SUPPORTING INFORMATION

Hydroxylated Derivatives of NPC1161: Theoretical Insights into Their Potential Toxicity and the Feasibility and Regioselectivity of Their Formation

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S1. Details of Conformational Analysis of NPC1161a. A Monte Carlo random conformational search at the MMFF94s level of theory was performed, using Schrödinger’s MacroModel software package, yielding 453 conformers within an energy window of 130 kJ/mol (Figure S1). The 25 conformers with the lowest energies within an energy cut-off of 8.4 kJ/mol (~2 kcal/mol) were submitted to full hybrid density-functional theory (DFT) geometry optimization, leading to 20 conformers located at the B3LYP/6-31G** level in the gas phase (Figure 1). Harmonic vibrational frequencies were calculated at the same level to confirm that they are minima on the potential energy surface, and single point energies were computed at the B3LYP/6-311++G**/B3LYP/6-31G** level in the gas phase and at the B3LYP-SCRF(PCM)/6-311++G**/B3LYP/6-31G** level in water. Conformational analysis (Table S2) at the B3LYP/6-31G** level in the gas phase afforded a broad distribution of 5, 6, 14, 14, 7, 5, 12, 13, 9, and 6% for the conformers 01, 11, 02, 20, 03, 12, 05, 14, 10, and 19, respectively, in total accounting for 92% of the Boltzmann-weighted population. The rotation of the phenolic group about C5-O (C1″) is the geometric difference between the conformers 1-10 and 11-20, in which the dihedral angles of C6-C5-O-C1″ are about -77 and 77, respectively. And this rotation is the only geometric difference between the conformers 01 and 11, 02 and 20, 03 and 12, 05 and 14, and 10 and 19. Among the above conformers, weak CH—N and/or NH—N hydrogen bonds were found in the conformers 03, 05, 10, 12, 14, and 19. The distances of N6′...H (C7) and N6′...H(C2′) in conformers 03 and 12 are 2.67 and 2.54 Å, those of N6′...H(N1′), N6′...H(C3′), and N1′...H(C5′) in 05 and 14 are 2.23, 2.65, and 2.62 Å, and those of N6′...H(C2′) in 10 and 19 are 2.42 Å, respectively. These conformers with hydrogen bonds accounted for 52% of the Boltzmann-weighted population at the B3LYP/6-31G** level in the gas phase, but this was reduced to 24% and 4% at the B3LYP/6-311++G**/B3LYP/6-31G** level in the gas phase and at the B3LYP-SCRF(PCM)/6-311++G**/B3LYP/6-31G** level in water.
water, respectively, indicating that they are neither predominant in water nor even in the gas phase as evidenced at the more accurate B3LYP/6-311++G**//B3LYP/6-31G** level. The conformers with the most extended side chain, specifically 02 and 20, were found to be the major conformers, accounting in total for 43% in the gas phase at the B3LYP/6-311++G**//B3LYP/6-31G** level and for 45% at the B3LYP-SCRF(PCM)/6-311++G**//B3LYP/6-31G** level in water.
S2. Details of Ionization Potentials of NPC1161a. The geometries of all conformers found in water with a Boltzmann contribution greater than 2% were employed as the starting points to locate the ionized conformers of NPC1161a at the B3LYP/6-31G** level in the gas phase. Single point energies and the conformational distribution were computed by using the same protocols described above in S1. The ionized conformers 02 and 20 were again found to be the major ones, with contributions of 31% and 34% at the B3LYP/6-311++G**//B3LYP/6-31G** level in the gas phase, and of 26% and 28% in water at the B3LYP-SCRF(PCM)/6-311++G**//B3LYP/6-31G** level. The ionization potentials were calculated at the above levels and are listed in Table S3. The biggest differences of IP values were found to be only 8.81, 7.20 and 3.14 kJ/mol at the B3LYP/6-31G**, B3LYP/6-311++G**//B3LYP/6-31G**, and B3LYP-SCRF(PCM, H2O)/6-311++G**//B3LYP/6-31G** levels, respectively, indicating that the IPs in this case are relatively insensitive to conformation, especially in water.
Figure S1. Random conformational search of NPC1161a within an energy cut-off of 130 kJ/mol.
**Figure S2.** Potential energy surface scanned at the AM1 level in the gas phase about the dihedral angle H-O-C2-N1 in the hydroxylated metabolite of conformer NPC1161a02 at the C2 position.
Figure S3. Potential energy surface scanned at the AM1 level in the gas phase about the dihedral angle H-O-C7-C8 in the hydroxylated metabolite of conformer NPC1161a02 at the C7 position.
Figure S4. Potential energy surface scanned at the AM1 level in the gas phase about the dihedral angle H-O-N1'-C8 in the hydroxylated metabolite of conformer NPC1161a02 at the N1' position.
### Table S1. Total Energies of Neutral and Ionized Conformers of NPC1161a (a.u.).

| Conformer | E<sup>a</sup> | E<sup>b</sup> | E<sup>c</sup> | E<sup>d</sup> | E<sup>e</sup> | E<sup>f</sup> |
|-----------|---------------|---------------|---------------|---------------|---------------|---------------|
| 01        | -2088.083357  | -2088.850243  | -2088.862935  | -2087.860457  | -2088.618058  | -2088.679903  |
| 02        | -2088.084306  | -2088.850846  | -2088.864054  | -2087.861127  | -2088.618647  | -2088.681368  |
| 03        | -2088.083640  | -2088.849131  | -2088.861082  |               |               |               |
| 04        | -2088.081198  | -2088.848109  | -2088.862106  | -2087.856142  | -2088.614042  | -2088.679134  |
| 05        | -2088.084120  | -2088.849444  | -2088.860242  |               |               |               |
| 06        | -2088.080531  | -2088.846048  | -2088.860382  |               |               |               |
| 07        | -2088.082199  | -2088.846666  | -2088.856816  |               |               |               |
| 08        | -2088.082413  | -2088.84738   | -2088.863495  | -2087.856418  | -2088.613705  | -2088.680764  |
| 09        | -2088.080771  | -2088.847058  | -2088.861163  |               |               |               |
| 10        | -2088.083854  | -2088.849742  | -2088.860646  |               |               |               |
| 11        | -2088.083550  | -2088.850260  | -2088.862826  | -2087.860361  | -2088.618133  | -2088.679965  |
| 12        | -2088.083257  | -2088.848836  | -2088.860848  |               |               |               |
| 13        | -2088.080577  | -2088.846130  | -2088.858911  |               |               |               |
| 14        | -2088.084200  | -2088.849189  | -2088.860438  |               |               |               |
| 15        | -2088.080715  | -2088.847134  | -2088.861256  | -2087.854461  | -2088.611989  | -2088.678886  |
| 16        | -2088.082418  | -2088.849441  | -2088.863619  | -2087.856359  | -2088.614356  | -2088.680777  |
| 17        | -2088.081269  | -2088.848255  | -2088.862610  | -2087.855679  | -2088.613479  | -2088.679549  |
| 18        | -2088.080415  | -2088.846260  | -2088.860350  |               |               |               |
| 19        | -2088.083547  | -2088.849453  | -2088.860459  |               |               |               |
| 20        | -2088.084308  | -2088.850890  | -2088.863995  | -2087.861159  | -2088.618735  | -2088.681460  |

<sup>a, b, c</sup> Electronic and zero-point energies of individual conformers of neutral NPC1161a at the B3LYP/6-31G** level in the gas phase, and their single-point energies at the B3LYP/6-311++G**//B3LYP/6-31G** and B3LYP-SCRF(PCM)/6-311++G**//B3LYP/6-31G** in the gas phase and water, respectively.  
<sup>d, e, f</sup> Electronic and zero-point energies of individual conformers of ionized NPC1161a at the B3LYP/6-31G** level in the gas phase, and their single-point energies at the B3LYP/6-311++G**//B3LYP/6-31G** and B3LYP-SCRF(PCM)/6-311++G**//B3LYP/6-31G** in the gas phase and water, respectively.
Table S2. Conformational Analysis of Neutral and Ionized NPC1161a.

| Conformer# | $\Delta E^a$ | P$^b$ | $\Delta E^c$ | P$^d$ | $\Delta E^e$ | P$^f$ | $\Delta E^g$ | P$^h$ | $\Delta E^i$ | P$^i$ | $\Delta E^j$ | P$^j$ |
|------------|-------------|------|-------------|------|-------------|------|-------------|------|-------------|------|-------------|------|
| 01         | 2.50        | 5.2  | 1.70        | 11.1 | 2.94        | 7.0  | 1.84        | 16.4 | 1.78        | 16.5 | 4.09        | 5.4  |
| 02         | 0.01        | 14.3 | 0.12        | 21.0 | 0.00        | 23.0 | 0.08        | 33.4 | 0.23        | 30.8 | 0.24        | 25.5 |
| 03         | 1.75        | 7.1  | 4.62        | 3.4  | 7.80        | 1.0  |             |      |             |      |             |      |
| 04         | 8.17        | 0.5  | 7.30        | 1.2  | 5.11        | 2.9  | 13.17       | 0.2  | 12.32       | 0.2  | 6.11        | 2.4  |
| 05         | 0.49        | 11.8 | 5.11        | 2.8  | 10.01       | 0.4  |             |      |             |      |             |      |
| 06         | 9.92        | 0.3  | 11.77       | 0.2  | 9.64        | 0.5  |             |      |             |      |             |      |
| 07         | 5.54        | 1.5  | 11.09       | 0.3  | 19.00       | 0.0  |             |      |             |      |             |      |
| 08         | 4.98        | 1.9  | 5.65        | 2.3  | 1.47        | 12.7 | 12.45       | 0.2  | 13.21       | 0.2  | 1.83        | 13.5 |
| 09         | 9.29        | 0.3  | 10.06       | 0.4  | 7.59        | 1.1  |             |      |             |      |             |      |
| 10         | 1.19        | 8.9  | 3.01        | 6.5  | 8.95        | 0.6  |             |      |             |      |             |      |
| 11         | 1.99        | 6.4  | 1.65        | 11.3 | 3.22        | 6.3  | 2.10        | 14.8 | 1.58        | 17.9 | 3.93        | 5.8  |
| 12         | 2.76        | 4.7  | 5.39        | 2.5  | 8.42        | 0.8  |             |      |             |      |             |      |
| 13         | 9.80        | 0.3  | 12.50       | 0.1  | 13.50       | 0.1  |             |      |             |      |             |      |
| 14         | 0.28        | 12.8 | 4.46        | 3.6  | 9.49        | 0.5  |             |      |             |      |             |      |
| 15         | 9.43        | 0.3  | 9.86        | 0.4  | 7.35        | 1.2  | 17.59       | 0.0  | 17.71       | 0.0  | 6.76        | 1.8  |
| 16         | 4.96        | 1.9  | 3.81        | 4.7  | 1.14        | 14.5 | 12.60       | 0.2  | 11.50       | 0.3  | 1.79        | 13.7 |
| 17         | 7.98        | 0.6  | 6.92        | 1.3  | 3.79        | 5.0  | 14.39       | 0.1  | 13.80       | 0.1  | 5.02        | 3.7  |
| 18         | 10.22       | 0.2  | 12.15       | 0.2  | 9.73        | 0.5  |             |      |             |      |             |      |
| 19         | 2.00        | 6.4  | 3.77        | 4.8  | 9.44        | 0.5  |             |      |             |      |             |      |
| 20         | 0.00        | 14.4 | 0.00        | 22.0 | 0.15        | 21.6 | 0.00        | 34.6 | 0.00        | 33.8 | 0.00        | 28.2 |

$^a$ Relative energies (kJ/mol) of individual conformers of neutral NPC1161a at the B3LYP6-31G** and B3LYP6-311++G**/B3LYP6-31G** levels in the gas phase, and at the B3LYP-SCRF(PCM)/6-311++G**/B3LYP6-31G** level in water, respectively.

$^b$ Relative energies (kJ/mol) of individual conformers of radical NPC1161a at the B3LYP6-31G** and B3LYP6-311++G**/B3LYP6-31G** levels in the gas phase, and at the B3LYP-SCRF(PCM)/6-311++G**/B3LYP6-31G** level in water, respectively.

$^c$ Conformational distribution of neutral NPC1161a calculated at the above levels, respectively.

$^d$ Conformational distribution of radical NPC1161a calculated at the above levels, respectively.

$^e$ Zero point energy at the B3LYP6-31G** level in the gas phase was included in the calculations at the above levels.
Table S3. Theoretically Calculated Absolute and Relative Ionization Potentials (kJ/mol) of Predominant Conformers of NPC1161a.

| Conformer# | |IP<sub>a</sub>| |Δ|IP<sub>b</sub>| |IP<sub>c</sub>| |Δ|IP<sub>d</sub>| |IP<sub>e</sub>| |Δ|IP<sub>f</sub>| |
|---|---|---|---|---|---|---|---|---|---|
| 01 | 585.22 | 0.00 | 611.48 | 0.29 | 482.42 | 2.74 |
| 02 | 585.96 | 0.74 | 611.38 | 0.19 | 481.38 | 1.70 |
| 04 | 590.88 | 5.66 | 615.82 | 4.63 | 481.67 | 1.99 |
| 08 | 593.35 | 8.13 | 617.94 | 6.75 | 480.62 | 0.94 |
| 11 | 585.98 | 0.76 | 612.17 | 0.98 | 482.82 | 3.14 |
| 15 | 594.03 | 8.81 | 618.24 | 7.05 | 479.68 | 0.00 |
| 16 | 593.52 | 8.30 | 618.39 | 7.20 | 481.23 | 1.55 |
| 17 | 592.29 | 7.07 | 617.20 | 6.01 | 481.42 | 1.74 |
| 20 | 585.88 | 0.66 | 611.19 | 0.00 | 480.91 | 1.23 |

<sup>a,b</sup> Absolute and relative ionization potentials calculated at the B3LYP/6-311G** level in the gas phase. <sup>c,d</sup> Absolute and relative ionization potentials calculated at the B3LYP/6-311++G**/B3LYP/6-31G** level in the gas phase. <sup>e,f</sup> Absolute and relative ionization potentials calculated at the B3LYP-SCRF(PCM)//B3LYP/6-31G** level in water. The zero point energy at the B3LYP/6-31G** level in the gas phase was included in the calculations at the above levels.
Table S4. Total Energies of Neutral and Ionized Metabolites of Conformer NPC1161a02 Hydroxylated at All Possible Positions (a.u.).

| OH@ | E₀ | E₁ | E₂ | E₃ | E₄ | E₅ | E₆ |
|-----|----|----|----|----|----|----|----|
| 2   | -2163.312881 | -2164.111301 | -2164.126376 | -2163.092388 | -2163.881062 | -2163.946163 |
| 3   | -2163.297783 | -2164.095473 | -2164.112079 | -2163.076913 | -2163.864753 | -2163.929991 |
| 4Me | -2163.287935 | -2164.090004 | -2164.106539 | -2163.058784 | -2163.851275 | -2163.919988 |
| 2"  | -2163.299367 | -2164.096301 | -2164.110588 | -2163.071401 | -2163.860053 | -2163.928308 |
| 5"  | -2163.301690 | -2164.099362 | -2164.113692 | -2163.079842 | -2163.867987 | -2163.930938 |
| 6"  | -2163.301522 | -2164.099101 | -2164.112725 | -2163.073842 | -2163.862516 | -2163.929824 |
| 6OMe| -2163.297604 | -2164.098053 | -2164.114474 | -2163.072478 | -2163.863393 | -2163.930953 |
| 7   | -2163.294155 | -2164.099462 | -2164.115179 | -2163.078151 | -2163.867142 | -2163.931912 |
| 1'  | -2163.236844 | -2164.031970 | -2164.046408 | -2163.027503 | -2163.812543 | -2163.880808 |
| 2'  | -2163.301198 | -2164.098736 | -2164.113497 | -2163.076702 | -2163.864724 | -2163.927406 |
| 2'Me| -2163.290492 | -2164.090764 | -2164.105704 | -2163.067660 | -2163.859852 | -2163.922113 |
| 3'R | -2163.300325 | -2164.099462 | -2164.115179 | -2163.078151 | -2163.867142 | -2163.931912 |
| 3'S | -2163.297735 | -2164.096099 | -2164.111521 | -2163.078729 | -2163.867052 | -2163.929128 |
| 4'R | -2163.297788 | -2164.098002 | -2164.114131 | -2163.082972 | -2163.872371 | -2163.931687 |
| 4'S | -2163.297991 | -2164.096839 | -2164.114182 | -2163.081515 | -2163.870616 | -2163.932241 |
| 5'R | -2163.300698 | -2164.101056 | -2164.118572 | -2163.074522 | -2163.865535 | -2163.935187 |
| 5'S | -2163.300718 | -2164.100453 | -2164.117415 | -2163.076222 | -2163.866657 | -2163.934032 |
| 6'  | -2163.246656 | -2164.043742 | -2164.059079 | -2163.022330 | -2163.810349 | -2163.876316 |

*a The position at which the hydroxylation occurs. b, c, d Electronic and zero-point energies of neutral metabolites of NPC1161a02 hydroxylated at different positions at the B3LYP/6-31G** level in the gas phase, and their single-point energies at the B3LYP/6-311++G**/B3LYP/6-31G** and B3LYP-SCRF(PCM)/6-311++G**//B3LYP/6-31G** levels in the gas phase and water, respectively. e, f, g Electronic and zero-point energies of ionized metabolites of NPC1161a02 hydroxylated at different positions at the B3LYP/6-31G** level in the gas phase, and their single-point energies at the B3LYP/6-311++G**/B3LYP/6-31G** and B3LYP-SCRF(PCM)/6-311++G**//B3LYP/6-31G** in the gas phase and water, respectively.
Table S5. Relative Energies of Neutral and Ionized Metabolites of Conformer NPC1161a02 Hydroxylated at All Possible Positions (kJ/mol).

| OH@<sup>a</sup> | ΔE<sub>b</sub> | ΔE<sub>c</sub> | ΔE<sub>d</sub> | ΔE<sub>e</sub> | ΔE<sub>f</sub> | ΔE<sub>g</sub> |
|----------------|-------------|-------------|-------------|-------------|-------------|-------------|
| 2              | 0.00        | 0.00        | 0.00        | 0.00        | 0.00        | 0.00        |
| 3              | 39.64       | 41.56       | 37.54       | 40.63       | 42.82       | 42.46       |
| 4Me            | 65.50       | 55.92       | 52.08       | 88.23       | 78.21       | 68.72       |
| 2"             | 35.48       | 39.38       | 41.45       | 55.10       | 55.16       | 46.88       |
| 5"             | 29.38       | 31.35       | 33.30       | 32.94       | 34.33       | 39.97       |
| 6"             | 29.82       | 32.03       | 35.84       | 48.69       | 48.69       | 42.90       |
| 6OMe           | 40.11       | 34.78       | 31.25       | 52.27       | 46.39       | 39.93       |
| 7              | 49.17       | 50.56       | 55.31       | 49.61       | 54.11       | 59.16       |
| N1'            | 199.64      | 206.16      | 207.83      | 178.68      | 187.46      | 188.53      |
| 2'             | 30.67       | 32.99       | 33.81       | 41.18       | 42.90       | 49.25       |
| 2'Me           | 58.78       | 53.92       | 54.27       | 64.92       | 55.69       | 63.14       |
| 3'R            | 32.97       | 31.08       | 29.40       | 37.38       | 36.55       | 37.42       |
| 3'S            | 39.77       | 39.91       | 39.00       | 35.86       | 36.79       | 44.73       |
| 4'R            | 39.63       | 34.92       | 32.15       | 24.72       | 22.82       | 38.01       |
| 4'S            | 39.09       | 37.97       | 32.01       | 28.55       | 27.43       | 36.55       |
| 5'R            | 31.99       | 26.90       | 20.49       | 46.91       | 40.77       | 28.82       |
| 5'S            | 31.93       | 28.48       | 23.53       | 42.44       | 37.82       | 31.85       |
| N6'            | 173.87      | 177.38      | 176.69      | 184.20      | 185.66      | 183.38      |

<sup>a</sup> The position at which the hydroxylation occurs. Numbering is as shown for NPC1161a in Chart 1. <sup>b, e</sup> Relative energies (kJ/mol) of neutral and ionized hydroxylated metabolites of conformer NPC1161a02 at different positions at the B3LYP/6-31G** level in the gas phase, respectively. <sup>c, f</sup> Relative energies (kJ/mol) of neutral and ionized hydroxylated metabolites of conformer NPC1161a02 at different positions at the B3LYP/6-311++G** level in the gas phase, respectively. <sup>d, g</sup> Relative energies (kJ/mol) of neutral and ionized hydroxylated metabolites of conformer NPC1161a02 at different positions at the B3LYP/SCRF(PCM)/6-311++G** level in water, respectively. The zero point energy at the B3LYP/6-31G** level in the gas phase was included in the calculations at all levels.
Table S6. Absolute and Relative Ionization Potentials of Metabolites of Conformer NPC1161a02 Hydroxylated at All Possible Positions (kJ/mol).

| OH@\(^a\) | IP\(^b\) | ΔIP\(^b\) | IP\(^c\) | ΔIP\(^c\) | IP\(^d\) | ΔIP\(^d\) |
|----------|-------|--------|-------|--------|-------|--------|
| Parent   | 585.96 | 0.00   | 611.38| 0.00   | 481.38| 0.00   |
| 2        | 578.90 | -7.05  | 606.37| -5.01  | 475.03| -6.36  |
| 3        | 579.89 | -6.06  | 607.44| -3.94  | 479.76| -1.63  |
| 4Me      | 601.64 | 15.68  | 627.15| 15.77  | 490.16| 8.78   |
| 2''      | 598.52 | 12.57  | 621.37| 9.98   | 479.67| -1.71  |
| 5''      | 582.46 | -3.49  | 609.03| -2.35  | 481.37| -0.01  |
| 6''      | 597.77 | 11.82  | 622.05| 10.67  | 481.11| -0.28  |
| 6OMe     | 591.07 | 5.11   | 617.58| 6.20   | 483.32| 1.93   |
| 7        | 579.35 | -6.61  | 609.16| -2.22  | 478.11| -3.27  |
| N1'      | 549.62 | -36.33 | 578.33| -33.05 | 437.01| -44.37 |
| 2'       | 589.41 | 3.46   | 614.79| 3.41   | 488.98| 7.59   |
| 2'Me     | 585.05 | -0.91  | 606.18| -5.20  | 481.94| 0.55   |
| 3'R      | 583.32 | -2.64  | 610.93| -0.45  | 482.14| 0.76   |
| 3'S      | 575.00 | -10.96 | 603.03| -8.35  | 480.54| -0.84  |
| 4'R      | 564.00 | -21.96 | 595.93| -15.45 | 482.54| 1.16   |
| 4'S      | 568.36 | -17.60 | 596.10| -15.28 | 479.84| -1.54  |
| 5'R      | 593.83 | 7.87   | 619.87| 8.49   | 482.98| 1.60   |
| 5'S      | 589.41 | 3.46   | 615.58| 4.20   | 483.22| 1.83   |
| N6'      | 589.23 | 3.27   | 614.37| 2.99   | 481.44| 0.06   |

\(^a\) The position at which the hydroxylation occurs. Numbering is as shown for NPC1161a in Chart 1. \(^b\), \(^c\), \(^d\) Ionization potentials of hydroxylated metabolites of Conformer NPC1161a02 (kJ/mol) at the B3LYP/6-31G** and B3LYP/6-311++G**/B3LYP/6-31G** levels in the gas phase, and at the B3LYP-SCRF(PCM)/6-311++G**/B3LYP/6-31G** level in water, respectively. The zero point energy at the B3LYP/6-31G** level in the gas phase was included in the calculations at the above levels.
Table S7. Total Energies of the Derivatives of Conformer NPC1161a02 (parent)

Dehydrogenated at All Possible Positions (a.u.) and the energy of the H atom in its ground state.

| deH@ |
|------|
| Eb   |
| Ec   |
| Ed   |
| Ee   |
| 2    | -2087.841778 | -2087.416785 | -2087.480373 | -2088.171208 |
| 3    | -2087.832815 | -2087.407889 | -2087.471646 | -2088.161734 |
| 4Me  | -2087.872056 | -2087.447672 | -2087.511339 | -2088.201844 |
| 2"   | -2087.831060 | -2087.406107 | -2087.469871 | -2088.160190 |
| 5"   | -2087.831774 | -2087.406837 | -2087.470419 | -2088.160381 |
| 6"   | -2087.828817 | -2087.403877 | -2087.467447 | -2088.157615 |
| 6OMe | -2087.856770 | -2087.432961 | -2087.496495 | -2088.186436 |
| 7    | -2087.834189 | -2087.409511 | -2087.473234 | -2088.163179 |
| 1'   | -2087.864098 | -2087.440343 | -2087.503768 | -2088.191365 |
| 2'   | -2087.870171 | -2087.445831 | -2087.507860 | -2088.198640 |
| 2'Me | -2087.848074 | -2087.425067 | -2087.488401 | -2088.178271 |
| 3'   | -2087.854228 | -2087.430952 | -2087.494994 | -2088.184011 |
| 4'   | -2087.854857 | -2087.431660 | -2087.495951 | -2088.184825 |
| 5'   | -2087.866976 | -2087.442801 | -2087.506113 | -2088.196968 |
| 6'   | -2087.857349 | -2087.434504 | -2087.497672 | -2088.184074 |
| parent | -2088.522253 | -2088.084305 | -2088.147076 | -2088.850846 |
| H    | -0.500273    | -0.500273    | -0.510927    | -0.502257    |

a The position at which the dehydrogenation occurs. b, c, d Electronic, electronic and zero-point, and Gibbs free energies of dehydrogenated NPC1161a02 at different positions at the B3LYP/6-31G** level in the gas phase. e Single-point energies at the B3LYP/6-311++G**/B3LYP/6-31G** in the gas phase.
Table S8. Calculated C-H/N-H Homolytic Bond Dissociation Energies (HBDEs, kJ/mol) in Conformer NPC1161a02 at all possible positions.

| H@a | HBDEb | ∆HBDEb | HBDEc | ∆HBDEc | HBDEd | ∆HBDEd | HBDEe | ∆HBDEe |
|-----|--------|---------|--------|---------|--------|---------|--------|---------|
| 4Me | 393.62 | 0.00    | 358.01 | 0.00    | 327.69 | 0.00    | 385.28 | 0.00    |
| 2'  | 398.58 | 4.96    | 362.85 | 4.84    | 336.82 | 9.13    | 393.69 | 8.41    |
| 5'  | 406.96 | 13.34   | 370.80 | 12.79   | 341.41 | 13.72   | 398.08 | 12.80   |
| N1' | 414.52 | 20.90   | 377.26 | 19.25   | 347.57 | 19.88   | 412.79 | 27.51   |
| N6' | 432.24 | 38.62   | 392.59 | 34.58   | 363.57 | 35.88   | 431.93 | 46.65   |
| 6OMe| 433.76 | 40.14   | 396.64 | 38.63   | 366.66 | 38.97   | 425.73 | 40.45   |
| 3'  | 440.43 | 46.81   | 401.91 | 43.90   | 370.60 | 42.91   | 432.10 | 46.82   |
| 4'  | 438.78 | 45.16   | 400.05 | 42.04   | 368.09 | 40.40   | 429.96 | 44.68   |
| 2'Me| 456.59 | 62.97   | 417.36 | 59.35   | 387.91 | 60.22   | 447.17 | 61.89   |
| 2   | 473.12 | 79.50   | 439.11 | 81.10   | 408.99 | 81.30   | 465.71 | 80.43   |
| 7   | 493.05 | 99.43   | 458.20 | 100.19  | 427.73 | 100.04  | 486.79 | 101.51  |
| 3   | 496.65 | 103.03  | 462.46 | 104.45  | 431.90 | 104.21  | 490.59 | 105.31  |
| 5"  | 499.39 | 105.77  | 465.23 | 107.22  | 435.12 | 107.43  | 494.14 | 108.86  |
| 2"  | 501.26 | 107.64  | 467.14 | 109.13  | 436.56 | 108.87  | 494.64 | 109.36  |
| 6"  | 507.15 | 113.53  | 473.00 | 114.99  | 442.93 | 115.24  | 501.40 | 116.12  |

a The positions at which the hydrogen is attached; b,c,d HBDEs and relative HBDEs calculated using total energies, total energies with zero point energies, and Gibbs free energies at the B3LYP/6-31G** level in the gas phase, respectively; e HBDEs and relative HBDEs calculated using single point energies at the B3LYP/6-311+G**//B3LYP/6-31G** level in the gas phase.