 7.52 Hz), δ 3.76 (H-2, H-6, 4H, s), δ 2.46 (N-CH_3, 3H, s), δ 2.38 (CH_3, 3H, s). ^{13}C NMR: δ 186.96 (C-4), δ 139.30 (C-5), δ 132.49 (C-1'), δ 132.42 (C-4'), δ 129.30 (C-3' and C-5'), δ 120.50 (C-2' and C-6'), δ 57.17 (C-6) and δ 45.88 (N-C); IR (KBr disc) (cm^{-1}): 3040 (sp^2 C-H), 2972 (sp^3 C-H), 1670 (C=O), 1603 (C=C), 1580 and 1510 (aromatic skeletal bands), 815 (para substitution); Mass: $[M+H]^+$ = m/z 317.9000; UV (MeOH) λ_{max} (nm): 341 and 336 nm.



SI-Figure 22: ^{1}H NMR spectrum of 3,5-Bis ((E)-4-methylbenzylidene)-N-methyl-4-piperidone.



SI-Figure 23: ^{13}C NMR spectrum of 3,5-Bis ((E)-4-methylbenzylidene)-N-methyl-4-piperidone.

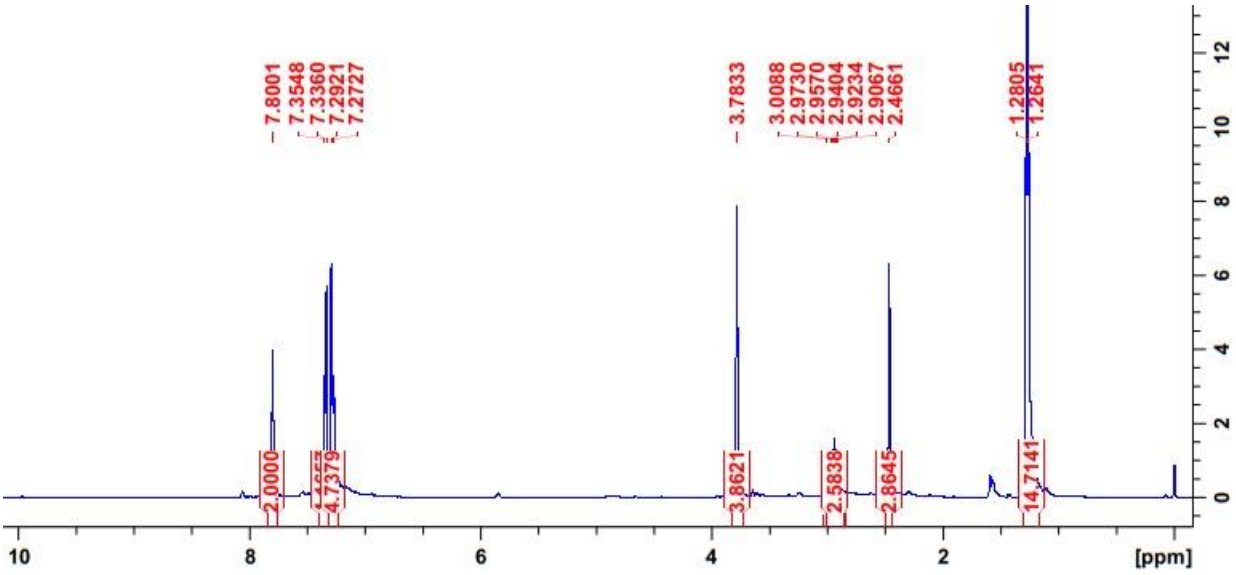


Mass: $[M+H]^+$ = m/z 317.9000

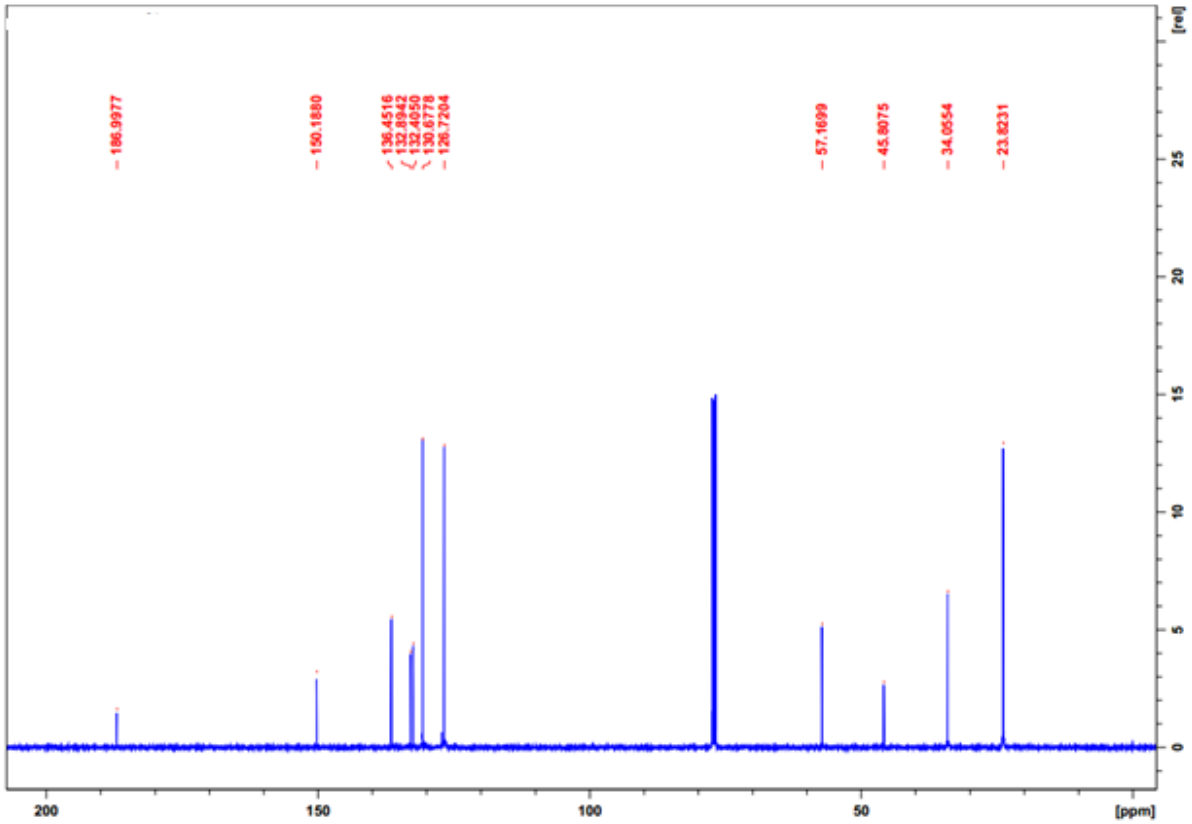
SI-Figure 24: Mass spectrum of 3,5-Bis ((E)-4-methylbenzylidene)-N-methyl-4-piperidone.

Ii) 3,5-Bis ((E)-4-isopropylbenzylidene)-N-methyl-4-piperidone

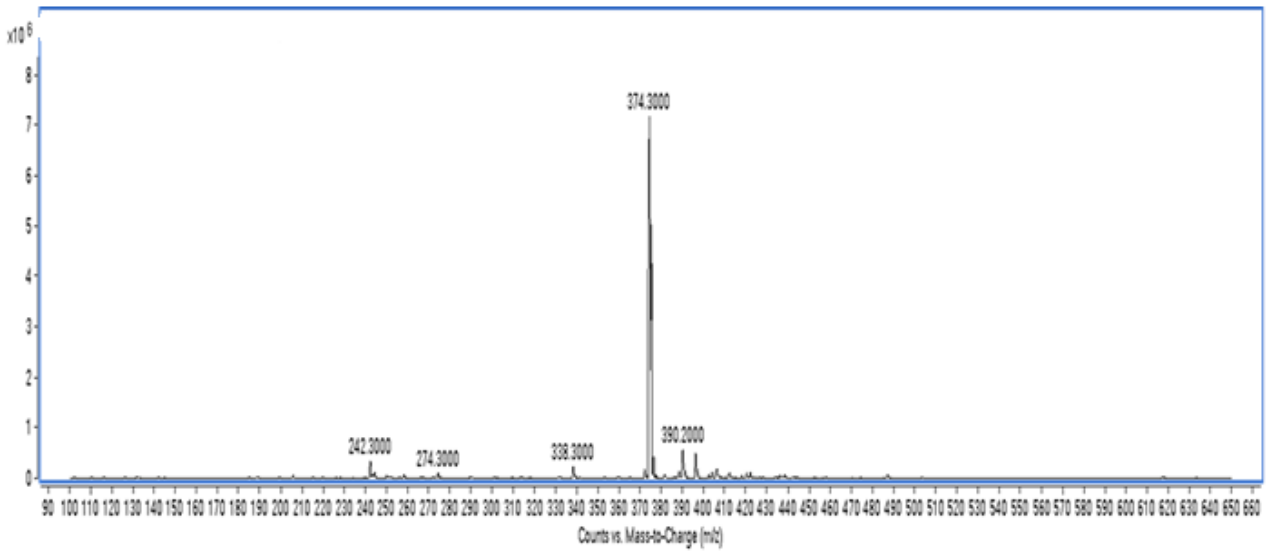
Yield: 74.5%. Yellow powder. Recrystallized from 1.chloroform-methanol 2.chloroform-pet ether, and 3.methanol. mp: 105.5-108.5^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80 (H-7, 2H, s), δ 7.34 (H-2' and H-6', 4H, d, J = 7.52 Hz), δ 7.28 (H-3' and 5', 4H, d, J = 7.52 Hz), δ 3.78 (H-2, H-6, 4H, s), δ 2.95 (CH, 1H, sep, J = 13.44 Hz), δ 2.46, (N-CH_3, 3H, s). δ 1.28 (CH-C$\underline{H}_3$, 3H, d, J = 6.56 Hz). ^{13}C NMR (400 MHz, $CDCl_3$): δ 186.99 (C-4), δ 150.18 (C-4'), δ 136.4 (C-5), δ 132.89 (C-7), δ 132.4 (C-1'), δ 126.72 (C-3'), δ 57.16 (C-6) and δ 45.80 (N-C), δ 34.05 (C-7'); IR (KBr disc) (cm^{-1}): 3050 (sp^2 C-H), 2970 (sp^3 C-H), 1670 (C=O), 1610 (C=C), 1515 and 1475 (aromatic skeletal stretch), 990 and 920 (C-H alkene out of plane bend), 825 (aromatic C-H out of plane bend); Mass: $[M+H]^+$ = m/z 374.3000; UV (MeOH) λ_{max} (nm): 342 and 235 nm.



SI-Figure 25: ^{1}H NMR spectrum of 3,5-Bis ((E)-4-isopropylbenzylidene)-N-methyl-4-piperidone.



SI-Figure 26: ^{13}C NMR spectrum of 3,5-Bis ((E)-4-isopropylbenzylidene)-N-methyl-4-piperidone.

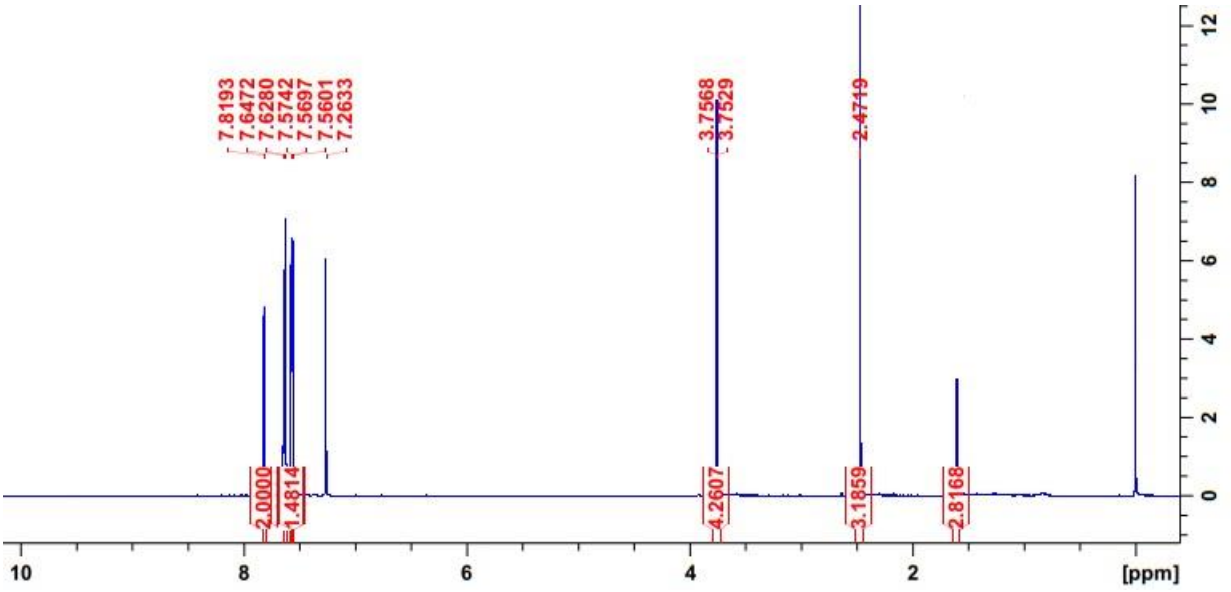


Mass: $[M+H]^+$ = m/z 374.3000

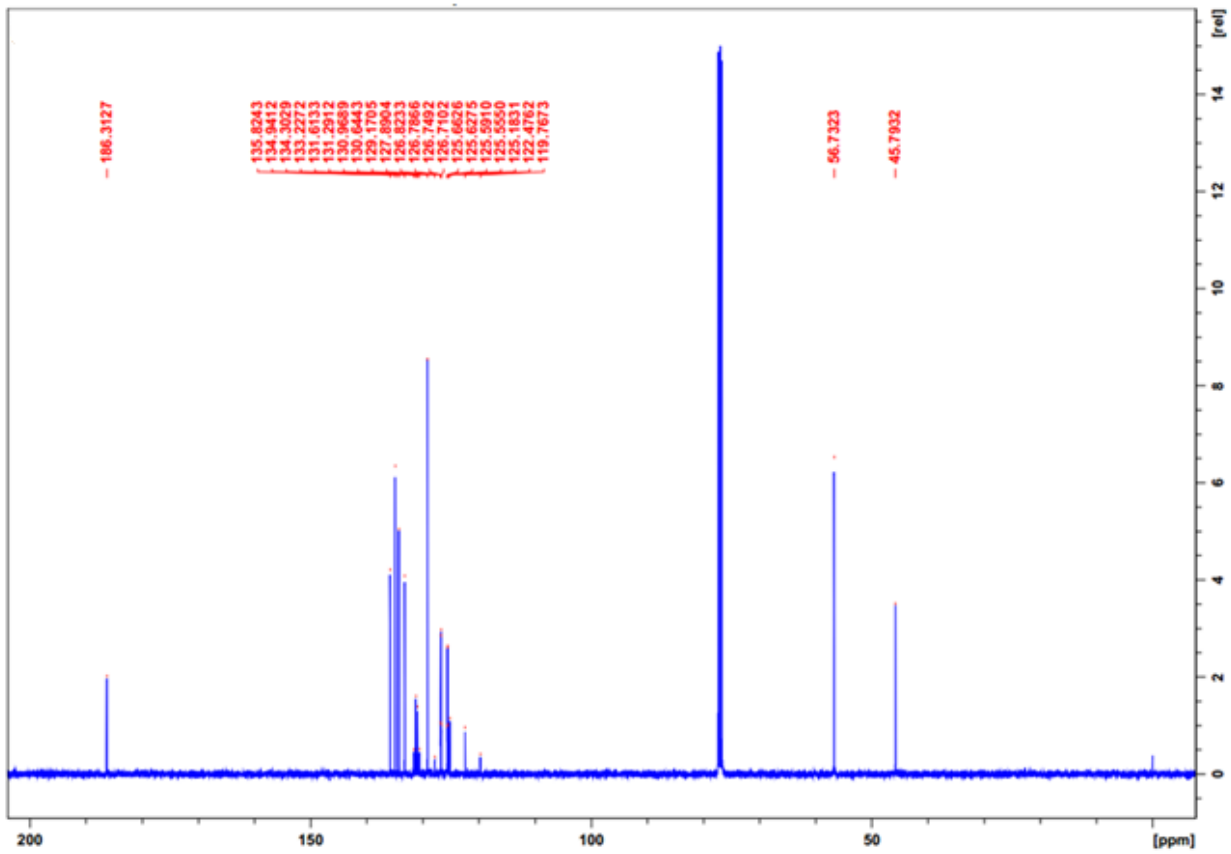
SI-Figure 27: Mass spectrum of 3,5-Bis ((E)-4-isopropylbenzylidene)-N-methyl-4-piperidone.

Ij) 3,5-Bis ((E)-3-trifluorobenzylidene)-N-methyl-4-piperidone

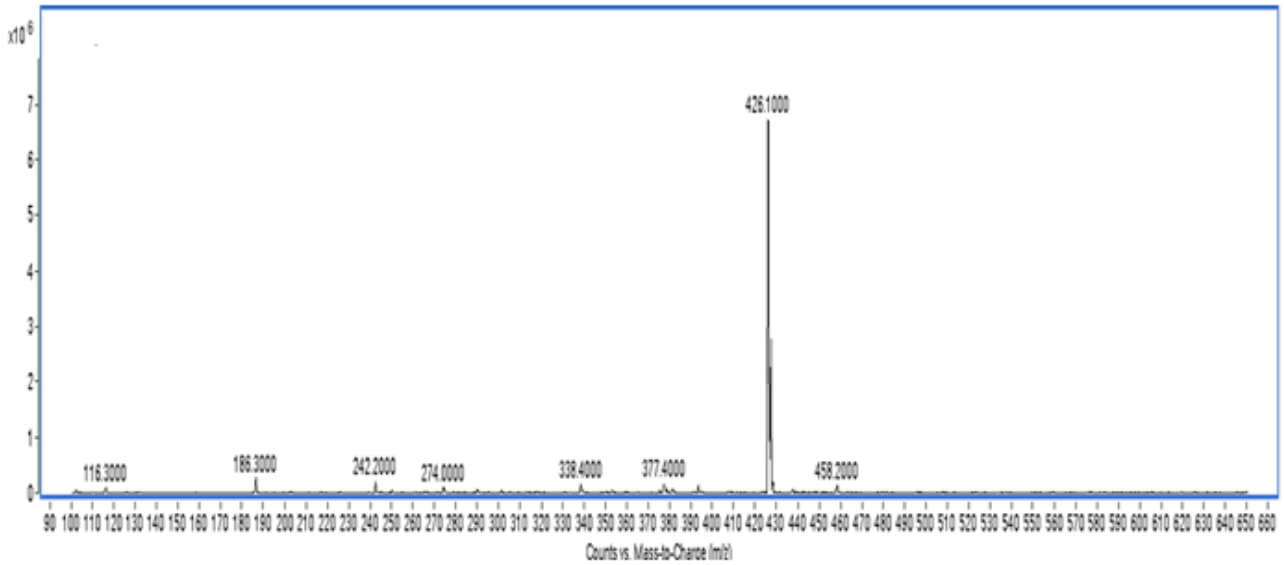
Yield: 51.6%.Yellow crystals. Recrystallized from pet ether-chloroform. mp: 143-145.2^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) : δ 7.81 (H-7, 2H, s), δ 7.64-7.62 (H-4', H-6', 4H, two doublets overlapping), δ 7.57 (H-5', 2H, t), δ 7.56 (H-2', 2H, s), δ 3.75 (H-2, H-6, 4H, s), δ 2.47, (NCH_3, 3H, s). ^{13}C NMR (400 MHz, $CDCl_3$): δ 186.31 (C-4), δ 135.82 (C-5), δ 134.92 (C-7), δ 134.3 (C-1'), δ 133.22 (C-6'), δ 131.61 (C-3'), δ 129.2 (C-5'), δ 126.76 (C-2') , δ 125.6 (C-4') , δ 123.82 (C-F), δ 56.73 (C-6) and δ 45.7 (N-C); IR (KBr disc) (cm^{-1}): 3050 (sp^2 C-H stretch), 2950 (sp^3 C-H stretch), 1680 (C=O stretch), 1575 and 1495 (Ar skeletal bands), 1280 and 1155 (C-O stretch), 980 (C-H alkene out of plane bend), 830 and 800 (mono substituted aromatic ring); Mass: m/z 426.1000; UV (MeOH) λmax (nm): 315 and 228.



SI-Figure 28: ^{1}H NMR spectrum of 3,5-Bis ((E)-3-trifluorobenzylidene)-N-methyl-4-piperidone.



SI-Figure 29: ^{13}C NMR spectrum of 3,5-Bis ((E)-3-trifluorobenzylidene)-N-methyl-4-piperidone.

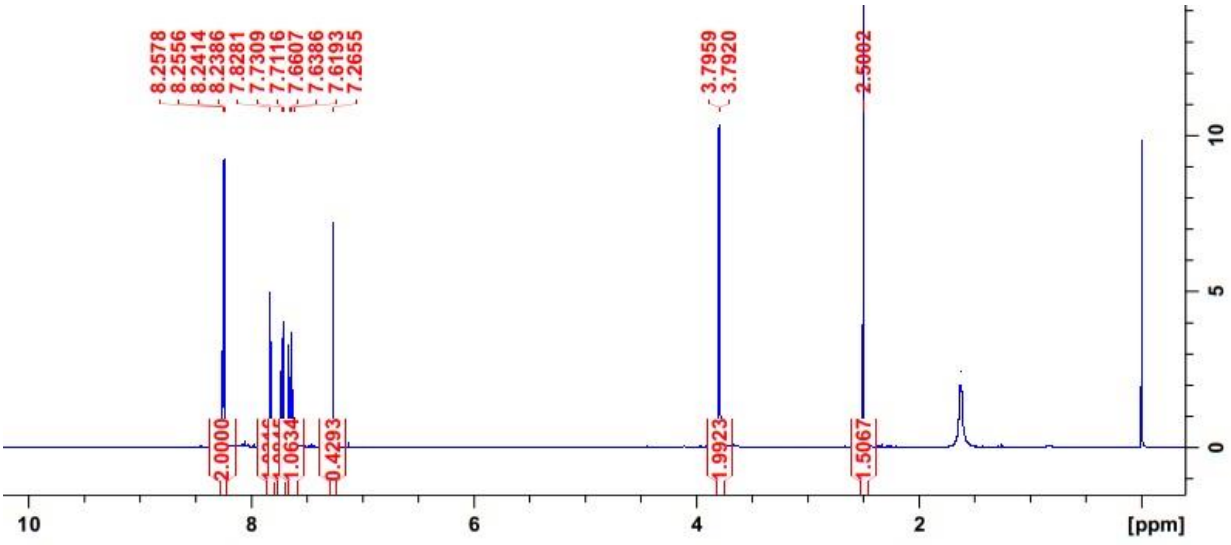


Mass: m/z 426.1000

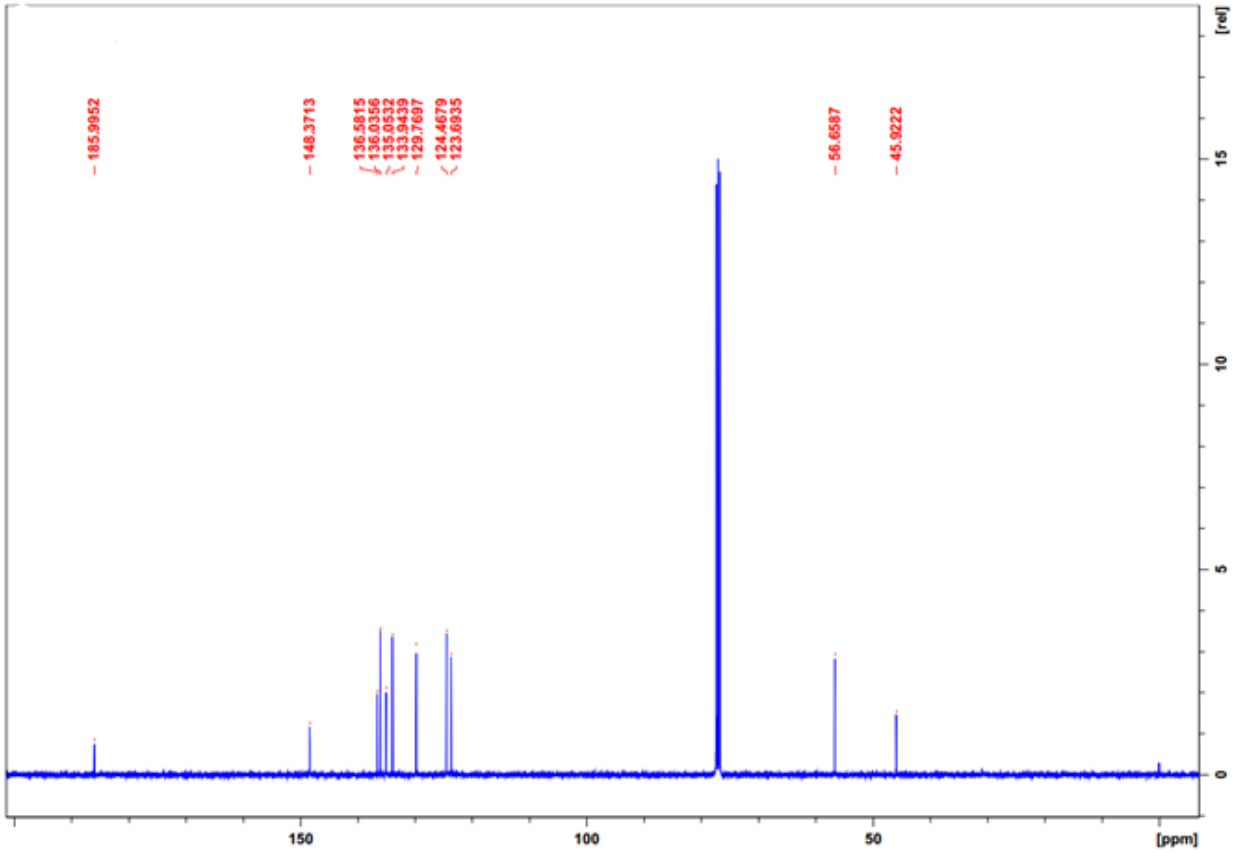
SI- Figure 30: Mass spectrum of 3,5-Bis ((E)-3-trifluorobenzylidene)-N-methyl-4-piperidone.

Ik) 3,5-Bis ((E)-3-nitro benzylidene)-N-methyl-4-piperidone

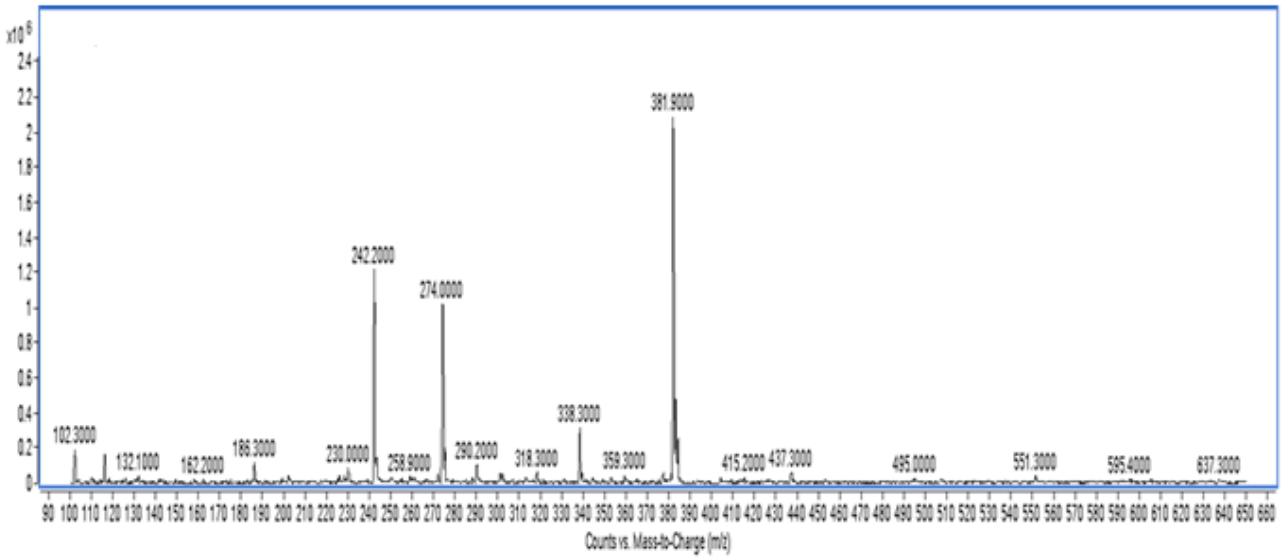
Yield: 97.8%. It was obtained as yellow powder after recrystallization from chloroform-methanol. mp: 185-188.5^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.25 (H-2', 2H, d, J = 8.84 Hz), δ 8.24 (H-4', 2H, s), δ 7.82 (H-7, 2H), δ 7.72 (H-6', 2H, d, J = 8.84 Hz), δ 7.64 (H-5', 2H, t, J = 8.84 Hz), δ 3.79 (H-2, H-6, 4H, s), δ 2.50, (N-CH, 3H, s). ^{13}C NMR (400 MHz, $CDCl_3$): δ 185 (C-4), δ 148.37 (C-3'), δ 136.56 (C-1'), δ 136.03 (C-7), δ 135.05 (C-5), δ 133.84 (C-2'), δ 129.76 (C-4'), δ 124.4 (C-6'), δ 123.8 (C-5'), δ 56 (C-6) and δ 45 (N-C); IR (KBr disc) (cm^{-1}): 3080 (sp^2 C-H), 2935 (sp^3 C-H), 1670 (C=O), 1610 (C=C), 1590 (aromatic skeletal stretch), 1550 and 1350 (NO_2 stretch), 935 (C-H alkene out of plane bend), 895, 800 and 750 (aromatic C-H out of plane bend); Mass: $[M+H]^+$ = m/z 381.3000; UV (MeOH) λ_{max} (nm): 311, 274 and 221 nm.



SI-Figure 31: ^{1}H NMR spectrum of 3,5-Bis ((E)-3-nitro benzylidene)-N-methyl-4-piperidone.



SI-Figure 32: ^{13}C NMR spectrum of 3,5-Bis ((E)-3-nitro benzylidene)-N-methyl-4-piperidone.

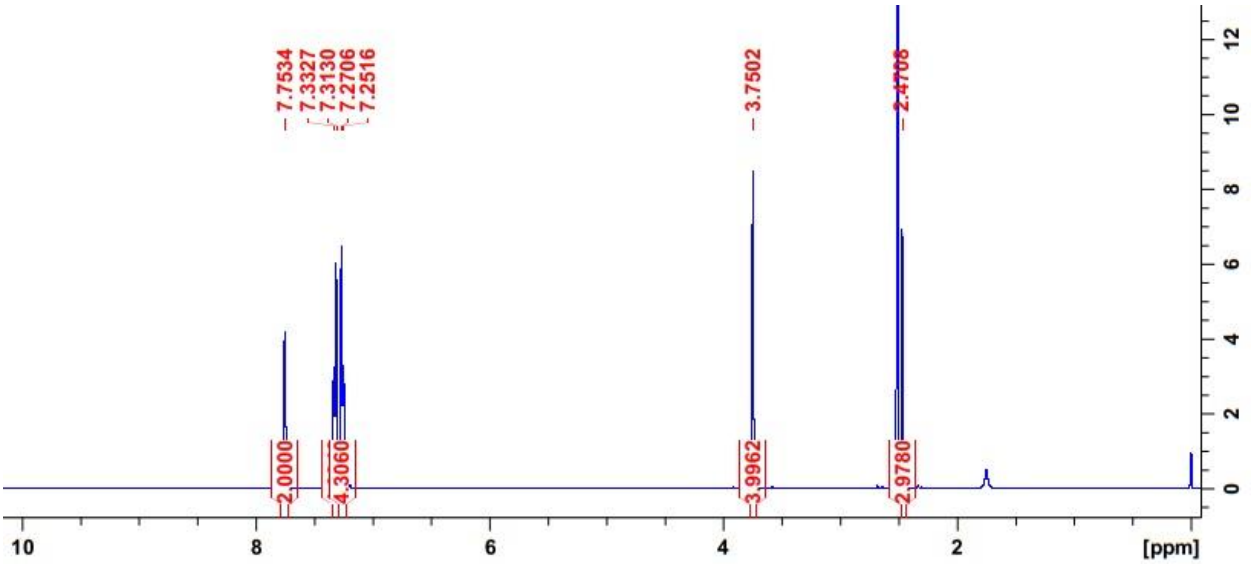


Mass: $[M+H]^+$ = m/z 381.3000

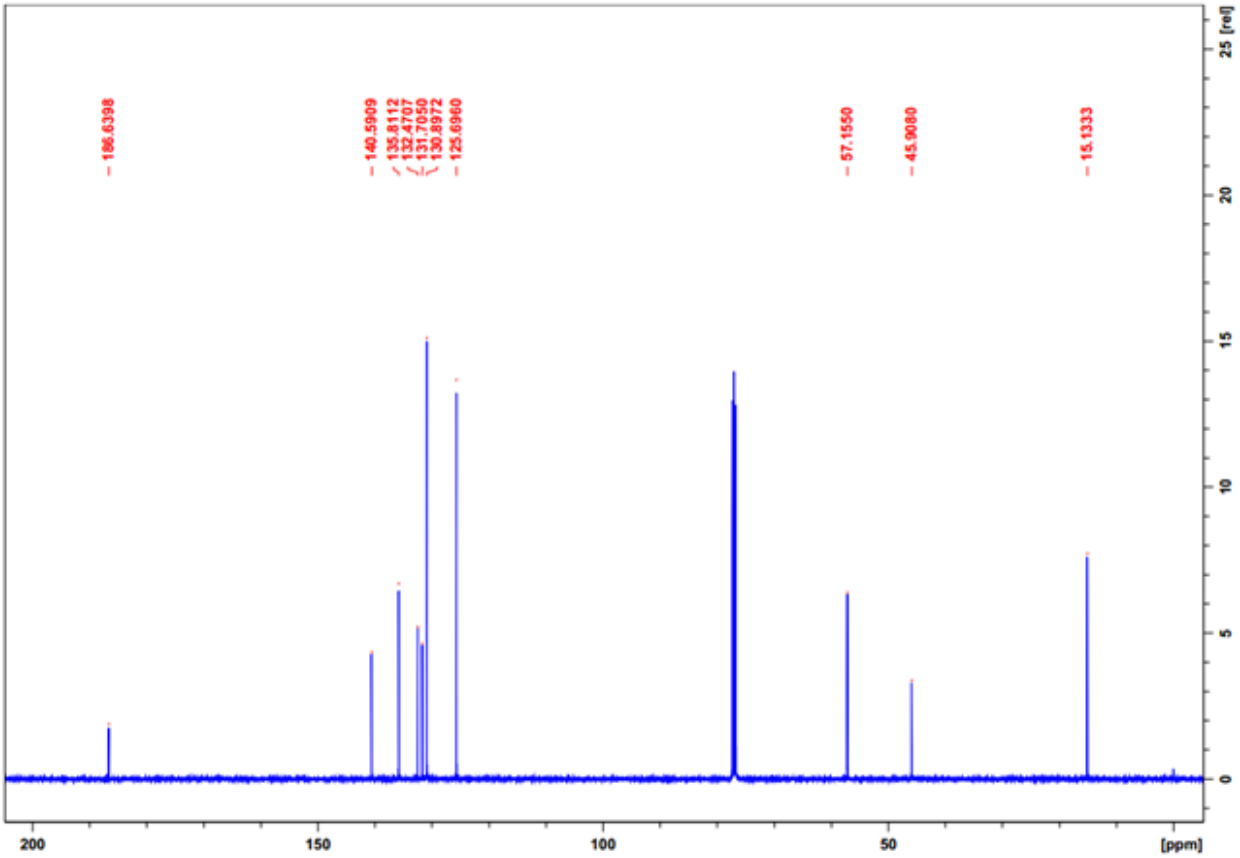
SI-Figure 33: Mass spectrum of 3,5-Bis ((E)-3-nitro benzylidene)-N-methyl-4-piperidone.

Il) 3,5-Bis ((E)-4-methylthiobenzylidene)-N-methyl-4-piperidone

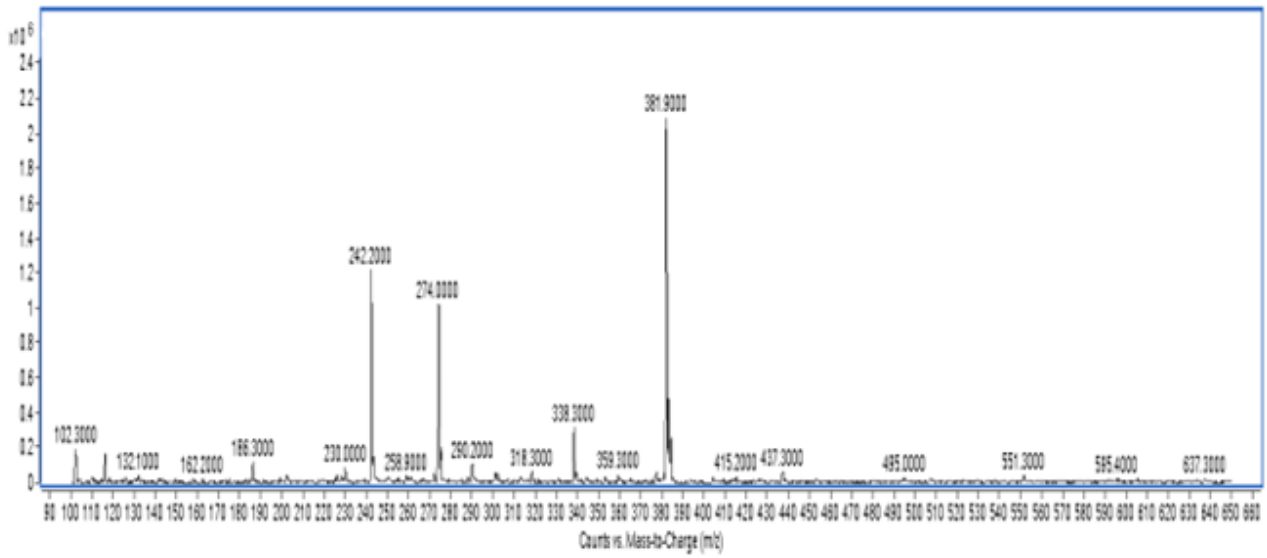
Yield: 82.8%. Yellow solid. Recrystallized from methanol-chloroform. mp: 172-174^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (H-7, 2H, s), δ 7.32 (H-3' and H-5' 4H, d, J = 7.60 Hz), δ 7.25 (H-2' and H-6', 4H, d, J = 7.60 Hz), δ 3.75 (H-2, H-6, 4H, s), δ 2.47, (N-CH_3, 3H, s). ^{13}C NMR (400 MHz, $CDCl_3$): δ 186.6 (C-4), δ 140.59 (C-4'), δ 135.81 (C-5), δ 132.81 (C-7), δ 131.7 (C-1'), δ 130.89 (C-2'), δ 125.69 (C-3'), δ 57.15 (C-6) and δ 45.90 (N-C), δ 15.13 (S-C); IR (KBr disc) (cm^{-1}): 3014 (sp^2 C-H), 2920 (sp^3 C-H), 1669 (C=O), 1587 and 1546 (aromatic skeletal stretch), 1607 (C=C), 821(para- substitution); Mass: m/z 381.9000; UV (MeOH) λ_{max} (nm): 382 and 260 nm.



SI-Figure 34: ^{1}H NMR spectrum of 3,5-Bis ((E)-4-methylthiobenzylidene)-N-methyl-4-piperidone.



SI-Figure 35: ^{13}C NMR spectrum of 3,5-Bis ((E)-4-methylthiobenzylidene)-N-methyl-4-piperidone.

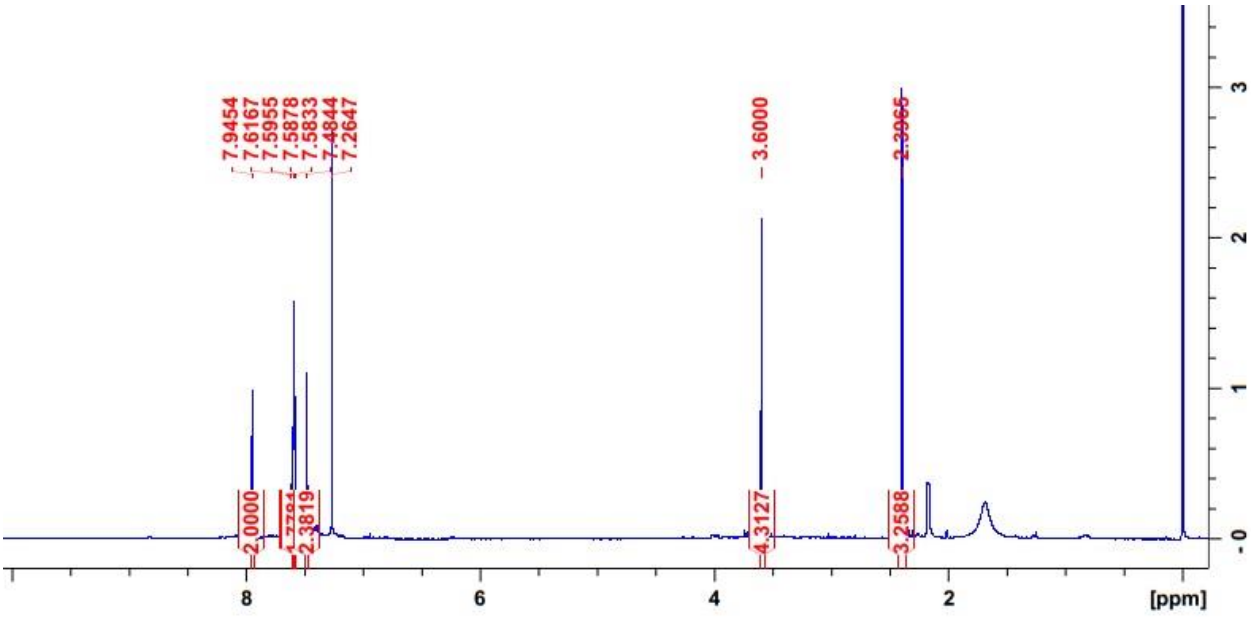


Mass: m/z 381.9000

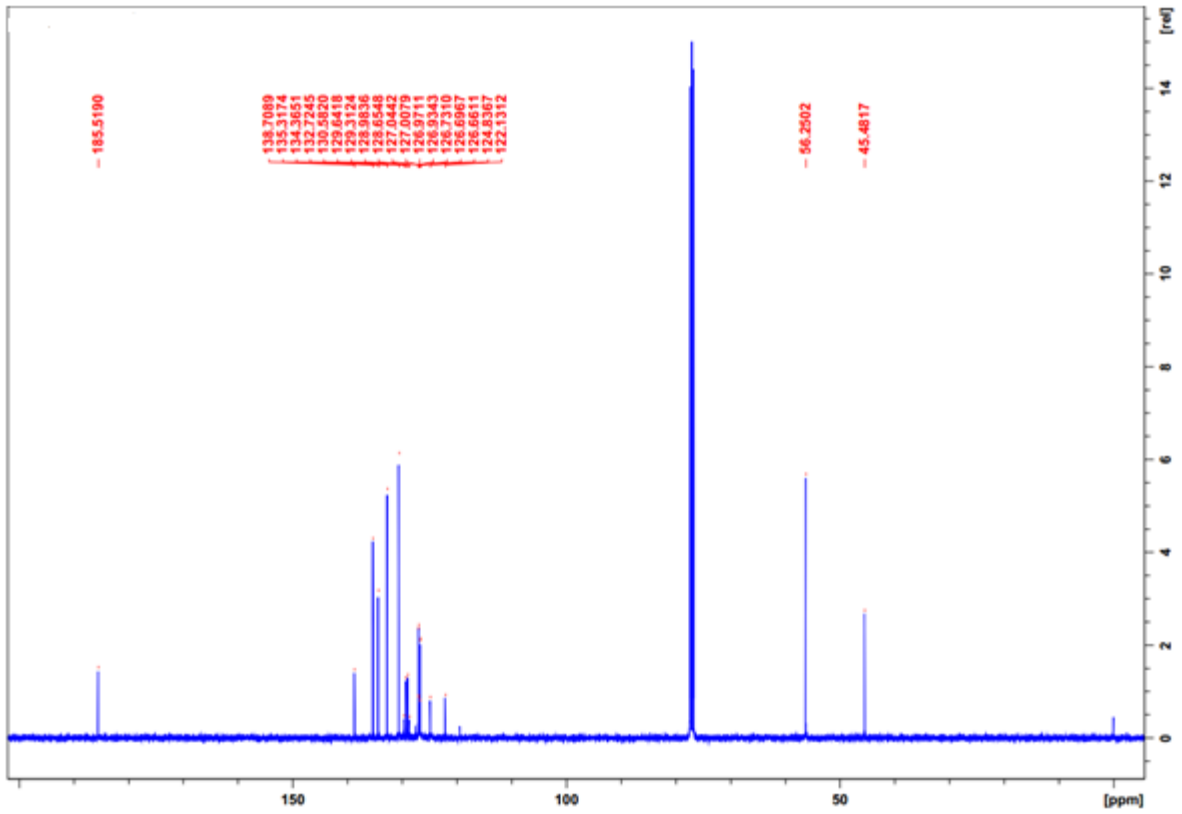
SI-Figure 36: Mass spectrum of 3,5-Bis ((E)-4-methylthiobenzylidene)-N-methyl-4-piperidone.

Im) 3,5-Bis ((E)-2-chloro-5-(trifluoro)benzylidene)-N-methyl-4-piperidone

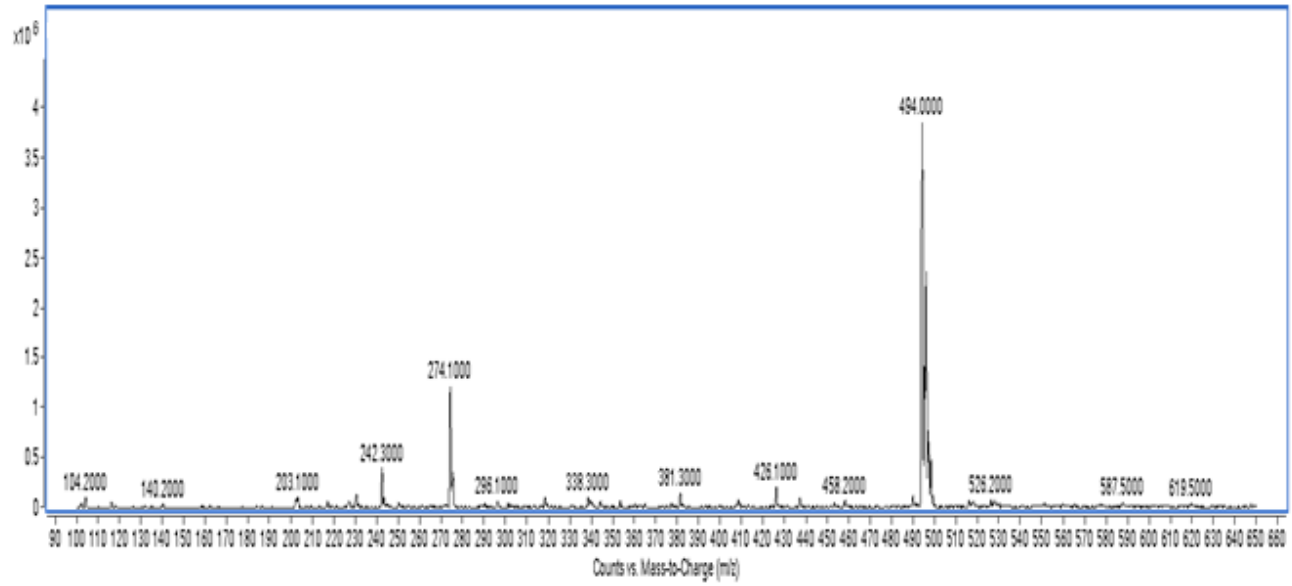
Yield: 81%. Yellow crystals. Recrystallized from chloroform-methanol. mp: 154.4-157.2^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.94 (H-7, 2H, s), δ 7.59 (H-6', 2H, s), δ 7.58 (H-3', 2H, d, overlapping signals), δ 7.48 (H-4', 2H, d, overlapping signals) δ 3.6 (H-2, H-6, 4H, s), δ 2.39, (N-CH_3, 3H, s). ^{13}C NMR (400 MHz, $CDCl_3$): δ 185.51 (C-4), δ 138.70 (C-2'), δ 135.51 (C-5), δ 134.56 (C-1'), δ 132.72 (C-7), δ 130.58 (C-3'), δ 129.14 (C-5'), δ 126.98 (C-6'), δ 126.69 (C-4'), δ 123.52 (C-F), δ 56.25 (C-6) and δ 45.48 (N-C); IR (KBr disc) (cm^{-1}): 3000 (sp^2 C-H), 2950 (sp^3 C-H), 1685 (C=O), 1610 (C=C), 1480 (aromatic skeletal stretch), 1175 and 1120 (C-F), 1090 (aromatic C-Cl), 1000 and 910 (C-H alkene out of plane bend), 825 and 710 (aromatic C-H out of plane bend); Mass: $[M+H]^+$ = m/z 494.0000, $[M+2]^+$ = m/z 496; UV (MeOH) λ_{max} (nm): 301 nm.



SI-Figure 37: ^{1}H NMR spectrum of 3,5-Bis ((E)-2-chloro-5-(trifluoro)benzylidene)-N-methyl-4-piperidone.



SI-Figure 38: ^{13}C NMR spectrum of 3,5-Bis ((E)-2-chloro-5-(trifluoro)benzylidene)-N-methyl-4-piperidone.

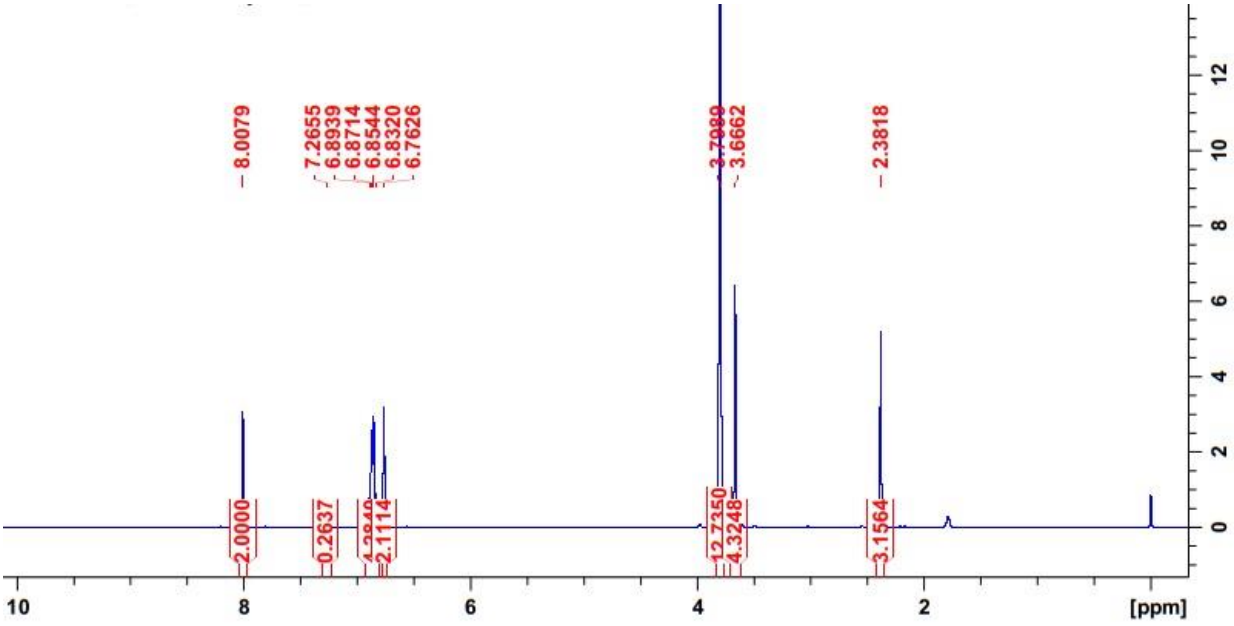


Mass: $[M+H]^+$ = m/z 494.0000

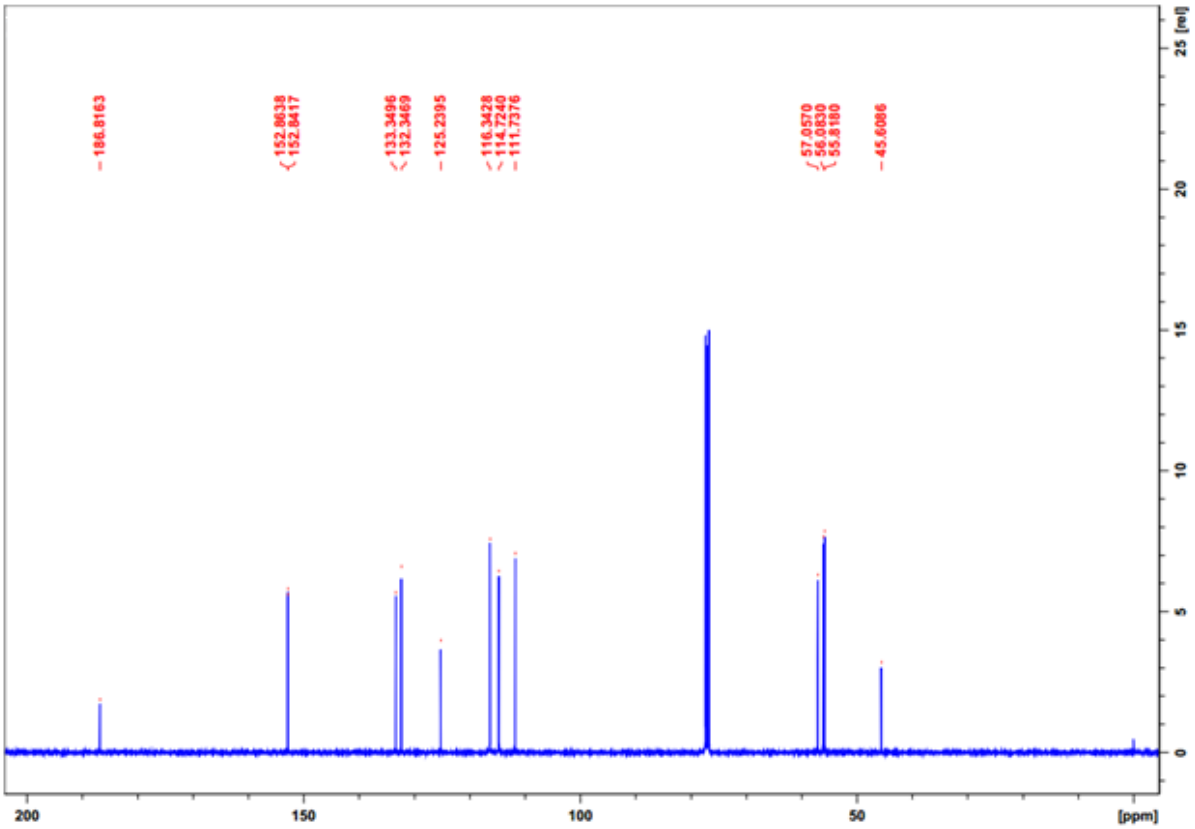
SI-Figure 39: Mass spectrum of 3,5-Bis ((E)-2-chloro-5-(trifluoro)benzylidene)-N-methyl-4-piperidone.

In) 3,5-Bis ((E)-2,5-dimethoxybenzylidene)-N-methyl-4-piperidone

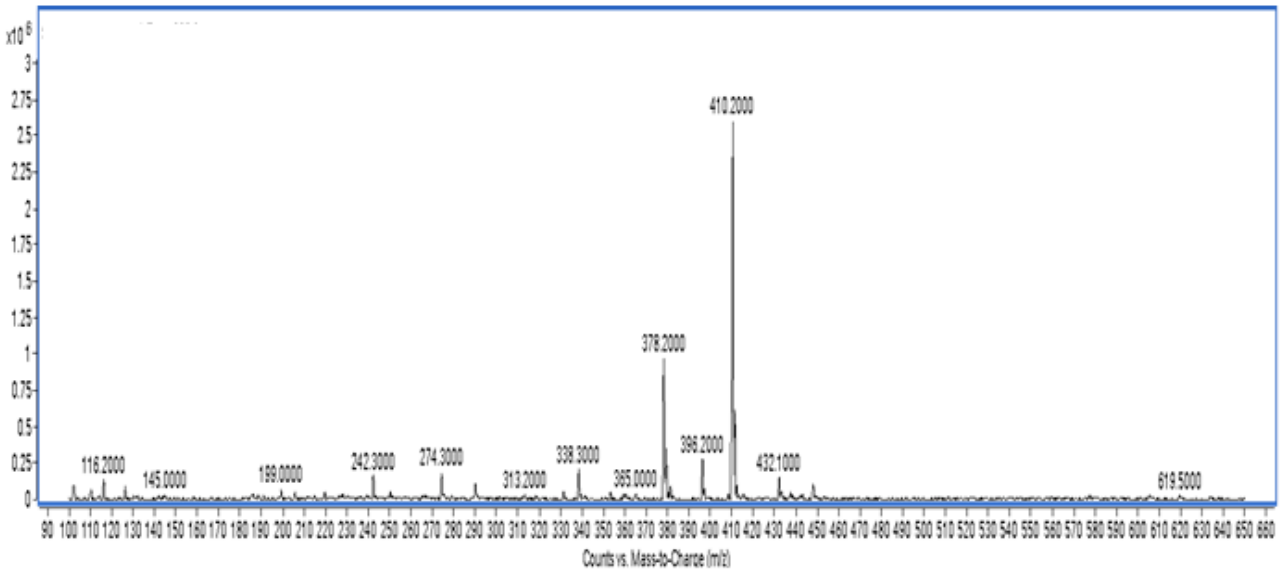
Yield: 75.2%. Yellow crystals. Recrystallized from 1. Chloroform-methanol 2. Pet ether-acetone; mp: 135-137.3^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.0 (H-7, 2H, s), δ 6.88 (H-4', 2H, s), δ 6.84 (H-3' 2H, d, J = 8.96 Hz), δ 6.76 (H-6', 2H, d, J = 8.96 Hz), δ 3.79 (O-CH, 3H, s), δ 3.66 (H-2, H-6, 4H, s), δ 2.38, (N-CH_3, 3H, s). ^{13}C NMR (400 MHz, $CDCl_3$): δ 186.81 (C-4), δ 152.86 (C-2'), δ 152.84 (C-5'), δ 133.34 (C-5), δ 132.34 (C-7), δ 125.23 (C-1'), δ 116.34 (C-6'), δ 114.72 (C-4'), δ 111.73 (C-3'), δ 57.05 (O-CH), δ 56.08 (C-6) and δ 45.60 (N-C); IR (KBr disc) (cm^{-1}): 3007 (sp^2 C-H), 2940 (sp^3 C-H), 1663 (C=O), 1578 and 1524 (aromatic skeletal stretch), 1604 (C=C); Mass: $[M+H]^+$ = m/z 410.2000, $[M-31]^+$ = m/z 378.2000; UV (MeOH) λ_{max} (nm): 286 and 310 nm.



SI-Figure 40: ^{1}H NMR spectrum of 3,5-Bis ((E)-2,5-dimethoxybenzylidene)-N-methyl-4-piperidone.



SI-Figure 41: ^{13}C NMR spectrum of 3,5-Bis ((E)-2,5-dimethoxybenzylidene)-N-methyl-4-piperidone.

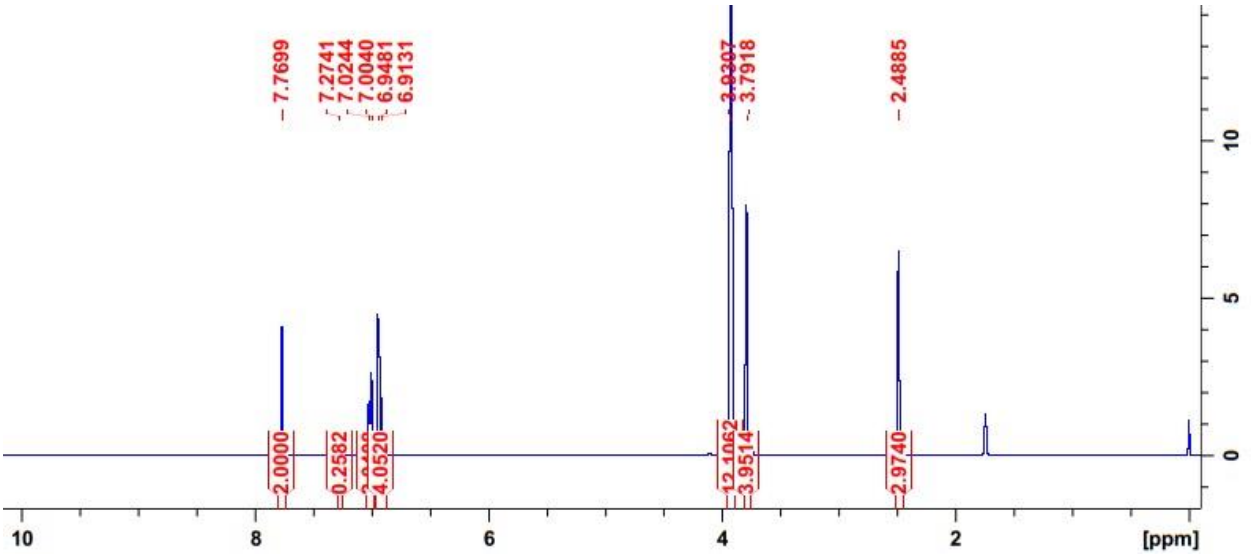


Mass: $[M+H]^+$ = m/z 410.2000

SI-Figure 42: Mass spectrum of 3,5-Bis ((E)-2,5-dimethoxybenzylidene)-N-methyl-4-piperidone.

Io) 3,5-Bis ((E)-3,4-dimethoxybenzylidene)-N-methyl-4-piperidone

Yield: 56.4%. Bright yellow crystals. Recrystallized from methanol-chloroform. mp: 167.2-169.7^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (H-7, 2H, s), δ 7.02 (H-6', 2H, d, J = 8.16 Hz), δ 6.94 (H-2', 2H, s), δ 6.92 (H-5', 2H, d, J = 8.16 Hz), δ 3.93 (O-CH_3 (3'), 3H, s), δ 3.91 (O-CH (4'), 3H, s), δ 3.79 (H-2, H-6, 4H, s), δ 2.48, (N-CH_3, 3H, s). ^{13}C NMR (400 MHz, $CDCl_3$): δ 186.68 (C-4), δ 149.91 (C-4'), δ 148.75 (C-3'), δ 136.31 (C-5), δ 131.54 (C-7), δ 128.25 (C-1'), δ 123.7 (C-6'), δ 113.85 (C-2'), δ 110.93 (C-5'), δ 111.73 (C-3'), δ 57.14 (O-CH), δ 55.94 (C-6) and δ 45.82 (N-C); IR (KBr disc) (cm^{-1}): 3063 (sp^2 CH), 2985 (sp^3 C-H), 1660 (C=O), 1600 (C=C), 1576 and 1514 (aromatic skeletal band), 1147 and 1022 (C-O); Mass: $[M+H]^+$ = m/z 410.2000 and $[M-OCH_3]^+$ = m/z 378.2000; UV (MeOH) λ_{max} (nm): 256 and 379 nm.



SI-Figure 43: ^{1}H NMR spectrum of 3,5-Bis ((E)-3,4-dimethoxybenzylidene)-N-methyl-4-piperidone.

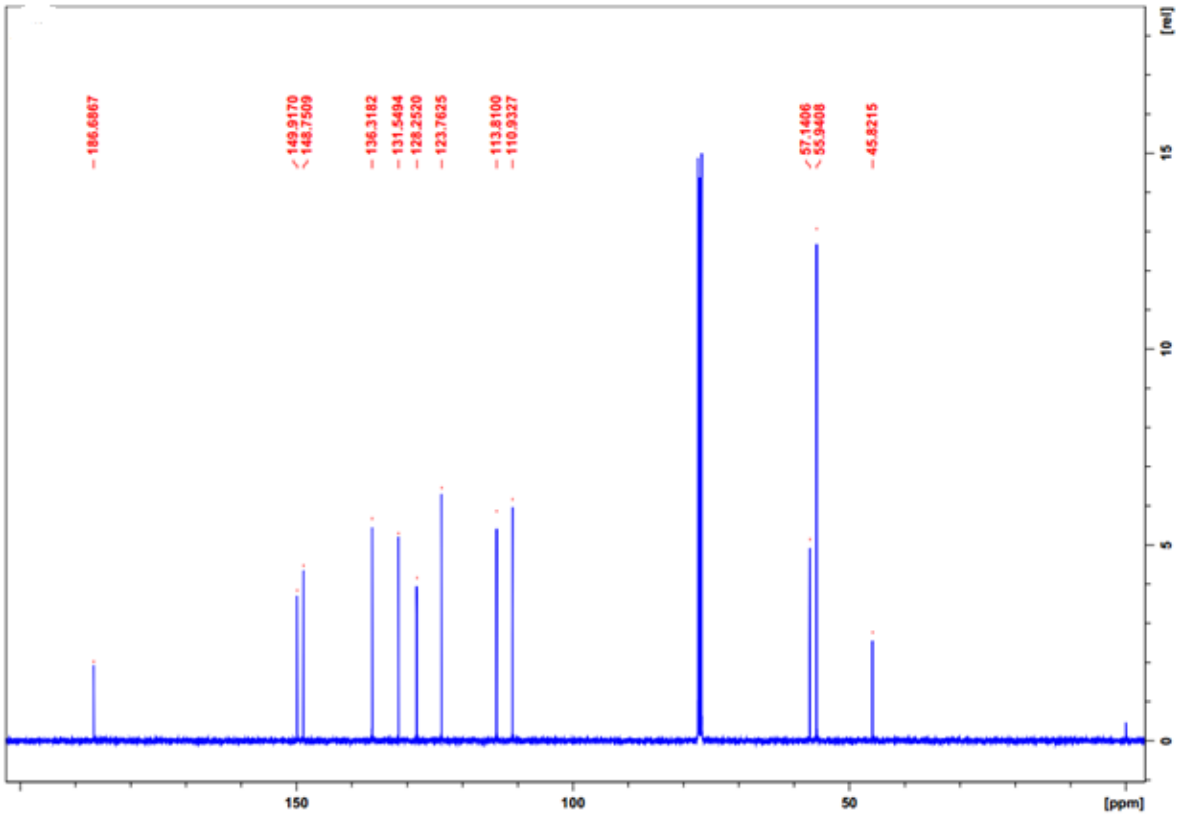
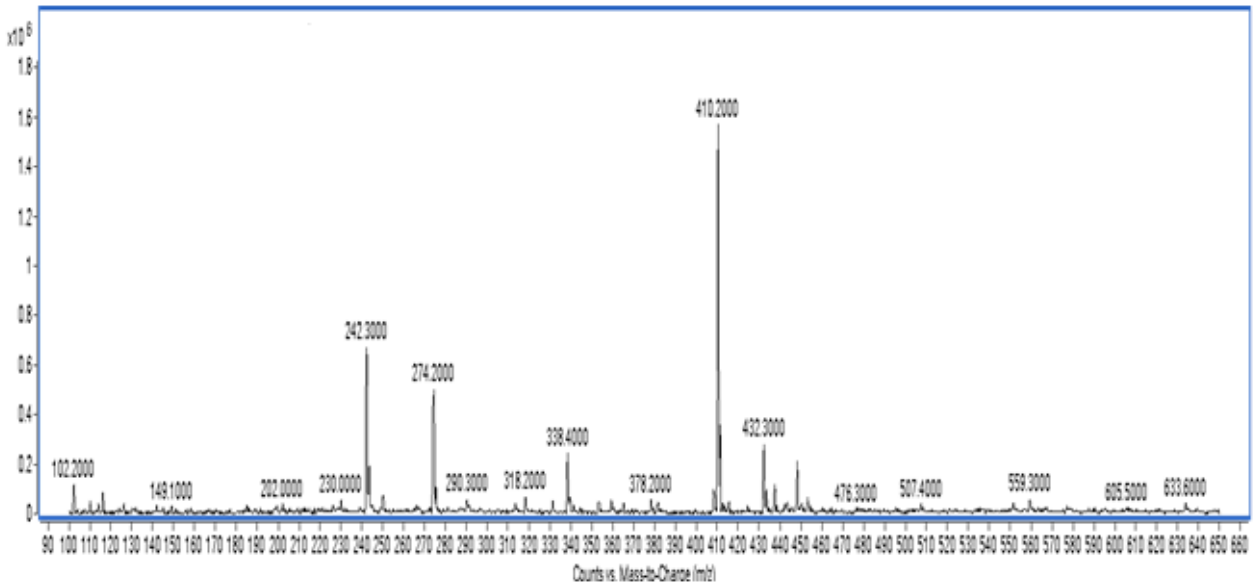


Figure 44: ^{13}C NMR spectrum of 3,5-Bis ((E)-3,4-dimethoxybenzylidene)-N-methyl-4-piperidone.

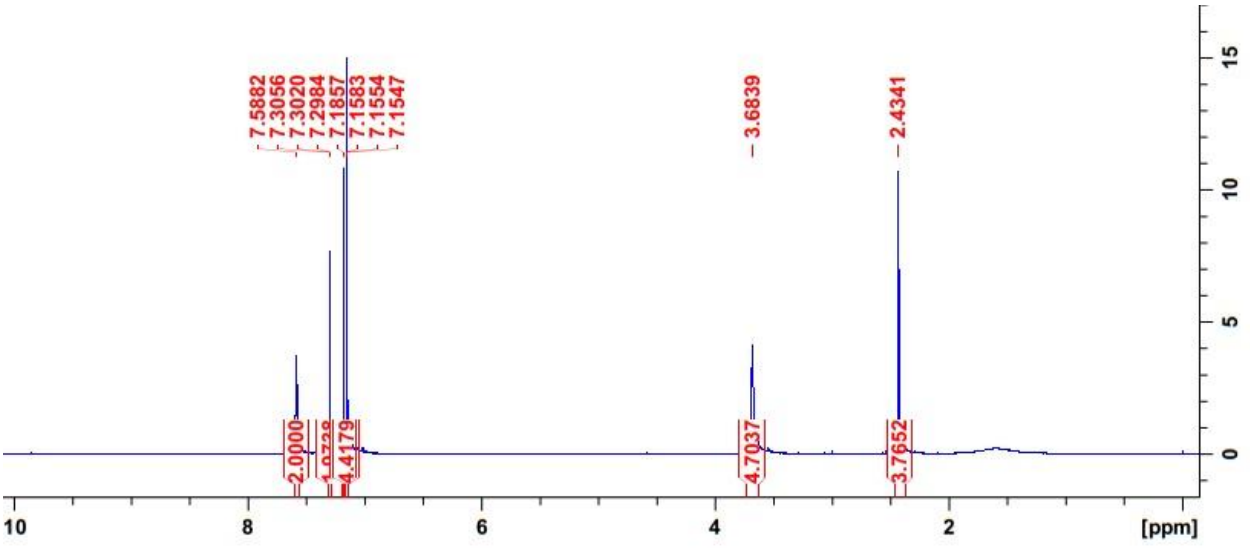


Mass: $[M+H]^+$ = m/z 410.2000

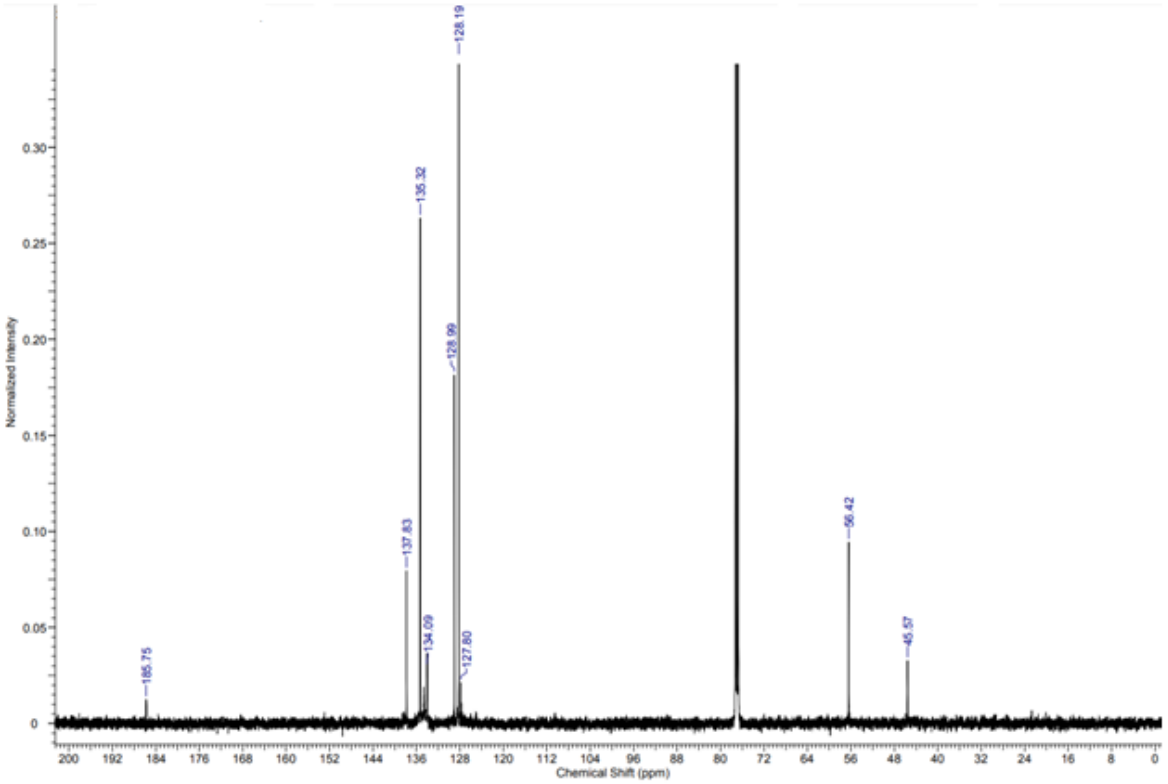
SI-Figure 45: Mass spectrum of 3,5-Bis ((E)-3,4-dimethoxybenzylidene)-N-methyl-4-piperidone.

Ip) 3,5-Bis ((E)-3,5-dichlorobenzylidene)-N-methyl-4-piperidone

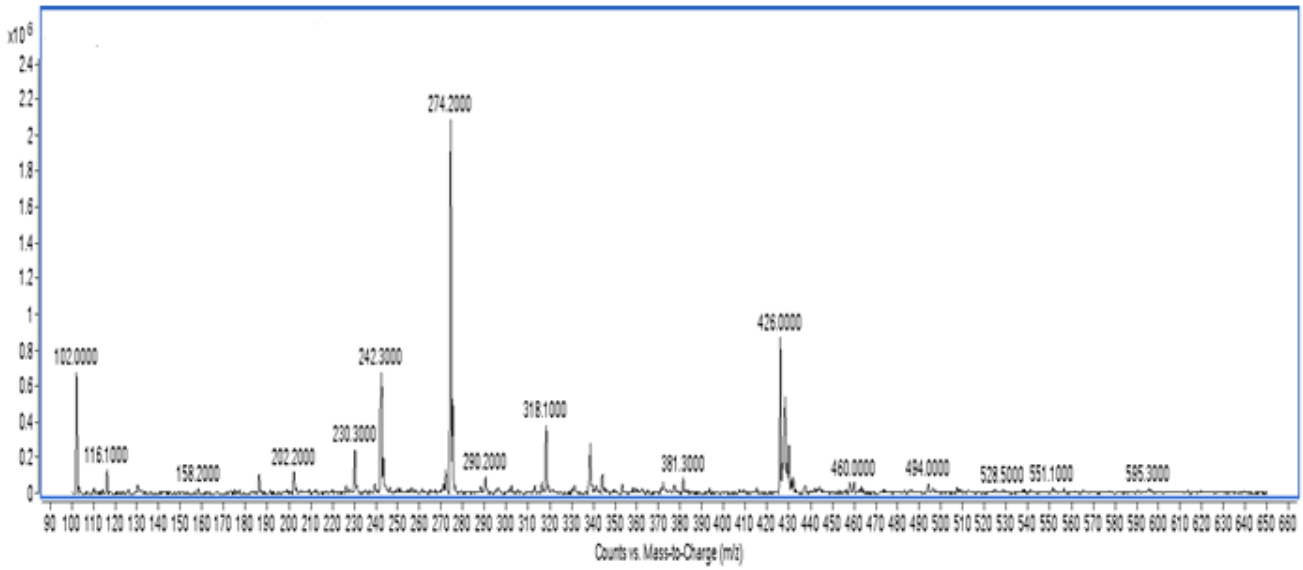
Yield: 75%. Yellow solid. Recrystallized from methanol-chloroform. mp: 186.3-188.6^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.58 (H-7, 2H, s), δ 7.30 (H-4', 2H, t, J = 1.44 Hz), δ 7.158 (H-6', 2H, d, J = 0.416 Hz), δ 7.155 (H-2', 2H, dd, J = 1.44 and 0.416 Hz), δ 3.73 (H-2, H-6, 4H, s), δ 2.47, (N-CH_3, 3H, s). ^{13}C NMR (400 MHz, $CDCl_3$): δ 185.75 (C-4), δ 137.83 (C-7), δ 135.32 (C-3' and 5'), δ 134.09 (C-3), δ 128.99 (C-4'), δ 128.19 (C-2'), δ 127.80 (C-1'), δ 56.42 (C-2 and C-6) and δ 45.57 (N-C); IR (KBr disc) (cm^{-1}): 3070 (sp^2 C-H), 2950 (sp^3 C-H), 1675 (C=O), 1615 (C=C), 1590 and 1570 (aromatic skeletal stretch), 1095 (aromatic C-Cl), 1005 and 930 (C-H alkene out of plane bend), 870 and 675 (aromatic C-H out of plane bend); Mass: $[M+H]^+$ = m/z 426.0000, $[M-HCl]^+$ = m/z 318.1000; UV (MeOH) λ_{max} (nm): 311 and 250 nm.



SI-Figure 46: ^{1}H NMR spectrum of 3,5-Bis ((E)-3,5-dichlorobenzylidene)-N-methyl-4-piperidone.



SI-Figure 47: ^{13}C NMR spectrum of 3,5-Bis ((E)-3,5-dichlorobenzylidene)-N-methyl-4-piperidone.

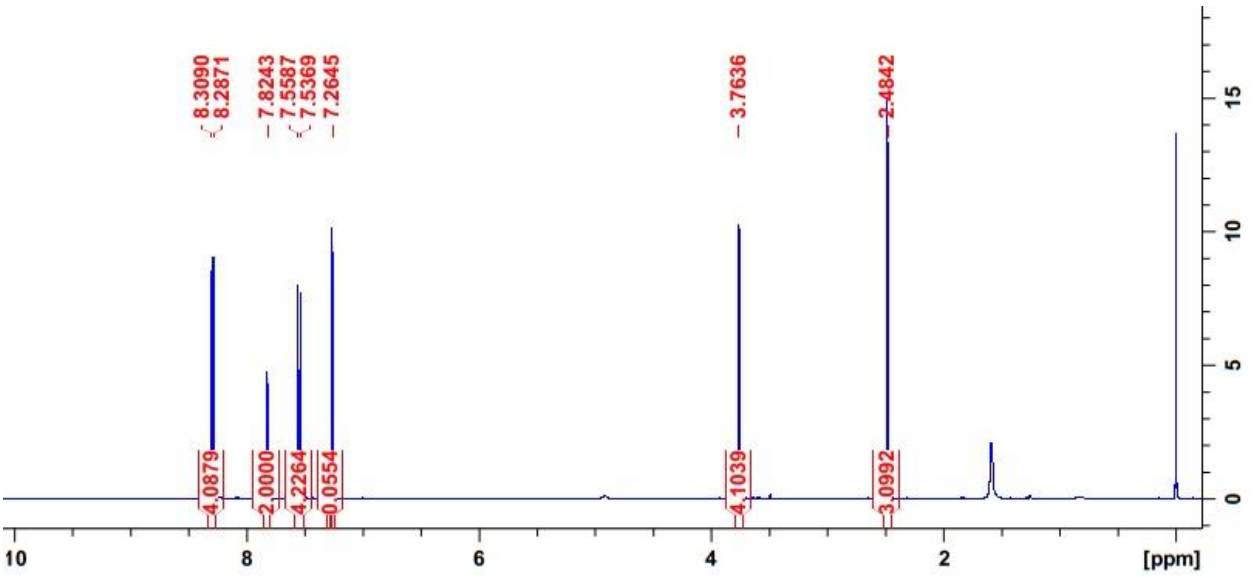


Mass: $[M+H]^+$ = m/z 426.0000

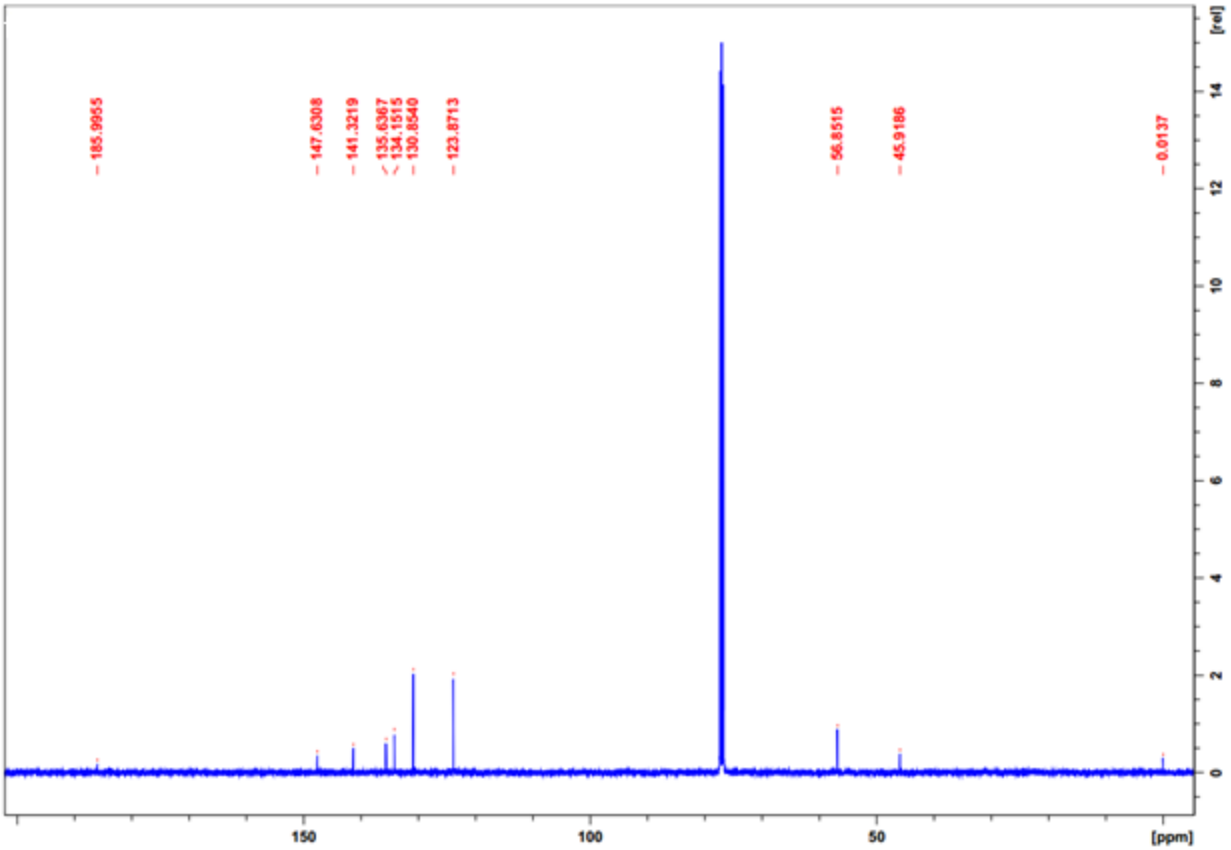
SI-Figure 48: Mass spectrum of 3,5-Bis ((E)-3,5-dichlorobenzylidene)-N-methyl-4-piperidone.

Iq) 3,5-Bis ((E) 4-nitrobenzylidene)-N-methyl-4-piperidone

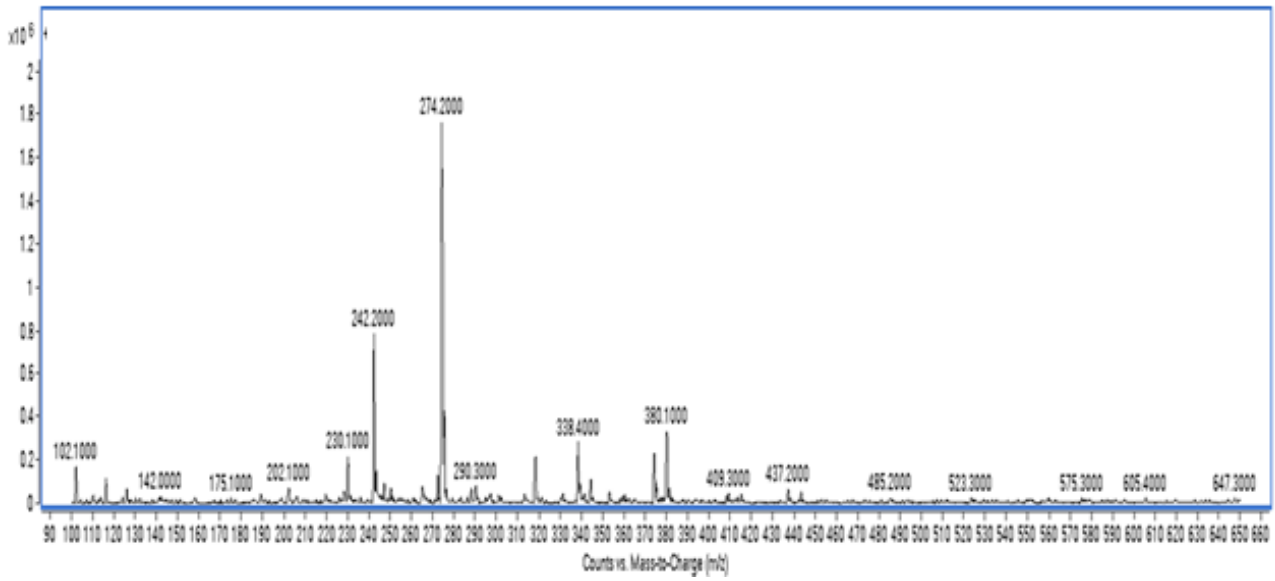
Yield: 65%. Brown powder. Recrystallized from chloroform- methanol. mp: 230.5-231.2^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) : δ 8.29 (H-3' and H-5', 4H, d, J = 8.72 Hz), δ 7.82 (H-7, 2H, s), δ 7.55 (H-2' and H-6', 4H, d, J = 8.72 Hz), δ 3.76 (H-2, H-6, 4H, s), δ 2.48 (N-CH_3, 3H, s). ^{13}C NMR (400 MHz, $CDCl_3$): δ 185.9 (C-4), δ 147.63 (C-4'), δ 141.3 (C-5), δ 135.63 (C-7), δ 134.15 (C-1'), δ 130.85 (C-3' and C-5'), δ 123.87 (C-2' and C-6'), δ 56 (C-6) and δ 45 (N-C). Mass: $[M+H]^+$ = m/z 380.1000; UV (MeOH) λ_{max} (nm): 331 and 272; IR (KBr disc) (cm^{-1}): 3070 (sp^2 C-H), 2928 (sp^3 C-H), 1670 (C=O), 1612 (C=C), 1592 (aromatic skeletal stretch), 1540 and 1350 (NO_2 stretch), 932 (C-H alkene out of plane bend), 895, 800 and 750 (aromatic C-H out of plane bend).



SI-Figure 49: ^{1}H NMR spectrum of 3,5-Bis ((E) 4-nitrobenzylidene)-N-methyl-4-piperidone.



SI-Figure 50: ^{13}C NMR spectrum of 3,5-Bis ((E) 4-nitrobenzylidene)-N-methyl-4-piperidone.

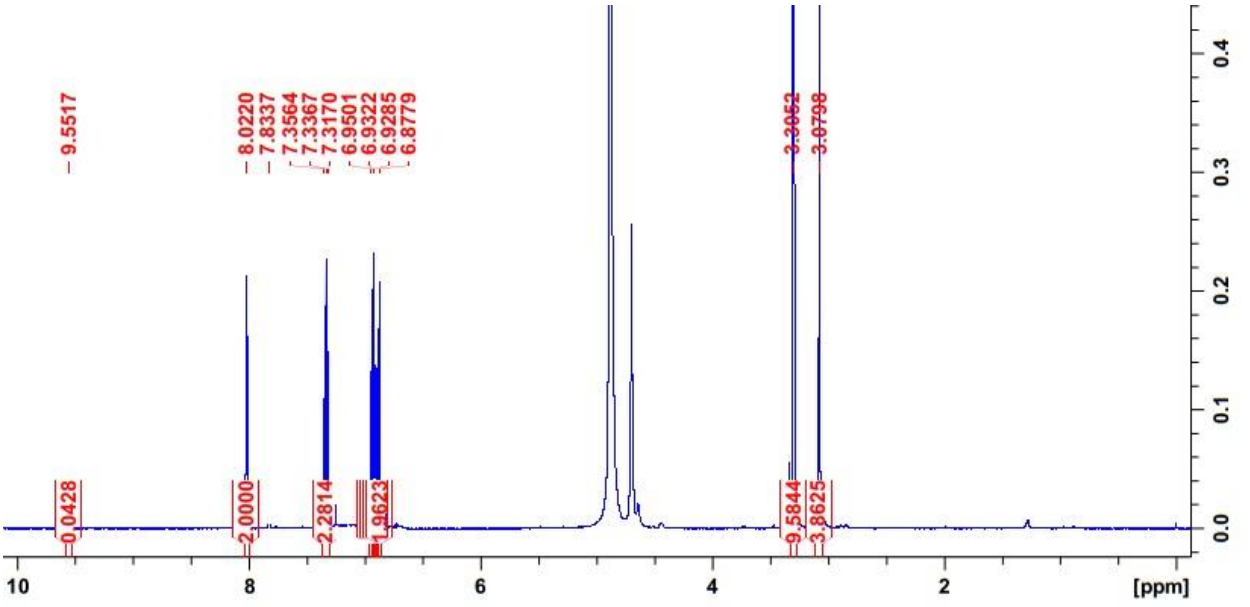


Mass: $[M+H]^+$ = m/z 380.1000

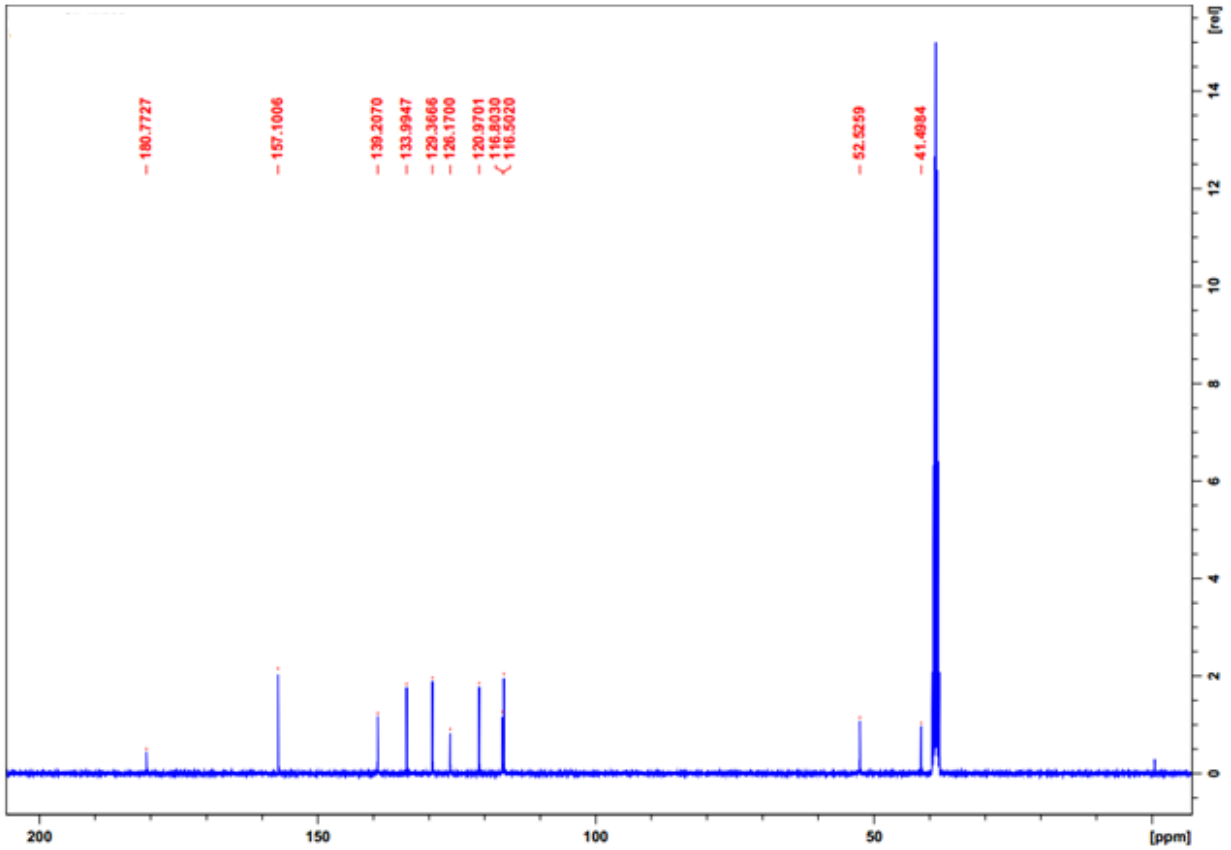
SI-Figure 51: Mass spectrum of 3,5-Bis ((E) 4-nitrobenzylidene)-N-methyl-4-piperidone.

Ir) 3,5-Bis ((E) 3-hydroxybenzylidene)-N-methyl-4-piperidone

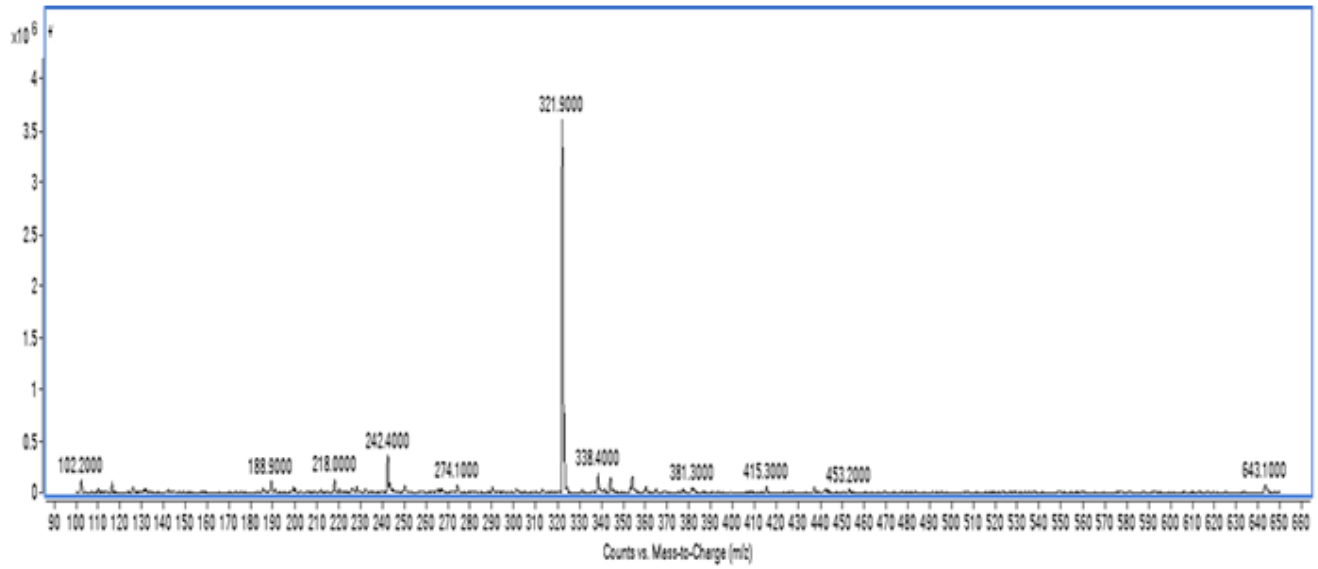
Yield: 83.8%. Light yellow powder. Chloroform and methanol were recrystallization solvents. mp: 284-286^{o}C. ^{1}H NMR (400 MHz, CD_3OH) : δ 9.55 (O-H) δ 7.8 (H-7, 2H, s), δ 7.33 (H-5$^{'}$, 2H, t, J = 7.88 Hz), δ 6.95-6.87 (H-2$^{'}$, H-4$^{'}$, H-6$^{'}$, 6H, overlapping signals), δ 3.30 (H-2, H-6, 4H,s), δ 3.07 (N-CH_3, 3H, s). ^{13}C NMR (400 MHz, CD_3OH) : δ 180.77 (C-4), δ 157.1 (C-3$^{'}$), δ 139.20 (C-5), δ 133.99 (C-5'), δ 129.36 (C-7), δ 126.17 (C-1$^{'}$), δ 120.97 (C-6'), δ 116.5 (C-2$^{'}$), δ 116.8 (C-4$^{'}$), δ 52.52 (C-6) and δ 41.4 (N-C); IR (KBr disc) (cm^{-1}): 3600-3200 (O-H), 3050 (sp^2 C-H), 2980 (sp^3 C-H), 1680 (C=O), 1610 (C=C), 1580 and 1480 (aromatic skeletal stretch), 1225 (C-O), 1005 and 995 (C-H alkene out of plane bend), 850 and 780 (aromatic C-H out of plane bend); Mass: $[M+H]^+$ = m/z 321.9000; UV (MeOH) λ_{max} (nm): 328 and 262 nm.



SI-Figure 52: ^{1}H NMR spectrum of 3,5-Bis ((E) 3-hydroxybenzylidene)-N-methyl-4-piperidone.



SI-Figure 53: ^{13}C NMR spectrum of 3,5-Bis ((E) 3-hydroxybenzylidene)-N-methyl-4-piperidone.

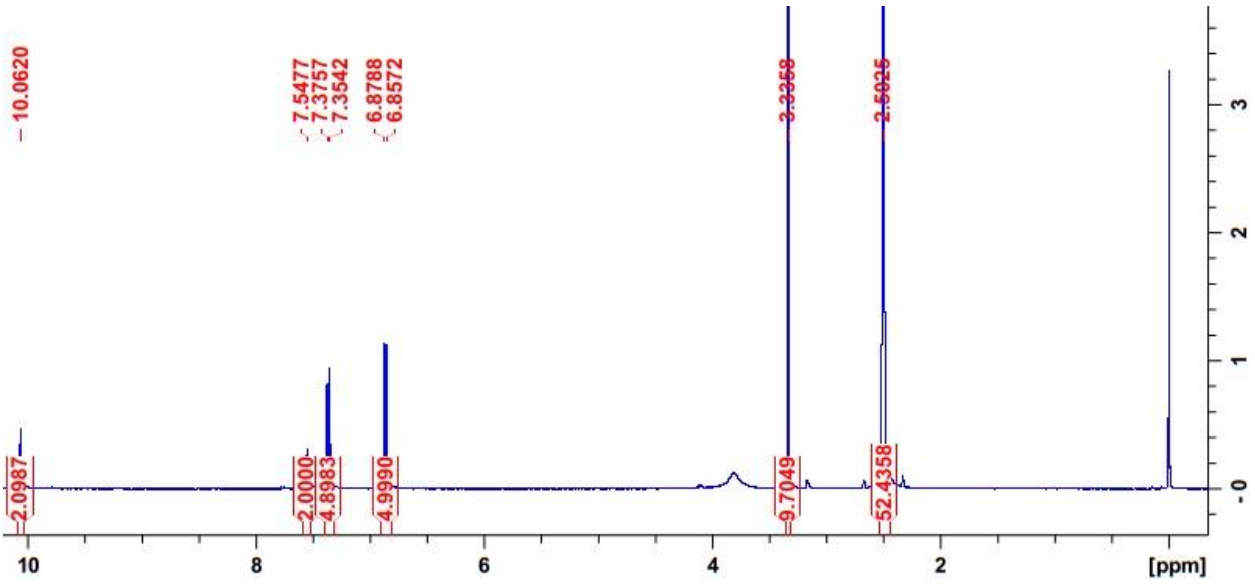


Mass: $[M+H]^+$ = m/z 321.9000

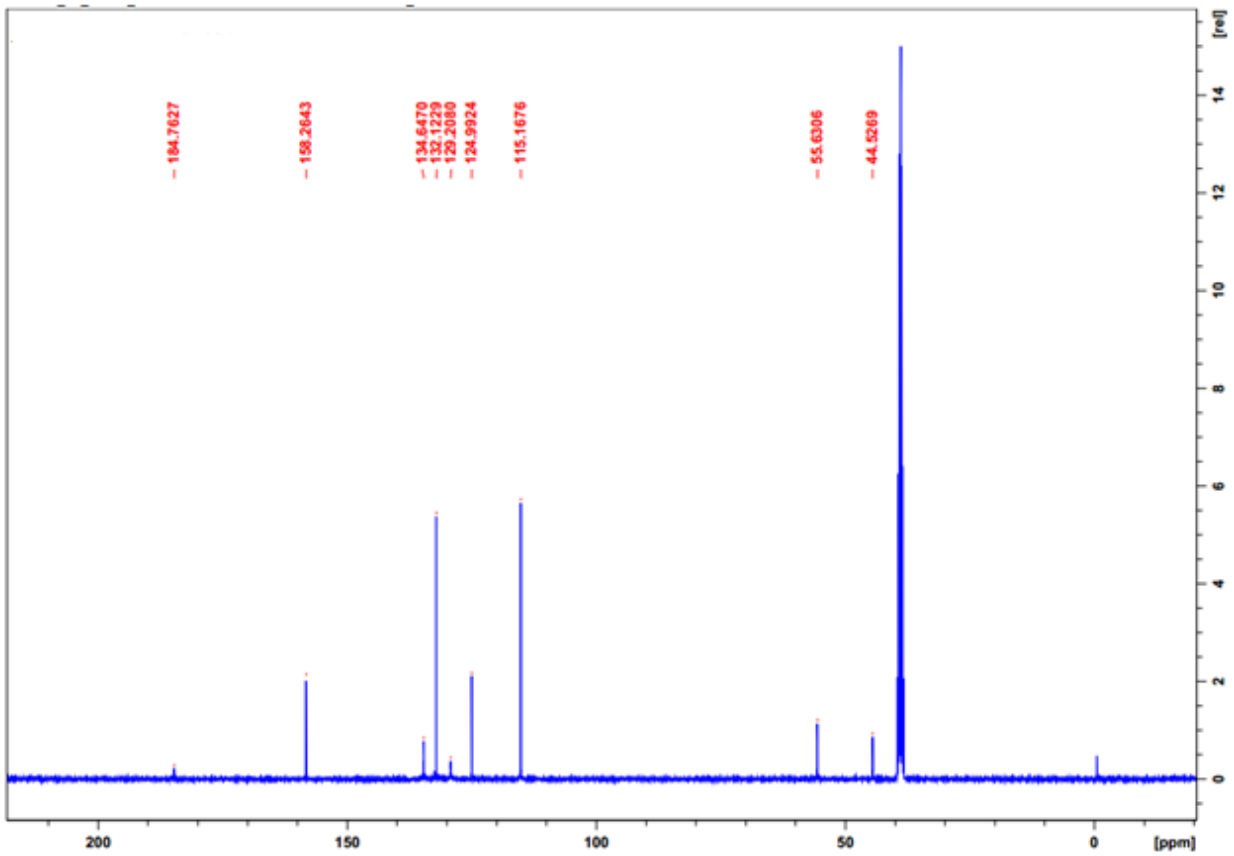
SI-Figure 54: Mass spectrum of 3,5-Bis ((E) 3-hydroxybenzylidene)-N-methyl-4-piperidone.

Is) 3,5-Bis ((E) 4-hydroxybenzylidene)-N-methyl-4-piperidone

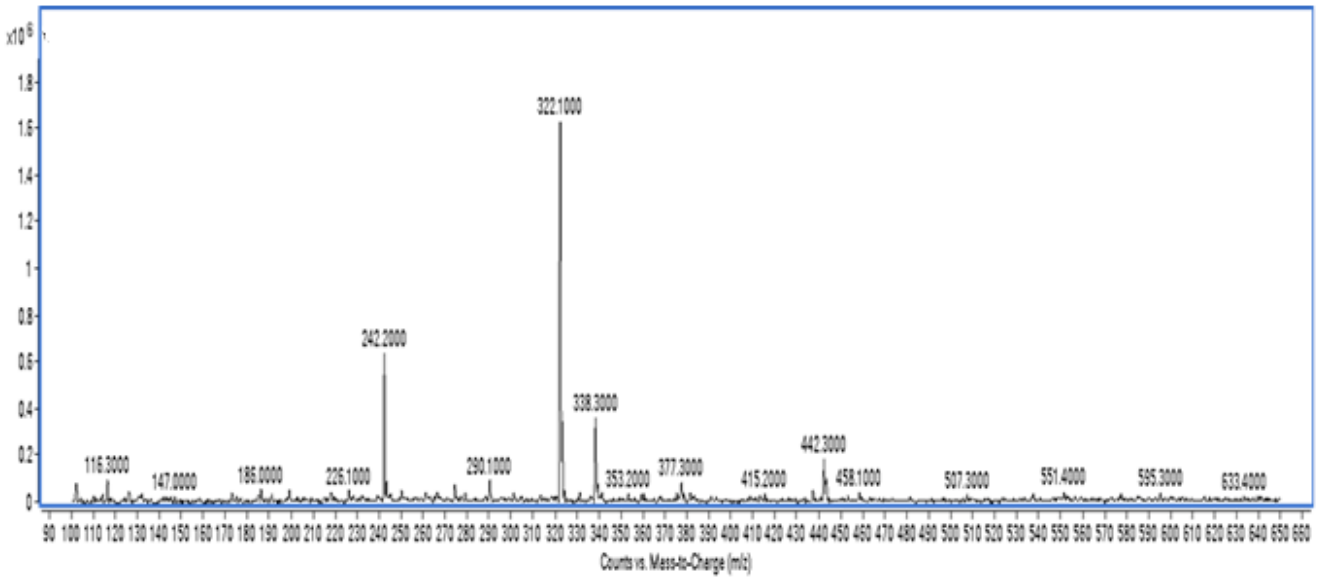
Yield: 84%. Orange powder. Recrystallized from chloroform-methanol. mp: 230.5-231.2^{o}C. ^{1}H NMR (400 MHz, DMSO-d6): δ 10.09 (O-H) δ 7.55 (H-7, 2H, s), δ 7.36 (H-2$^{'}$, H-6', 4H, d, J = 8.6 Hz), δ 6.87 (H-3$^{'}$, H-5', 4H, d, J = 8.6 Hz), δ 3.33 (H-2, H-6, 4H, s), δ 2.50, (N-CH_3, 3H, s). ^{13}C NMR (400 MHz, DMSO-d6): δ 184.7 (C-4), δ 158.26 (C-4$^{'}$), δ 134.6 (C-5), δ 132.12 (C-2$^{'}$ and 6$^{'}$), δ 129.20 (C-1$^{'}$), δ 124.9 (C-7), δ 115.16 (C-3$^{'}$ and 5$^{'}$), δ 55.6 (C-6) and δ 44.5 (N-C); IR (KBr disc) (cm^{-1}): 3600-3200 (O-H stretch), 3050 (sp^2 C-H stretch), 2990 (sp^3 C-H stretch), 1650 (C=O stretch), 1588 and 1572(Ar skeletal bands), 764 and 693 (mono substituted aromatic ring); Mass: $[M+H]^+$= m/z 322.1000; UV (MeOH) λmax (nm): 373 and 244.



SI-Figure 55: ^{1}H NMR spectrum of 3,5-Bis ((E) 4-hydroxybenzylidene)-N-methyl-4-piperidone.



SI-Figure 56: ^{13}C NMR spectrum of 3,5-Bis ((E) 4-hydroxybenzylidene)-N-methyl-4-piperidone.

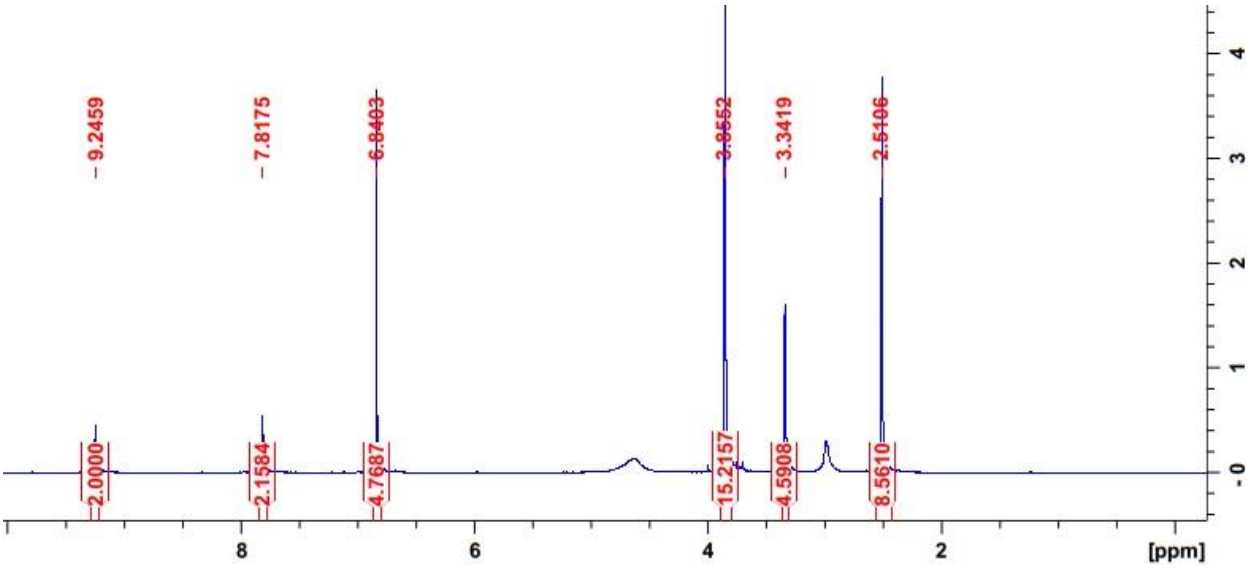


Mass: $[M+H]^+$= m/z 322.1000

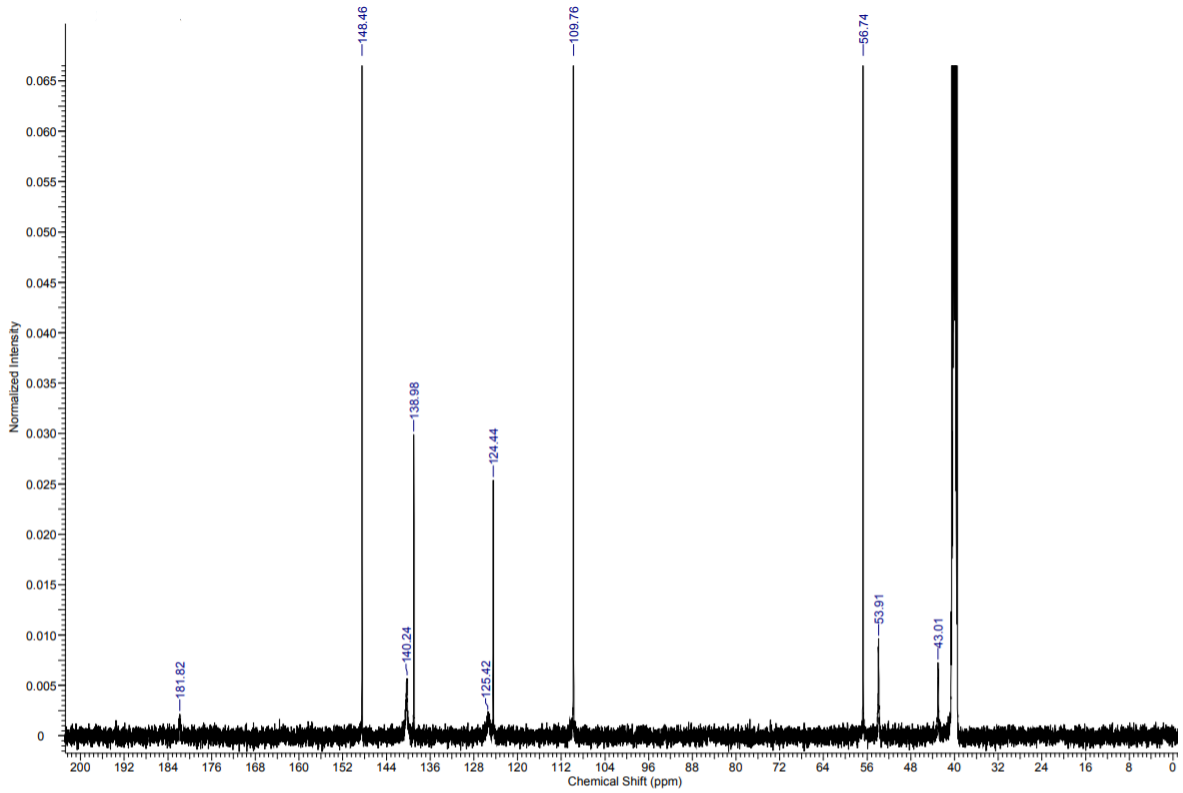
SI-Figure 57: Mass spectrum of 3,5-Bis ((E) 4-hydroxybenzylidene)-N-methyl-4-piperidone.

It) 3,5-Bis ((E) 4-hydroxy-3,5-dimethoxybenzylidene)-N-methyl-4-piperidone

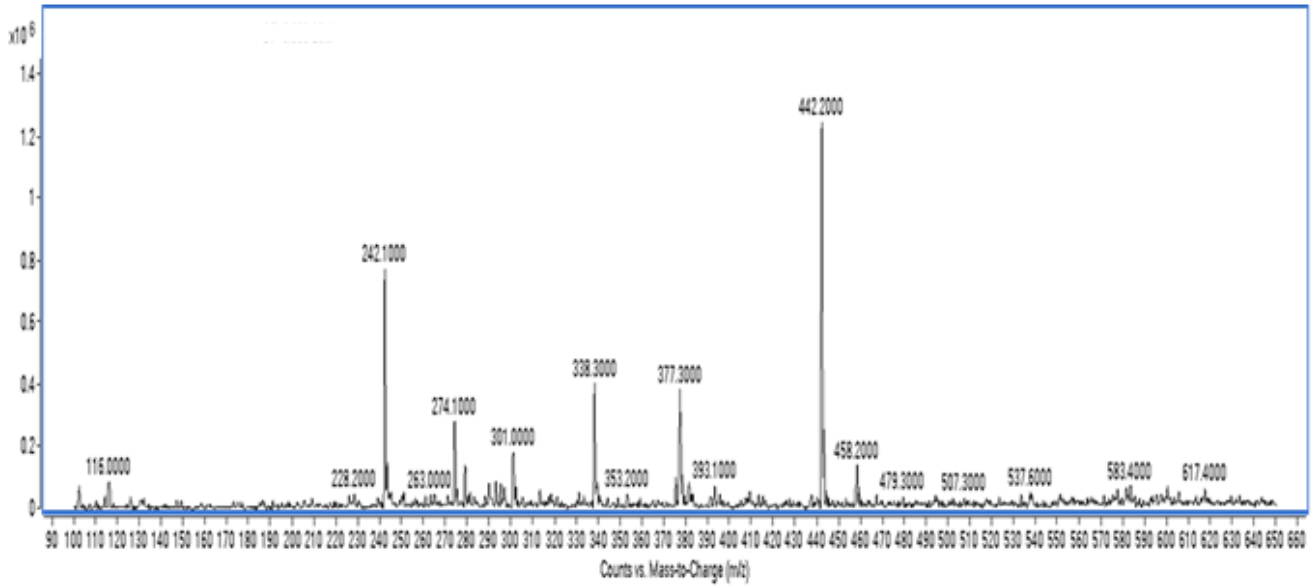
Yield: 67%. Obtained as red powder after recrystallization from methanol. mp: 237.2-238.4^{o}C. ^{1}H NMR (400 MHz, DMSO-d6): δ 9.24 (O-H) δ 7.81 (H-7, 2H, s), δ 6.84 (H-2$^{'}$, H-6', 4H), δ 3.85 (O-CH_3, 12H, s), δ 3.34 (H-2, H-6, 4H, s) δ 2.51, (N-CH_3, 3H, s). ^{13}C NMR (400 MHz, DMSO-d6): δ 181.82 (C-4), δ 148.46 (C-3' and 5$^{'}$), δ 140.24 (C-3), δ 138.98 (C-4$^{'}$), δ 125.42 (C-1'), δ 124.44 (C-7), δ 109.76 (C-2$^{'}$ and 6$^{'}$), δ 56.74 (O-CH_3), δ 53.91 (C-2 and C-6) and δ 43.01 (N-C); IR (KBr disc) (cm^{-1}): 3600-3200 (O-H), 3010 (sp^2 C-H), 2920 (sp^3 C-H), 1620 (C=O), 1600 (C=C), 1590 and 1570 (aromatic skeletal stretch), 1275 (C-O), 985 and 900 (C-H alkene out of plane bend), 820 and 790 (aromatic C-H out of plane bend).); Mass: $[M+H]^+$ = m/z 442.2000; UV (MeOH) λ_{max} (nm): 403 and 259 nm.



SI-Figure 58: ^{1}H NMR spectrum of 3,5-Bis ((E) 4-hydroxy-3,5-dimethoxybenzylidene)-N-methyl-4-piperidone.



SI-Figure 59: ^{13}C NMR spectrum of 3,5-Bis ((E) 4-hydroxy-3,5-dimethoxybenzylidene)-N-methyl-4-piperidone.

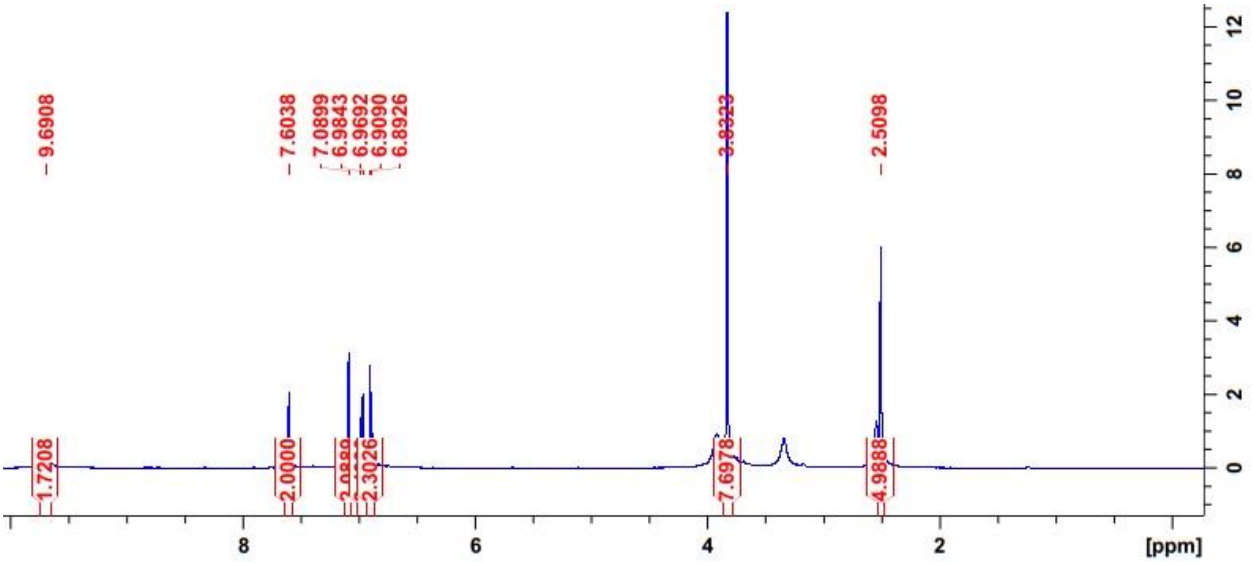


Mass: $[M+H]^+$ = m/z 442.2000

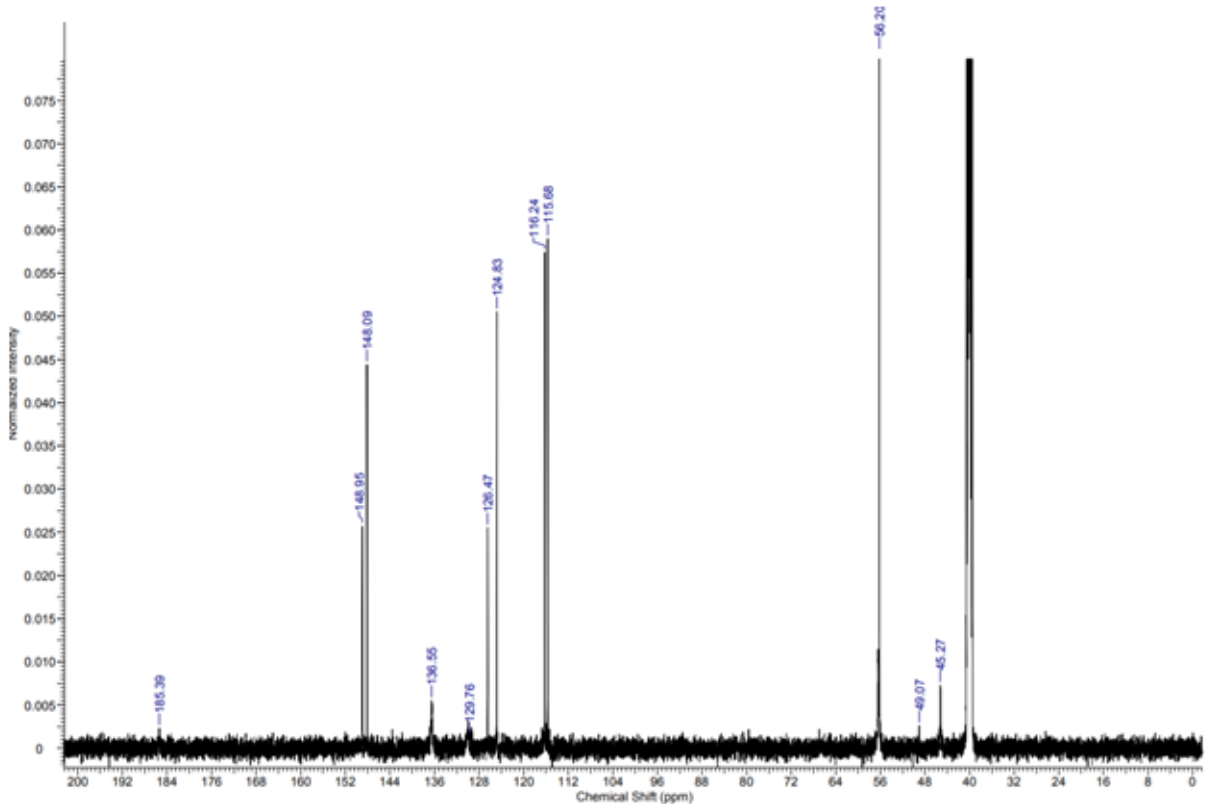
SI-Figure 60: Mass spectrum of 3,5-Bis ((E) 4-hydroxy-3,5-dimethoxybenzylidene)-N-methyl-4-piperidone.

Iu) 3,5-Bis ((E) 4-hydroxy-3-methoxybenzylidene)-N-methyl-4-piperidone

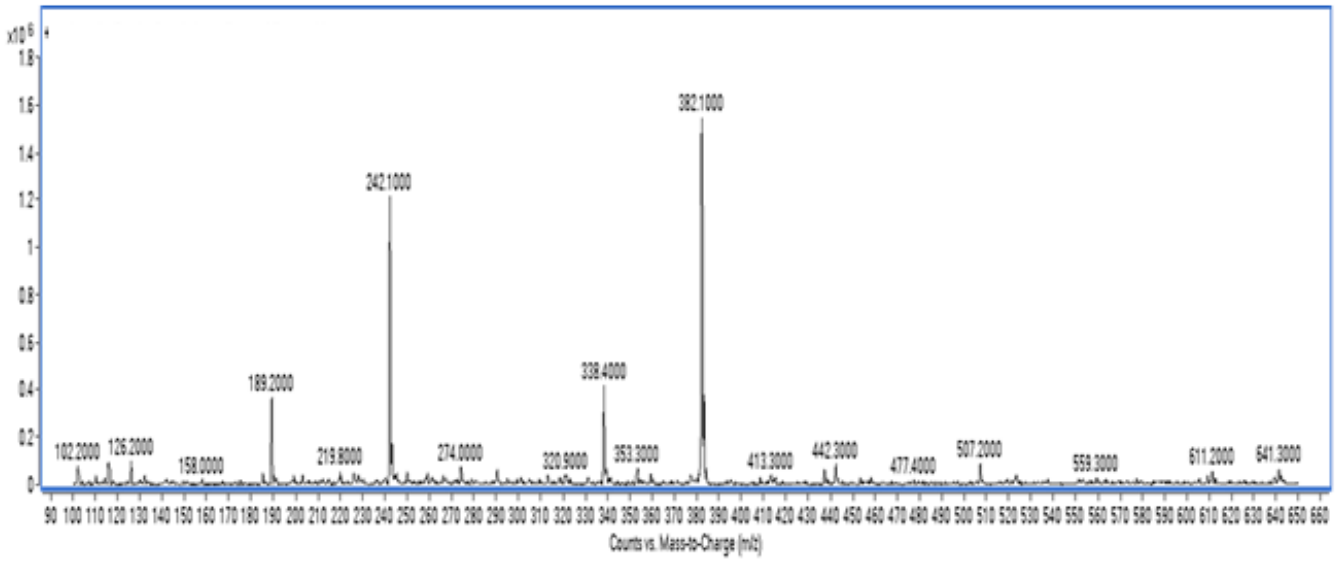
Yield: 30%. The compound after recrystallizing in methanol was obtained as dark green powder. mp: 217-220.5^{o}C. ^{1}H NMR (400 MHz, DMSO-d6): δ 9.69 (O-H) δ 7.60 (H-7, 2H, s), δ 7.08 (H-2$^{'}$, 2H), δ 6.97 (H-6$^{'}$, 2H, d, J = 6.04 Hz), δ 6.90 (H-5$^{'}$, 2H, d, J = 6.04 Hz), δ 3.83 (O-CH_3, 12H), δ 3.34 (H-2, H-6, 4H, s), δ 2.50, (N-CH_3, 3H, s). ^{13}C NMR (400 MHz, DMSO-d6): δ 185.39 (C-4), δ 148.95 (C-4'), δ 148.09 (C-3'), δ 136.55 (C-3), δ 129.76 (C-1'), δ 126.47 (C-7), δ 124.83 (C-6$^{'}$), δ 116.24 (C-2$^{'}$), δ 115.68 (C-5$^{'}$), δ 56.20 (O-CH_3), δ 49.07 (C-2 and C-6) and δ 45.27 (N-C); IR (KBr disc) (cm^{-1}): 3600-3200 (O-H), 3020 (sp^2 C-H), 2980 (sp^3 C-H), 1680 (C=O), 1600 (C=C), 1510 (aromatic skeletal stretch), 1280 (C-O), 990 and 910 (C-H alkene out of plane bend), 830 and 795 (aromatic C-H out of plane bend); Mass: $[M+H]^+$ = m/z 382.1000; UV (MeOH) λ_{max} (nm): 387, 265 and 257 nm.



SI-Figure 61: ^{1}H NMR spectrum of 3,5-Bis ((E) 4-hydroxy-3-methoxybenzylidene)-N-methyl-4-piperidone.



SI-Figure 62: ^{13}C NMR spectrum of 3,5-Bis ((E) 4-hydroxy-3-methoxybenzylidene)-N-methyl-4-piperidone.

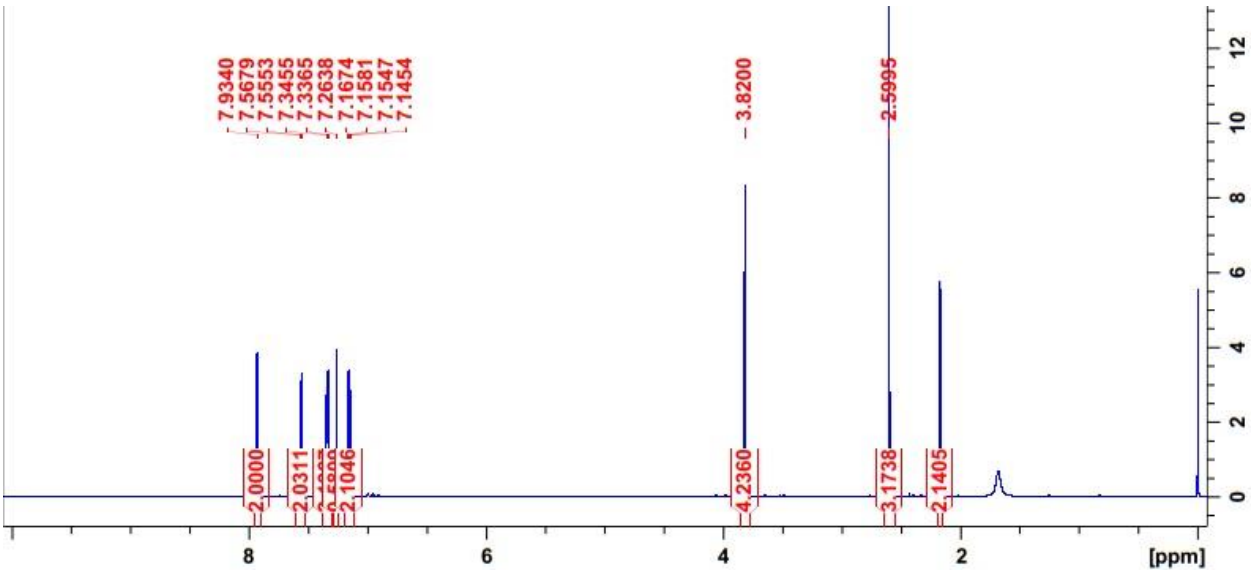


Mass: $[M+H]^+$ = m/z 382.1000

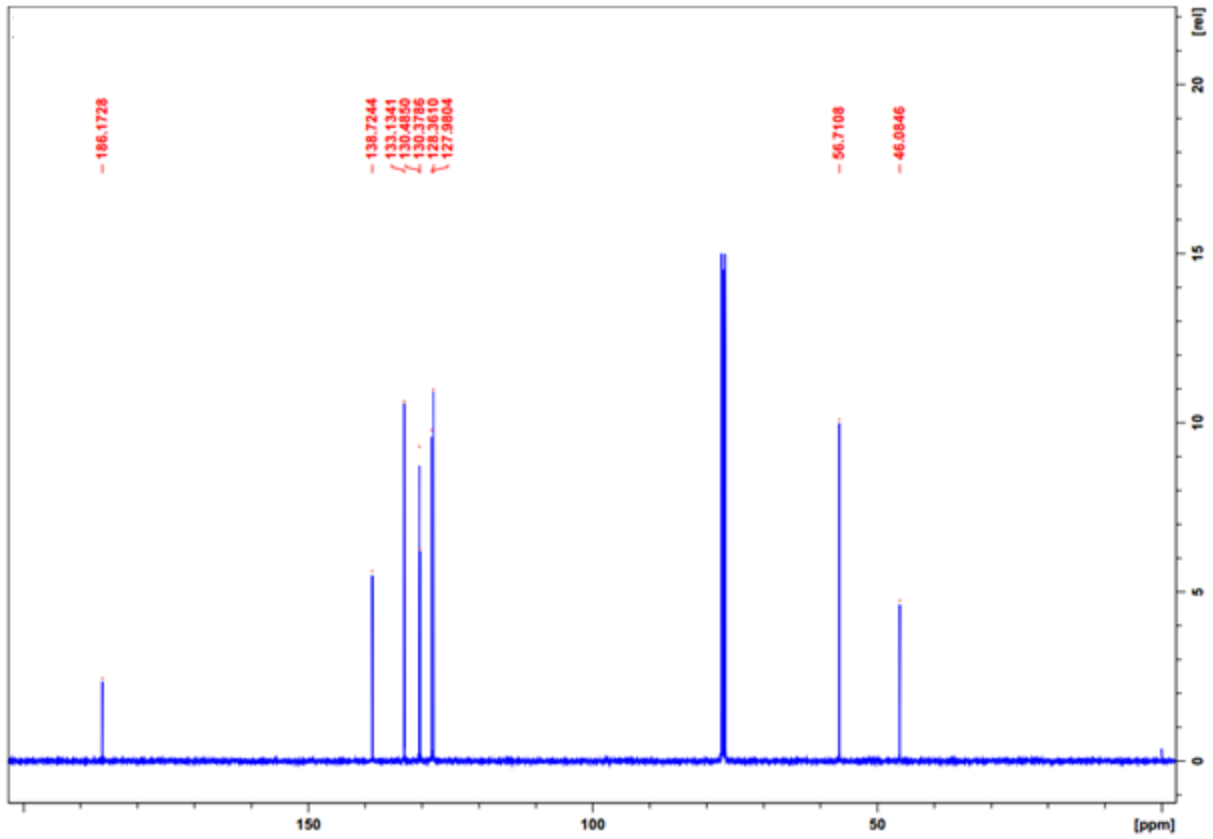
SI-Figure 63: Mass spectrum of 3,5-Bis ((E) 4-hydroxy-3-methoxybenzylidene)-N-methyl-4-piperidone.

IIa) 3,5-Bis ((E)-2-thiophenebenzylidene)-N-methyl-4-piperidone

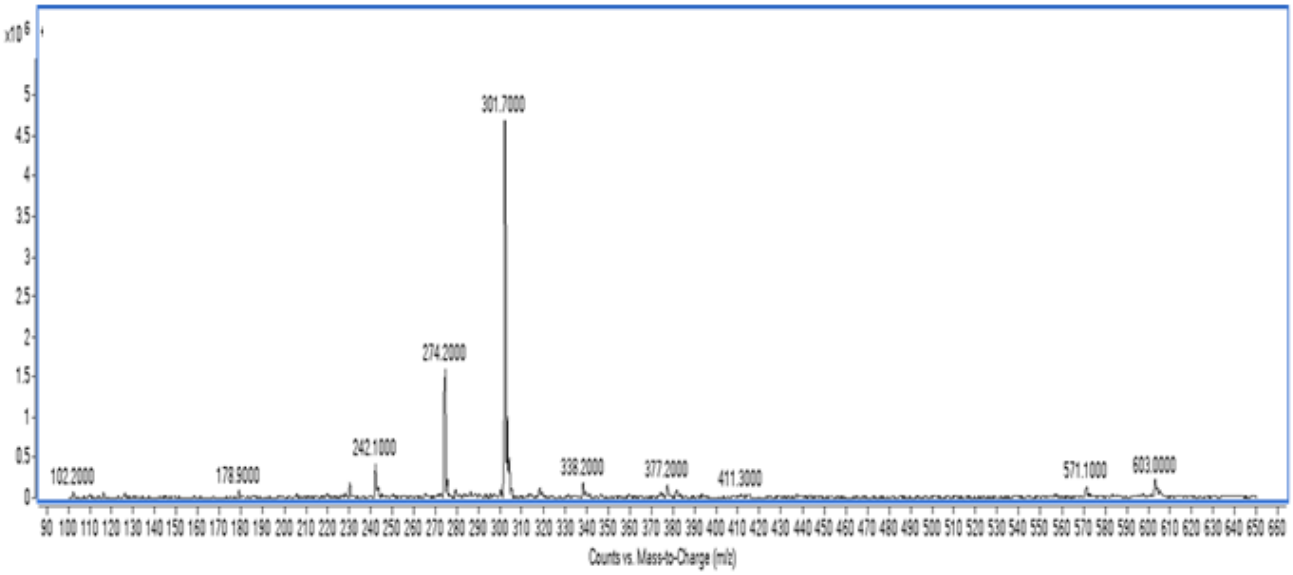
Yield: 81.5%. Light brown crystals. Recrystallized from methanol-chloroform. mp: 115-118.4^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93 (H-7, 2H, s), δ 7.56 (H-5', 2H, d, J = 3.6 Hz), δ 7.34 (H-3', 2H, d, J = 5.04 Hz), δ 7.15 (H-4', 2H, dd, J= 3.6 and 5.04 Hz), δ 3.82 (H-2, H-6, 4H, s), δ 2.59, (N-CH_3, 3H, s). ^{13}C NMR (400 MHz, $CDCl_3$): δ 186.17 (C-4), δ 138.72 (C-6), δ 133.13 (C-7), δ 130.48 (C-1'), δ 130.47 (C-2'), δ 128.36 (C-4'), δ 127.98 (C-3'), δ 56.71 (C-6) and δ 46.08 (N-C); IR (KBr disc) (cm^{-1}): 3080 (sp^2 C-H), 2968 (sp^3 C-H), 1663 (C=O), 1575 and 1505 (aromatic skeletal stretch), 1604 (C=C), 732 and 652 (C-H); Mass: m/z 301.7000, $[M+2]^+$ = m/z 303, $[M-123]^+$ = m/z 178.9000; UV (MeOH) λ_{max} (nm): 256 and 376 nm.



SI-Figure 64: ^{1}H NMR spectrum of 3,5-Bis ((E)-2-thiophenebenzylidene)-N-methyl-4-piperidone.



SI-Figure 65: ^{13}C NMR spectrum of 3,5-Bis ((E)-2-thiophenebenzylidene)-N-methyl-4-piperidone.

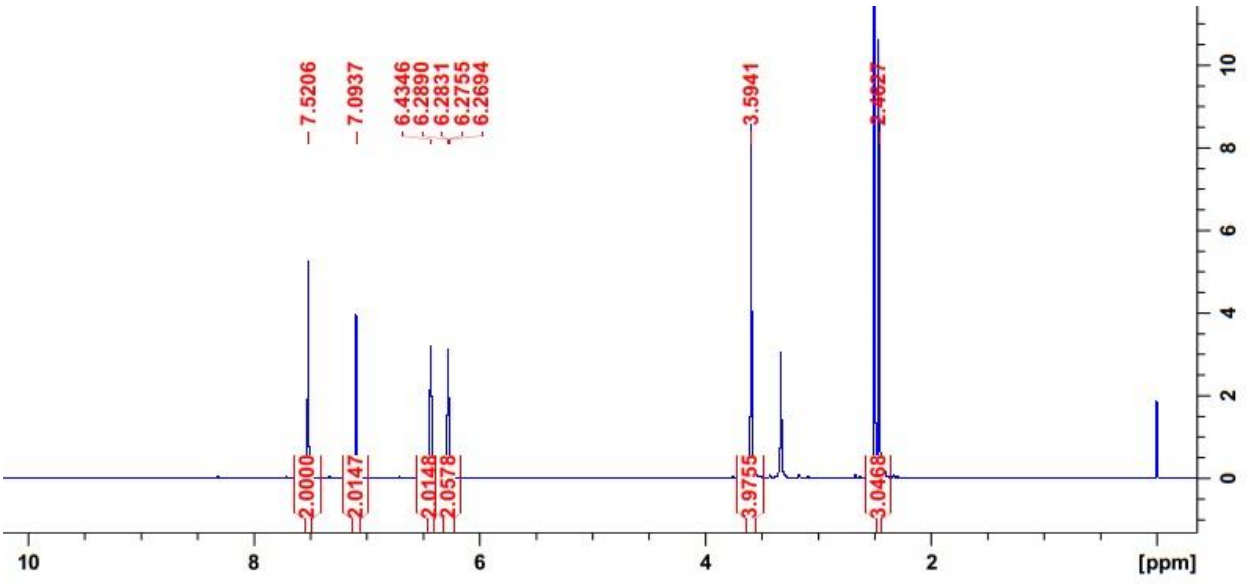


Mass: m/z 301.7000

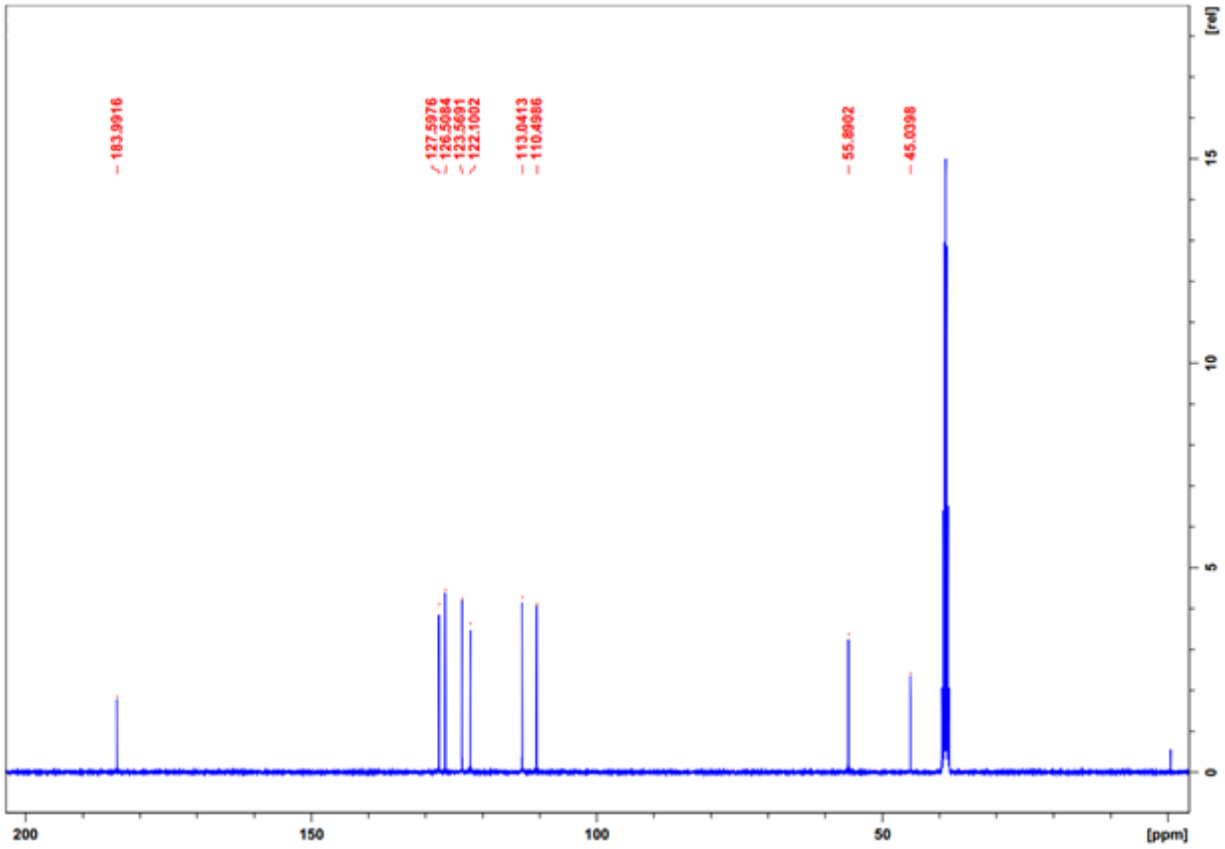
SI-Figure 66: Mass spectrum of 3,5-Bis ((E)-2-thiophenebenzylidene)-N-methyl-4-piperidone.

IIb) 3,5-Bis ((E) pyrrole-2-benzylidene)-N-methyl-4-piperidone

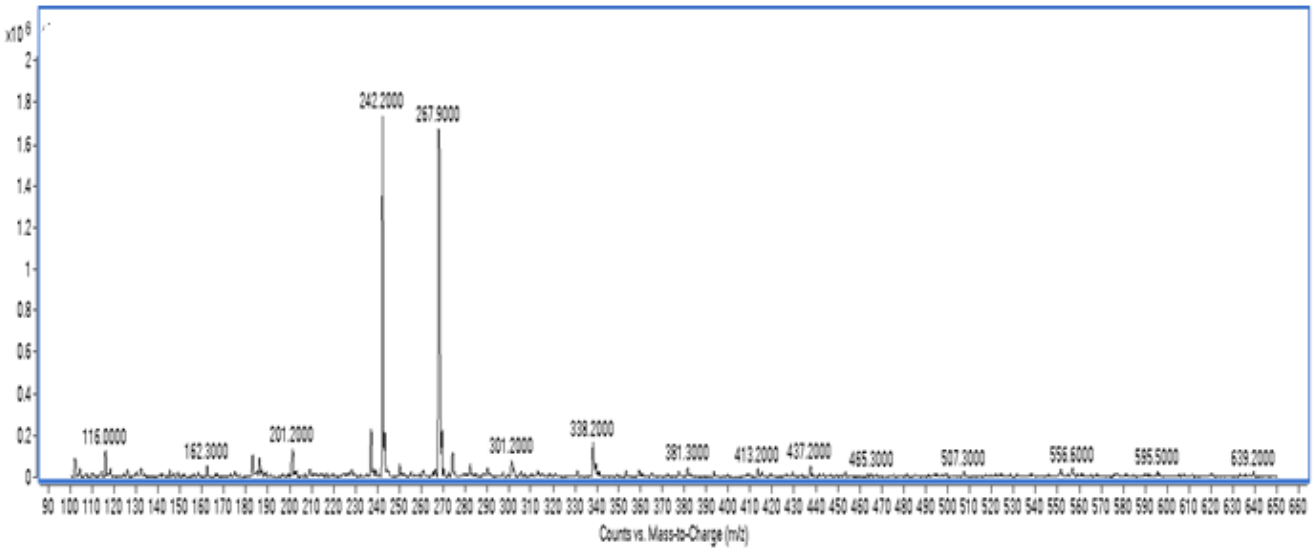
Yield: 58%. Red powder. Recrystallized from chloroform-methanol. mp: 214-215.4^{o}C. ^{1}H NMR (400 MHz, DMSO-d6): δ 7.52 (H-7, 2H, s), δ 7.09 (H-5', 2H, d), δ 6.43 (H-3', 2H, d), δ 6.28 (H-4', 2H, dd), δ 3.59 (H-2, H-6, 4H, s), δ 2.46, (N-CH_3, 3H, s). ^{13}C NMR (400 MHz, DMSO-d6): δ 183.99 (C-4), δ 127.59 (C-5), δ 126.50 (C-7), δ 123.56 (C-2'), δ 122.10 (C-5'), δ 113.04 (C-4'), δ 110.49 (C-3'), δ 55.80 (C-6) and δ 45.0 (N-C); IR (KBr disc) (cm^{-1}): 2995 (sp^2 C-H), 2950 (sp^3 C-H), 1635 (C=O), 1600 (C=C), 1550 (aromatic skeletal stretch), 1300 (C-N), 1000 and 920 (C-H alkene out of plane bend), 880 and 720 (aromatic C-H out of plane bend); Mass: m/z 267.9000; UV (MeOH) λ_{max} (nm): 436 and 252 nm.



SI-Figure 67: ^{1}H NMR spectrum of 3,5-Bis ((E) pyrrole-2-benzylidene)-N-methyl-4-piperidone.



SI-Figure 68: ^{13}C NMR spectrum of 3,5-Bis ((E) pyrrole-2-benzylidene)-N-methyl-4-piperidone.

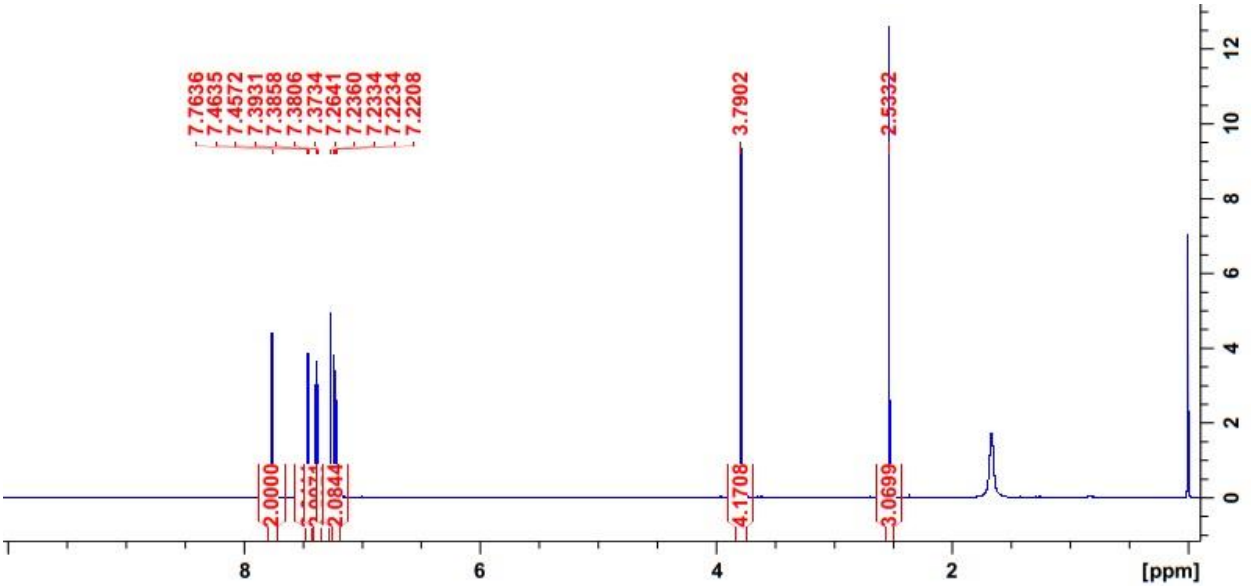


Mass: m/z 267.9000

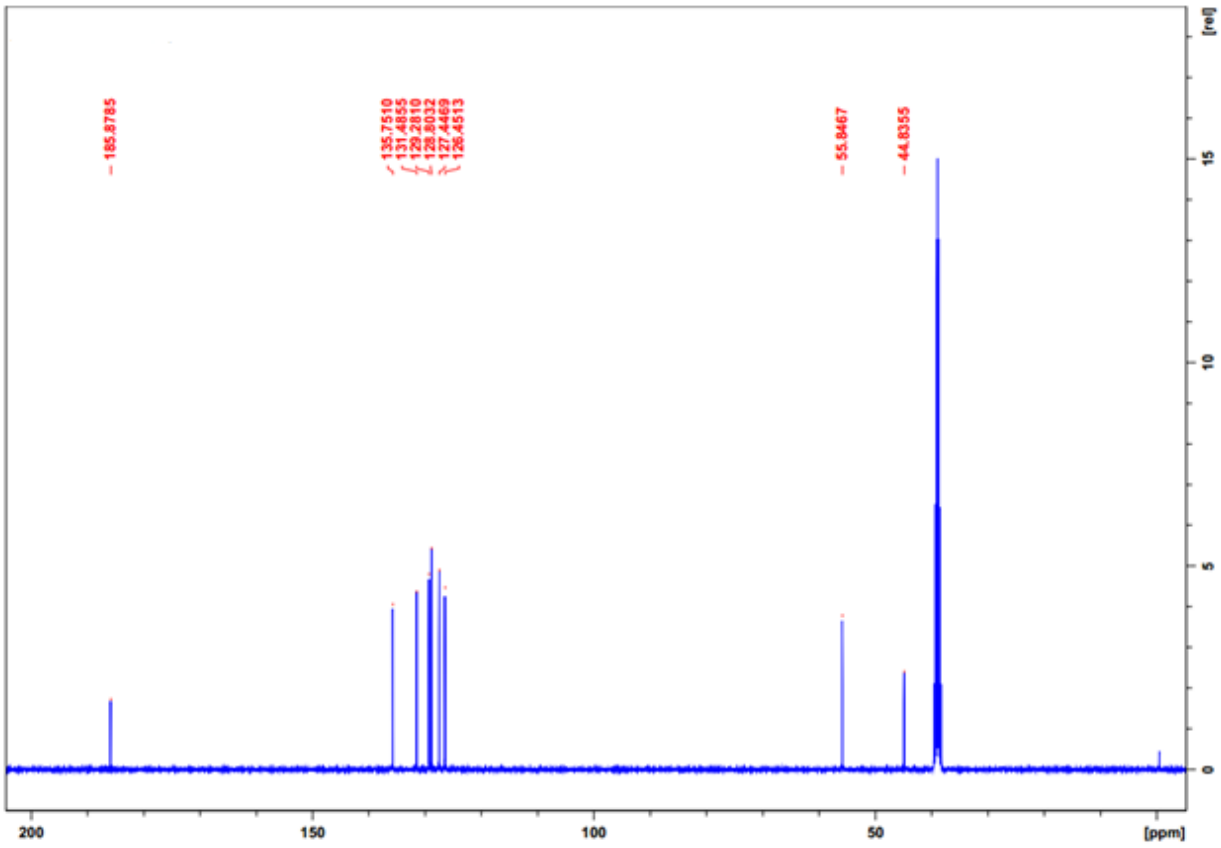
SI-Figure 69: Mass spectrum of 3,5-Bis ((E) pyrrole-2-benzylidene)-N-methyl-4-piperidone.

IIIa) 3,5-Bis ((E)-3-thiophenebenzylidene)-N-methyl-4-piperidone

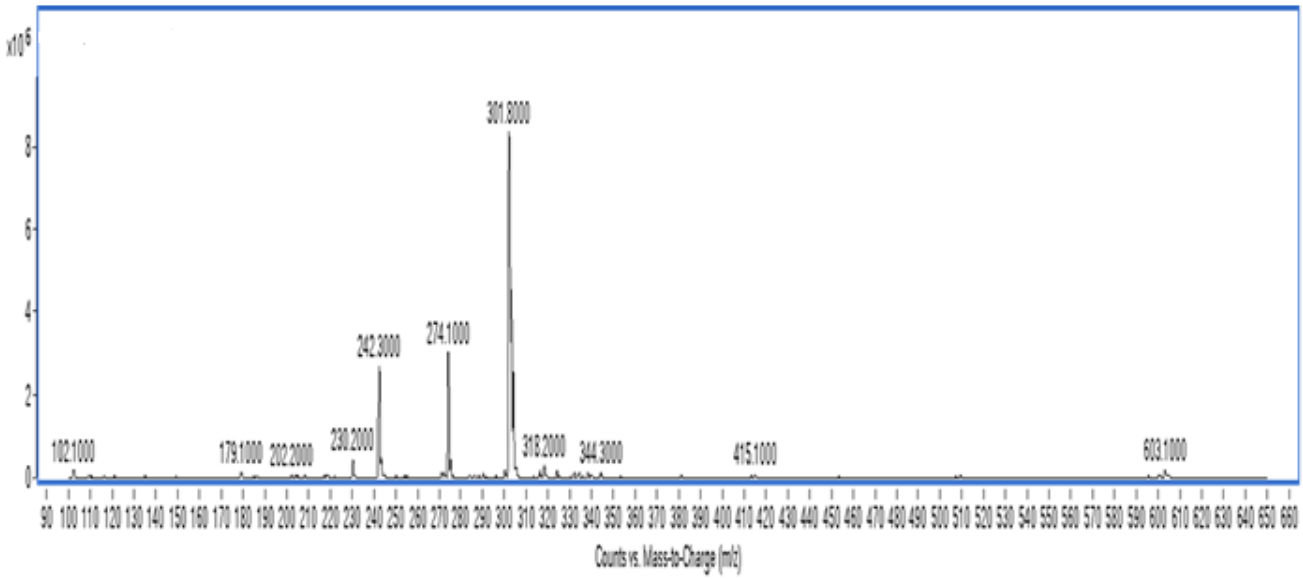
Yield: 54%. Brown crystals. Recrystallized from methanol. mp: 125.8-127.8^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (H-7, 2H, s), δ 7.46 (H-2', 2H, d, J = 2.52 Hz), δ 7.38 (H-5', 2H, dd, J = 2.52 and 5 Hz), δ 7.22 (H-4', 2H, d, J = 5 Hz), δ 3.79 (H-2, H-6, 4H, s), δ 2.53 (N-CH_3, 3H, s). ^{13}C NMR (400 MHz, $CDCl_3$): δ 185.87 (C-4), δ 135.75 (C-5), δ 131.48 (C-7), δ 129.28 (C-3'), δ 128.80 (C-2'), δ 127.49 (C-4'), δ 126.45 (C-5'), δ 55.84 (C-6) and δ 44.8 (N-C); IR (KBr disc) (cm^{-1}): 3095 (sp^2 C-H), 2970 (sp^3 C-H), 1675 (C=O), 1600 (C=C), 1515 and1475 (aromatic skeletal stretch), 1000 and 940 (C-H alkene out of plane bend), 890 and 800 (aromatic C-H out of plane bend); Mass: $[M+H]^+$ = m/z 301.8000; UV (MeOH) λ_{max} (nm): 354 and 212 nm.



SI-Figure 70: ^{1}H NMR spectrum of 3,5-Bis ((E)-3-thiophenebenzylidene)-N-methyl-4-piperidone.



SI-Figure 71: ^{13}C NMR spectrum of 3,5-Bis ((E)-3-thiophenebenzylidene)-N-methyl-4-piperidone.

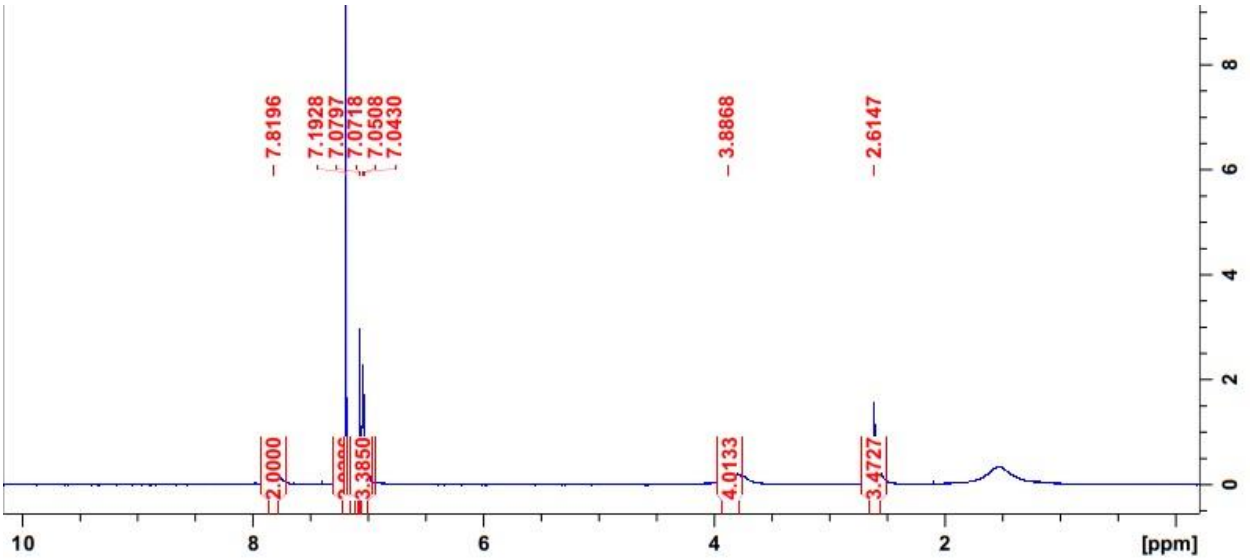


Mass: $[M+H]^+$ = m/z 301.8000

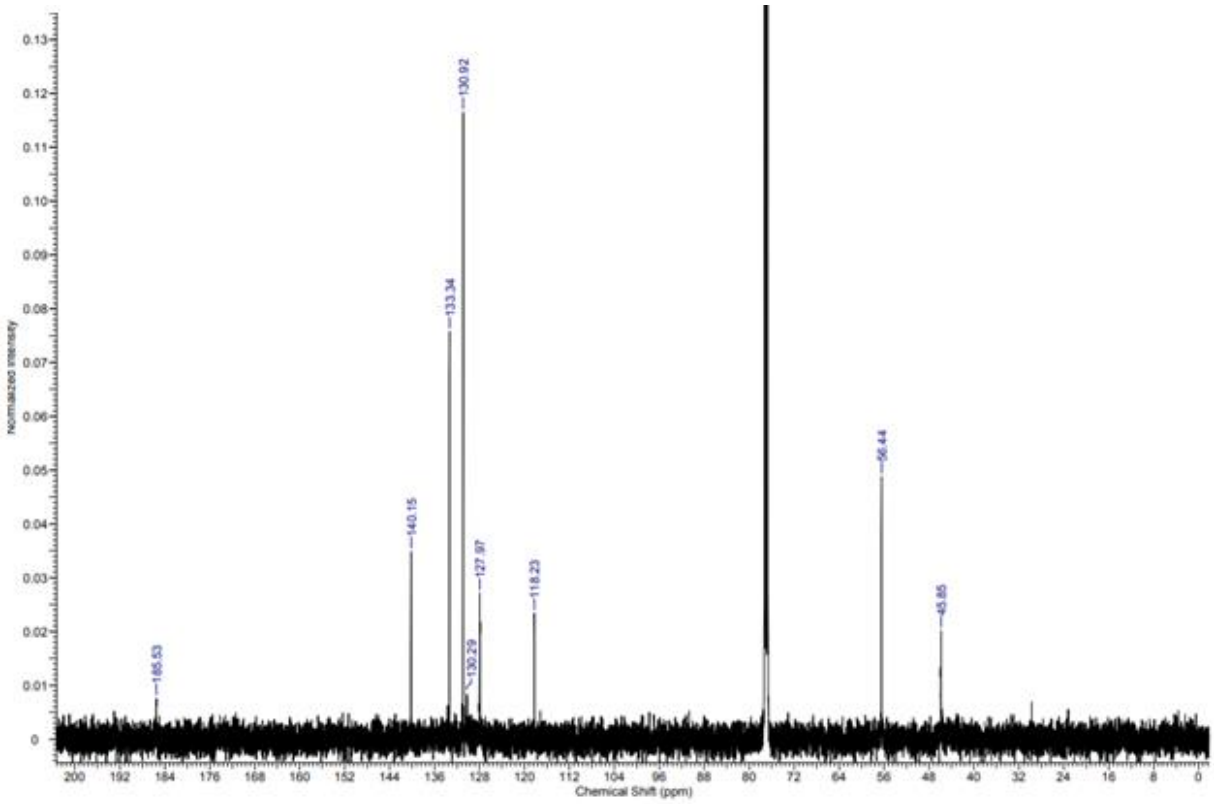
SI-Figure 72: Mass spectrum of 3,5-Bis ((E)-3-thiophenebenzylidene)-N-methyl-4-piperidone.

IVa) 3,5-Bis ((E)-5-bromo-2-thiophene benzylidene)-N-methyl-4-piperidone

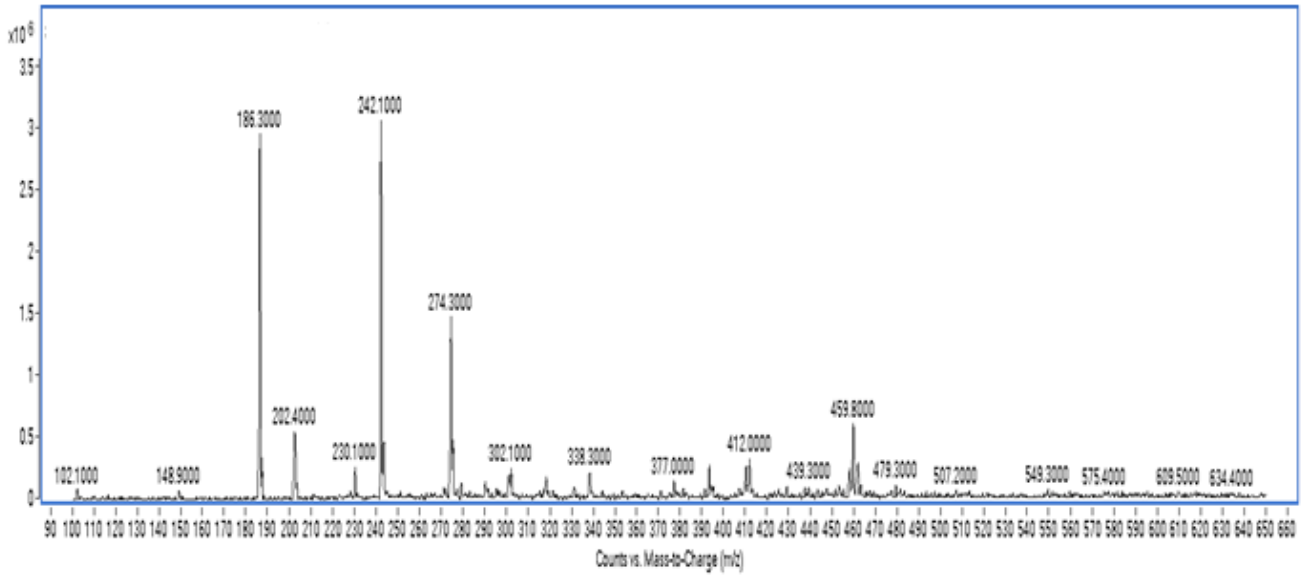
Yield: 87.8%. Yellow crystals. Recrystallized from chloroform and methanol. mp: 204.8-207^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.81 (H-7, 2H, s), δ 7.07 (H-3', 2H, d, J = 3.12 Hz), δ 7.04 (H-4', 2H, d, J = 3.12 Hz), δ 3.88 (H-2, H-6, 4H, s), δ 2.61, (N-CH_3, 3H, s). ^{13}C NMR (400 MHz, $CDCl_3$): δ 185.53 (C-4), δ 140.15 (C-7), δ 133.34 (C-3'), δ 130.92 (C-4'), δ 130.29 (C-3), δ 127.97 (C-2'), δ 118.23 (C-5'), δ 56.44 (C-2 and C-6) and δ 45.85 (N-C); IR (KBr disc) (cm^{-1}): 3085 (sp^2 C-H), 2960 (sp^3 C-H), 1630 (C=O), 1600 (C=C), 1575 and1498 (aromatic skeletal stretch), 1030 and 920 (C-H alkene out of plane bend), 800 and 720 (aromatic C-H out of plane bend); Mass: $[M+H]^+$ = m/z 459.8000, $[M+2]^+$ = m/z 461; UV (MeOH) λ_{max} (nm): 387 and 272 nm.



SI-Figure 73: ^{1}H NMR spectrum of 3,5-Bis ((E)-5-bromo-2- thiophene benzylidene)-N-methyl-4-piperidone.



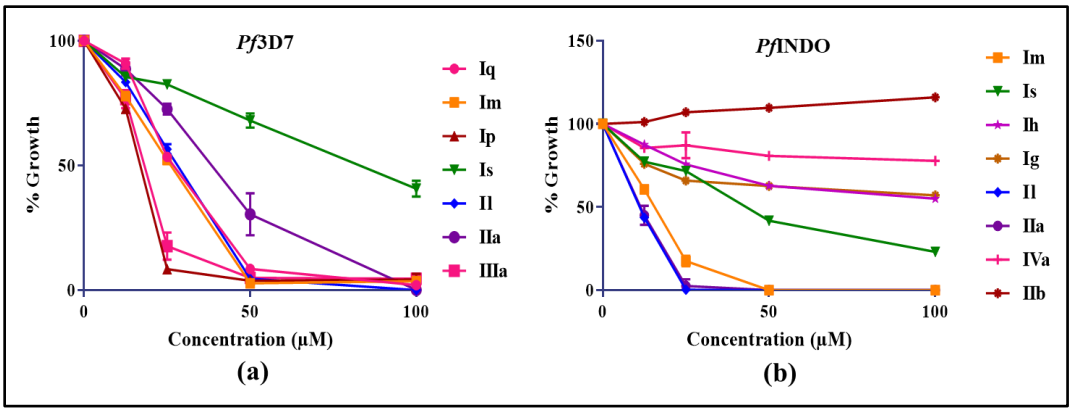
SI-Figure 74: ^{13}C NMR spectrum of 3,5-Bis ((E)-5-bromo-2-thiophene benzylidene)-N-methyl-4-piperidone.



Mass: $[M+H]^+$ = m/z 459.8000

SI-Figure 75: Mass spectrum of 3,5-Bis ((E)-5-bromo-2-thiophene benzylidene)-N-methyl-4-piperidone.

Anti-plasmodial studies:



SI-Figure 76: Anti-plasmodial screening of selected DANMPs at the concentrations between 100 and 10 µM: DANMPs showing >50% growth inhibition at 100 µM and <50% inhibition at 10 µM were selected for anti-plasmodial assay against rings of (a) *Pf*3D7 and (b) *Pf*INDO.

SI-Table 1: Comparison of melting points of DANMPs with literature values

Compound	Present study		Literature	
	Recrystallization solvent	melting point (°C)	Recrystallization solvent	melting point (°C)
Ia (R=H)	Petroleum ether-Chloroform	111.5-113.0	Ethanol	118.0[1]
Ib (R=2-Cl)	Chloroform-Methanol	145.0-147.8	Hexane-Ethyl acetate	135.8-136.8[2]
Ic (R=3-Cl)	Chloroform-Methanol	172.2-173.6	_#	_#
Id (R=4-Cl)	Ethyl acetate	176.0-177.5	Hexane- Ethyl acetate	180.0-182.0[3]
Ie (R=3-Br)	Petroleum ether-Acetone	123.0-125.0	Ethyl acetate	121.0-124.0[4]
If (R=4-Br)	Chloroform- Ethyl acetate	183.7-186.3	Ethanol	180.0-182.0[5]
Ig (R=4-OMe)	Chloroform-Methanol	201.0-203.2	Ethanol	202.0-204.0[4]
Ih (R=4-Me)	Chloroform-Methanol	200.0-202.0	Ethanol /H_2O	195.0-196.0[6]
Ii (R=4-Isopropyl)	Methanol	105.5-108.5	Ethanol	98.0-100.0[7]
Ij (R=3-CF_3)	Petroleum ether-Chloroform	143.0-145.2	Ethanol	142.0-143.0[7]

Ik (R=3-NO_2)	Chloroform-Methanol	185.0-188.5	Acetone	189.5-190.5[8]
Il (R=4-SCH_3)	Chloroform-Methanol	172.0-174.0	Ethanol	122.0-123.0[7]
Im (R=2-Cl-5-CF_3)*	Chloroform-Methanol	154.4-157.2	_	_
In (R=2,5-di-OMe)	Petroleum ether-Acetone	135.0-137.0	Ethanol	133.0-134.0[9]
Io (R=3,4-di-OMe)	Chloroform-Methanol	167.2- 169.7	Chloroform-Ethanol	157.3-159.4[2]
Ip (R=3,5-di-Cl)*	Chloroform-Methanol	186.3-188.6	_	_
Iq (R=4-NO_2)	Chloroform-Methanol	230.5-231.2	Butanol	204.0-205.0[6]
Ir (R=3-OH)	Chloroform-Methanol	284.0-286.0	Hexane- Ethyl acetate	136.0-136.8[10]
Is (R=4-OH)	Chloroform-Methanol	230.5-231.2	Ethanol	197.0-199.0[11]
It (R=4-OH-3,5-OMe)	Methanol	237.2-238.4	_#	_#
Iu (R=4-OH-3-OMe)	Methanol	217.0-220.5	Chloroform-Ethanol	195.0-197.0[11]
IIa (2-thiophene)	Chloroform-Methanol	115.0-118.0	Ethanol	118.0[1]
IIb (2-pyrrol)	Chloroform-Methanol	214.0-215.4	_#	_#
IIIa (3-thiophene)	Methanol	125.8-127.8	Ethanol and THF	102.0-104.0[12]
IVa (2-thiophene-5-Br)	Chloroform-Methanol	204.8-207.0	Ethanol and THF	128.0-130.0[12]

*Novel compounds; #Literature values are not known

For most compound, there are only some minor differences in melting points of our compounds when compared to the literature values because the solvent of recrystallization used by us is different from what has been used by previous investigators. Where there is considerable difference in melting points of our study and literature values, we have rechecked the melting points and obtained concurrent values. Therefore, our melting points are authentic. Besides both mass and NMR spectra are fully consistent with the structures given.

SI-Table 2: RPHPLC Retention times of the DANMPs

Molecule	Retention time (minutes)
Ia (R=H)	15.72
Ib (R=2-Cl)	18.01
Ic (R=3-Cl)	18.83
Id (R=4-Cl)	18.77
Ie (R=3-Br)	19.43
If (R=4-Br)	19.51
Ig (R=4-OMe)	13.95
Ih (R=4-Me)	17.55
Ii (R=4-Isopropyl)	18.60
Ij (R=3-CF_3)	15.56
Ik (R=3-NO_2)	14.80
Il (R=4-methyl thio)	11.94
Im (R=2-Cl-5-CF_3)	19.68
In (R=2,5-di-OMe)	10.93
Io (R=3,4-di-OMe)	9.56
Ip (R=3,5-di-Cl)	18.00
Iq (R=4-NO_2)	17.25
Ir (R=3-OH)	10.56
Is (R=4-OH)	8.85
It (R=4-OH-3,5-OMe)	9.33
Iu (R=4-OH-3-OMe)	9.47
IIa (2-thiophene)	10.22
IIb (2-pyrrol)	8.53
IIIa (3-thiophene)	10.70
IVa (2-thiophene-5-Br)	13.59

SI-Table 3: Crystallographic data of 3,5-Bis ((E)-4-chlorobenzylidene)-N-methyl-4-piperidone

Parameter	3,5-Bis ((E)-4-Chlorobenzylidene)-N-methyl-4-piperidone
Empirical formula	$C_{20}H_{17}Cl_2NO$
CCDC number	1960029
Formula weight	358.24
Crystal system	Monoclinic
Space group	$P2_1/c$
a (Å)	5.4902(4)
b (Å)	14.2399(8)
c (Å)	23.1491(14)
α (°)	90
β (°)	104.323(6)

γ (°)	90
V (Å^3)	1753.5(2)
Z	4
ρ_{calc} (g/cm^{-3})	1.357
Temperature (K)	293.0(2)
μ/ mm^{-1}	0.376
$2\theta_{min,\ max}$ (°)	5.722, 65.59
F (000)	744.0
$h_{min,max}$; $k_{min,max}$; $l_{min,max}$	-8, 7; -13, 21; -35, 21
Total no. of reflections	11576
R_{int}	0.0241
No. of unique reflections	5387
R_1 [I>2σ(I)]	0.0539
wR2 (all data)	0.1732
GooF on F^2	1.032
$\Delta\rho_{max,min}$/eÅ^{-3}	0.19/-0.36

***In silico* studies:**

Active site docking studies were done by selecting the pocket built up by amino acid residues D27, P52, K84, D105, S107, G156, G216, G217, F236, V237, G238, S239 in Pdx1 of the *Plasmodial* PLP synthase complex with bound phosphate ion (4ADS). All the compounds exhibited moderate to good energy scores within the range of -6.0 to -9.0 kcal mol^{-1} which are all > -5.5 kcal mol^{-1}, the value showed by R5P (SI-Table 4).This shows that all the DANMPs are binding strongly to the enzyme and may compete with R5P for binding to active site. The compounds **Ie** (IC_{50}: 0.35 μM), **Ir** (IC_{50}: 0.74 μM) and **If** (IC_{50}: 1.36 μM) which showed the best *in vitro* anti-plasmodial activities among all DANMPs against *Pf*MRA-1240 and *Pf*INDO also showed promising *in silico* energy scores of -7.0 and -8.1 kcal mol^{-1}. Interestingly, the less potent derivatives (**Ig**, **Ih**, **IIb**, **IIIa** and **IVa,** IC_{50} *Pf*3D7 16.5 to >100 μM) showed poorer docking energy scores (-6.0 to -6.9 kcal mol^{-1}) as compared to more potent derivatives **Ie, If, In** and **Ir (**IC_{50} *Pf*3D7 1.39 to 3.39 μM) with high docking scores (-7.0 to -7.3 kcal mol^{-1}).

SI-Table 4: Energy scores of the DANMPs with *Pf*PLP synthase with bound phosphate ion intermediate (4ADS).

Code	Energy Score (kcal mol^{-1})	Code	Energy Score (kcal mol^{-1})
Ia	-7.5	**In**	-7.3
Ib	-7.9	**Io**	-7.0
Ic	-7.9	**Ip**	-6.9
Id	-6.4	**Iq**	-6.9
Ie	-7.0	**Ir**	-8.1
If	-7.0	**Is**	-7.4
Ig	-6.4	**It**	-6.3
Ih	-6.4	**Iu**	-6.9
Ii	-7.3	**IIa**	-9.0
Ij	-8.0	**IIb**	-6.9
Ik	-7.9	**IIIa**	-6.3
Il	-6.1	**Iva**	-6.0
Im	-8.5		

Docking studies were also performed for these ligands on other Plasmodial proteins (Plasmepsin IV from *P. falciparum* (1LS5), Phosphatidylinositol 4-kinase III beta-PIK93 (4D0L), *P. falciparum* dihydroorotate dehydrogenase (6VTN)) and non-plasmodial proteins (COX-1 (3N8Y), COX-2 (3LN1), 5-LOX (3O8Y), Hen egg-white lysozyme (3WUN), and Human Hexokinase 2 (5HG1)). Molecular docking results (SI-Table 5) revealed that plasmepsin IV from *P. falciparum* could also be a good target for DANMP. However, the non-plasmodial proteins like 3O8Y displayed poor docking scores e.g -1.9 kcal mol^{-1} with **Ie** suggesting that docking with *plasmodial* proteins is not non-specific.

SI-Table 5: Docking scores of Potent DANMPs against *plasmodial* and non-*plasmodial* proteins

Compound	Plasmodial targets				Non-plasmodial targets				
	4ADS	**1LS5**	**4D0L**	**6VTN**	**3N8Y**	**3LN1**	**3O8Y**	**3WUN**	**5HG1**
Ie	-7.0	-8.6	-8.0	-9.6	-8.7	-6.5	-1.9	-7.3	-9.1
If	-7.0	-7.7	-8.4	-8.4	-8.7	-6.1	-3.8	-7.6	-8.1
Ic	-7.9	-8.6	-8.0	-9.3	-9.0	-6.7	-6.6	-7.3	-8.7
In	-7.3	-7.4	-7.8	- 4.0	-8.8	-6.5	-4.8	-7.2	-8.1
Ir	-8.1	-8.3	-8.3	-9.7	-8.5	-7.0	-8.1	-7.1	-8.7
It	-6.3	-7.0	-7.0	-6.7	-8.1	-6.9	-1.3	-6.6	-8.0
Ij	-8.0	-8.6	-9.2	-10.5	-10.2	-7.3	-6.3	-8.6	-9.9
Ia	-7.5	-8.7	-7.7	-8.8	-8.0	-6.3	-8.2	-6.7	-8.8

Protein-ligand interaction:

In a bid to find if with structural changes and the gain or loss of activity in test molecules, the interacting residues in PLP Synthase might be different; *in silico* identification of the interacting amino acid residues at the active site of the protein with most potent compounds among *para* and *meta* substituted DANMPs (SI-Table 6 and SI-Figure 77) was done using PyMol and BIOVIA Discovery Studio.

SI-Table 6: Anti-plasmodial potency, *Pf* Pyridoxal synthase (4ADS) *in silico* docking scores and amino acid residues interacting with the ligands

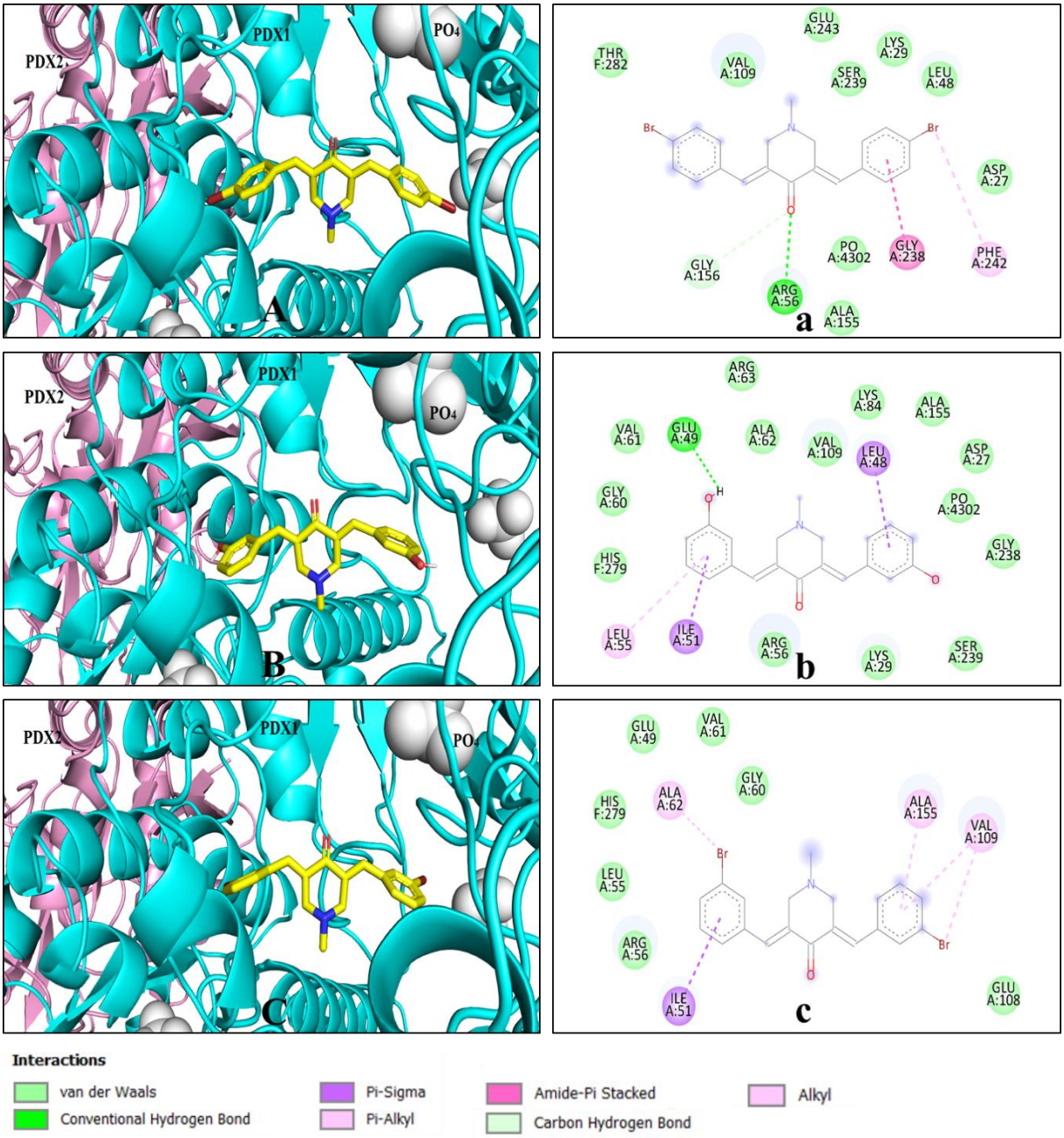
Interacting amino acid residues	**If** (-7.0)#	**Id** (-6.4)	**Ii** (-7.3)	**Ie** (-7.0)	**Ir** (-8.1)	**Ic** (-7.9)
IC_{50} (µM) *Pf*INDO	1.36	5.64	3.93	1.92	2.45	2.17
IC_{50} (µM) *Pf*3D7	3.39	3.32	3.68	1.39	1.44	1.95
IC_{50} (µM) *Pf*MRA	1.07	>100	3.19	0.35	0.74	0.74
D27*	+	+			+	+
K29	+	+			+	
L48	+	+			+	+
E49				+	+	+
I51		+	+	+	+	+
L55			+	+	+	+
R56	+	+	+	+	+	+
T58			+			
D59		+	+			
G60		+	+	+	+	+
V61				+	+	+
A62		+	+	+	+	+
R63					+	
K84*		+			+	+
E108				+		
V109	+	+		+	+	+
K152			+			
G153			+			
E154			+			
A155	+			+	+	+
G156*	+	+				
G238*	+	+			+	+
S239*	+	+			+	+

F242	+	+				
E243	+	+				
H279			+	+	+	+
T282	+	+				

*Catalytic residues from Ribose-5-phosphate substrate; Empty spaces represent the absence of the interaction, # values in parentheses represent docking scores with *Pf*PLP Synthase.

With the *meta* substituted derivatives, Ile 51, Ala 62, Ala 155, Val 109, GLU 49 and van der Walls forces of interactions in the active site are mainly responsible for their activity. The *meta* substituted compounds such as **Ie**, **Ir** and **Ic** formed pi-sigma interaction with Ile-51. Val 109 and Ala 155 showed pi-alkyl interactions with the arene rings of **Ie** while bromo groups in **Ie** exhibited interactions with the alkyl groups of Ala 62 and Val 106. These three interactions might be responsible for superior activity of **Ie**. Both pi-alkyl interactions with the arene rings as well as alkyl groups of the residues were not seen with **Ir**. However, **Ir** showed one hydrogen bond with Glu 49 and more van der Walls interactions as compared to **Ie** and **Ic**. These two interactions with **Ir** may be contributing towards its potential activity. The pi-alkyl interactions with the arene rings are missing in case of **Ic** but chloro groups in **Ic** interacted with the alkyl side chains of Ala 62, Val 109 and Ala 155. The compound **Ij** showed pi-alkyl interaction with a few of the above-mentioned residues e.g. Ala 62 and Ile 51. In contrast, **Ik**, which showed poor to moderate antiplasmodial activity, did not participate in any interactions with these key amino acids. This may explain the poor activity of **Ik**.

In case of the *para* substituted derivatives, Arg 56, Gly 156, Ala 62, Val 109, Ala 155 and van der Walls forces of interactions seemed to play a crucial role in their activity. The most active compound **If** among the para substituted derivatives displayed two hydrogen bonds between Arg 56 and Gly 156 and the carbonyl group of the molecule. The presence of pi-alkyl interaction with Ala 62, Val 109 and Ala 155, and many van der Walls interactions in case of **Ii** contributed to its potency. In contrast, **Id** showed one hydrogen bonding interaction with Arg 56 apart from some van der Walls interactions. Moderately active compound **Il** exhibited pi-alkyl interaction with Ala 62, Val 109 and Ala 155, and many van der Walls interactions. Less number of pi-alkyl interactions and van der Walls interactions with compounds **Is** and **Ig** may have resulted in the reduction of their potency.

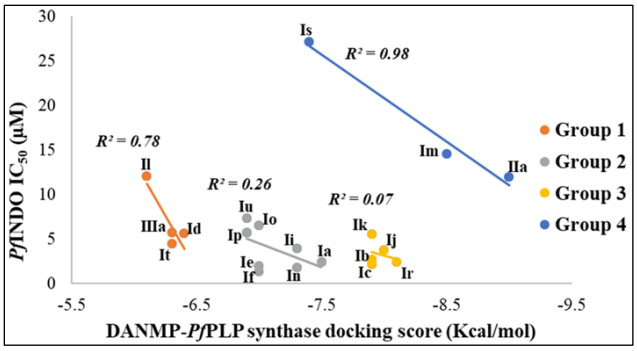


SI-Figure 77: The best docked poses of If (A), Ir (B), Ie (C) with *Pf*PLP synthase (PDB: 4ADS) and the 2-D representation of interacting amino acid residues **a-c.** Ligands are shown in stick format (yellow: carbon, red: oxygen and blue: Nitrogen).

Tanimoto Similarity Index (TSI):

A plot of the *in-silico* active site docking scores against IC_{50} (*Pf*INDO) for the derivatives with $IC_{50} < 30$ μM is shown in SI-Figure 78. The correspondence between good docking scores against PLP synthase and good *in vitro* anti-plasmodial activity against malaria parasite seems to suggest that this crucial enzyme may be one of the important targets the DANMPs action studied by us. Four inverse correlation patterns were found where an increase in docking energy (negative scale) was correlated with a decrease in IC_{50} values. In other words, compounds with high anti-plasmodial potency were found to be binding to PLP synthase with high docking scores. In addition, compounds within each group were compared with each other for their structural and functional similarities based on Tanimoto Similarity Index (TSI) calculated using Fingerprint Similarity tool (SI-Table 7) and ADME properties (SI-Table 8).

Since TSI of > 0.5 indicates significant similarities among compounds within each group, the observed TSI values of ≥ 0.6 except **IIIa** (TSI: 0.359) and **IIa** (TSI: 0.433) (SI-Figure 78) indicate good structural and functional similarity among the molecules in each of the four groups (SI-Table 7). The high TSI values for **It**, **Id** and **Il** in group 1 compounds may be related to the presence of +R substitution at 4^{th} position in all these compounds. **IIIa** of this group showed a low TSI perhaps because in contrast to all other members, which are benzene derivatives, **IIIa** happens to be a thienyl derivative. The common feature conferring high TSI to Group 2 compounds may be the fact that **Iu**, **Io** and **In** contain two oxygen bearing substituents in the aryl rings while **If**, **Ie** and **Ip** have halogens in the aryl rings. Incidentally, with the lone exception of **Ia** with a molar refractivity of 95, the molar refractivity for all members of this group also lay in the range 110-121. In contrast to Group 1 compounds characterized by a +R substitution, the unique feature of Group 3 compounds was –I substituents at the *meta* position on the aryl rings. The molar refractivity of Group 3 compounds lay in the range of 99-112. However, when different physicochemical and biological properties of compounds were compared within each group, Group 2 compounds (**Ia**, **Ie**, **If**, **Ii**, **In**, **Io**, **Ip** and **Iu**) showed maximum similarities such as the number of rotatable bonds, H-bond acceptors, GI absorption and BBB permeability (SI-Table 8). **Ir** with TSI of ~0.7 for Group 3 compounds, showed high GI absorption and BBB permeability similar to **Ie** and **If**. The above-mentioned similarities among potent compounds **Ie**, **If** and **Ir** were well supported by TSI values which were 0.69 (**Ie**), 0.71 (**If**) and 1.0 (**Ir**) (SI-Table 7).



SI-Figure 78: *In vitro Pf*INDO IC_{50} values vs *in silico Pf*PLP Synthase docking scores of DANMPs.

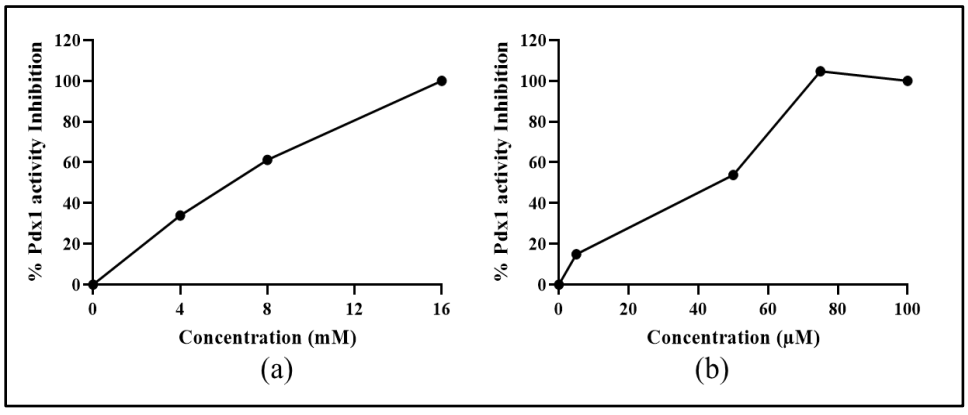
SI-Table 7: Tanimoto Similarity Index of DANMPs within each correlating group.

Compounds	TSI	Compounds	TSI
Group 1		**Group 3**	
It	1.000	**Ir**	1.000
Id	0.692	**Ik**	0.821
Il	0.654	**Ic**	0.807
IIIa	0.359	**Ib**	0.760
		Ij	0.736
Group 2		**Group 4**	
Iu	1.000	**Is**	1.000
Io	0.974	**Im**	0.672
In	0.829	**IIa**	0.433
Ia	0.770		
Ii	0.713		
If	0.713		
Ie	0.688		
Ip	0.680		

In nutshell, this correlation of high potency with high PLP synthase docking scores and similarities of ADME properties (SI-Table 8) among potent compounds further supports the hypothesis that one of the modes of action of DANMPs could be via targeting the PLP synthase of *P. falciparum*.

SI-Table 8: Different Lipinski parameters and ADME properties of 4 groups of compounds (selected from Figure 7): iLogP: Log $P_{o/w}$; GI absorption: Gestro-intestinal absorption; BBB permeant: Blood Brain Barrier permeant.

	DANMP	Properties										
		MW (g/mol)	Molar refractivity	Fraction Csp3	No.of rotatable bonds	H-bond acceptors	H-bond donors	iLogp	LogS	Log Kp	GI Absorption	BBB permeant
Line 1	Il	381.55	118.52	0.23	4	2	0	4.05	-5.22	-5.32	High	Yes
	IIIa	301.43	90.83	0.19	2	2	0	3.26	-3.84	-6.01	High	Yes
	Id	358.26	105.1	0.15	2	2	0	3.83	-5.38	-5.01	High	Yes
	It	441.47	125.09	0.29	6	8	2	3.81	-4.23	-7.0	High	No
Line 2	If	447.16	110.48	0.15	2	2	0	4.06	-6.01	-5.46	High	Yes
	Ie	447.16	110.48	0.15	2	2	0	4.02	-6.01	-5.46	High	Yes
	Ip	427.15	115.12	0.15	2	2	0	4.3	-6.57	-4.54	High	Yes
	Iu	381.42	112.11	0.23	4	6	2	3.55	-4.07	-6.59	High	No
	Io	409.47	121.04	0.29	6	6	0	4.16	-4.5	-6.3	High	Yes
	In	409.47	121.04	0.29	6	6	0	4.3	-4.5	-6.3	High	Yes
	Ii	373.53	124.24	0.35	4	2	0	4.69	-5.92	-4.4	High	Yes
	Ia	289.37	95.08	0.15	2	2	0	3.44	-4.19	-5.49	High	Yes
Line 3	Ic	358.26	105.1	0.15	2	2	0	3.77	-5.38	-5.01	High	Yes
	Ik	379.37	112.72	0.15	4	6	0	2.56	-4.32	-6.28	High	No
	Ib	358.26	105.1	0.15	2	2	0	3.69	-5.38	-5.01	High	Yes
	Ij	425.37	105.08	0.23	4	8	0	3.77	-5.91	-5.06	Low	No
	Ir	321.37	99.12	0.15	2	4	2	2.53	-3.91	-6.19	High	Yes
Line 4	Is	321.37	99.12	0.15	2	4	2	2.53	-3.91	-6.19	High	Yes
	Im	494.26	115.1	0.23	4	8	0	4.11	-7.11	-4.59	Low	No
	IIa	301.43	90.83	0.19	2	2	0	3.18	-3.88	-5.96	High	Yes



SI-Figure 79: IC_{50} curves of control E4P (a) and compound Ir (b) against Pdx1 enzyme activity inhibition. The IC_{50}s were calculated considering 100 % inhibition at the compound's maximum concentrations (16 mM for E4P and 100 µM for **Ir**.

References:

1 S. Z. Vatsadze, M. A. Manaenkova, N. V. Sviridenkova, N. V. Zyk, D. P. Krut'ko, A. V. Churakov, M. Y. Antipin, J. A. K. Howard and H. Lang, *Russ. Chem. Bull.*, 2006, **55**, 1184–1194.

2 J. Wu, Y. Zhang, Y. Cai, J. Wang, B. Weng, Q. Tang, X. Chen, Z. Pan, G. Liang and S. Yang, *Bioorganic Med. Chem.*, 2013, **21**, 3058–3065.

3 Y. Eryanti, R. Hendra, T. Herlina, A. Zamri and U. Supratman, *Indones. J. Chem.*, 2018, **18**, 362–366.

4 J. feng Sun, G. ge Hou, F. Zhao, W. Cong, H. juan Li, W. shuai Liu and C. Wang, *Chem. Biol. Drug Des.*, 2016, 534–541.

5 G. G. Lyle, J. J. Dziark, J. Connor and C. S. Huber, *Tetrahedron*, 1973, **29**, 4039–4044.

6 H. I. El-Subbagh, S. M. Abu-Zaid, M. A. Mahran, F. A. Badria and A. M. Al-Obaid, *J. Med. Chem.*, 2000, **43**, 2915–2921.

7 L. M. Deck, L. A. Hunsaker, T. A. Vander Jagt, L. J. Whalen, R. E. Royer and D. L. Vander Jagt, *Eur. J. Med. Chem.*, 2018, **143**, 854–865.

8 J. Leonard, M. Locke and J. Leonard, 1955, vol. 77, pp. 1852–1855.

9 B. Yadav, S. Taurin, R. J. Rosengren, M. Schumacher, M. Diederich, T. J. Somers-Edgar and L. Larsen, *Bioorganic Med. Chem.*, 2010, **18**, 6701–6707.

10 Y. Eryanti, A. Zamri, T. Herlina, U. Supratman, M. M. Rosli and H. K. Fun, *Acta Crystallogr. Sect. E Crystallogr. Commun.*, 2015, **71**, 1488–1492.

11 K. M. Youssef, M. A. El-Sherbeny, F. S. El-Shafie, H. A. Farag, O. A. Al-Deeb and S. A. A. Awadalla, *Arch. Pharm. (Weinheim).*, 2004, **337**, 42–54.

12 J. Dennis Bilavendran, A. Manikandan, P. Thangarasu and K. Sivakumar, *Bioorg. Chem.*, 2020, **94**, 103484.