

# Acceleration of 1,3-Dipolar Cycloadditions by Integration of Strain and Electronic Tuning

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**List of Abbreviations**

ABC	2-azabeno-8-chlorobenzocyclooctyne
ACN	acetonitrile
CPCM	conductor-like polarizable continuum model
DIBAC	dibenzoazacyclooctyne
DBO	dibenzocyclooctyne
DIC	<i>N,N'</i> -diisopropylcarbodiimide
DIFO	difluorocyclooctyne
diF-SNO-OCT	difluoro-sulfur, nitrogen, and oxygen-containing heterocyclic cyclooctyne
DMSO	dimethyl sulfoxide
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
HPLC	high-performance liquid chromatography
IEPCM	integral equation formalism of the polarizable continuum model
LiHMDS	lithium bis(trimethylsilyl)amide
NBO	natural bonding orbital
<i>n</i> -ABC	<i>n</i> -azabeno-benzocyclooctyne ( <i>n</i> = 2–6)
OCT	cyclooctyne
PBS	phosphate-buffered saline
PNBO	pre-orthogonal natural bonding orbital
SPAAC	strain-promoted azide–alkyne cycloaddition
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TS	transition state
UFF	universal force field

**Computational Details.** Geometry optimizations were performed with Gaussian 16 software<sup>S1</sup> at the M06-2X level of theory<sup>S2</sup> (including the IEFPCM dielectric continuum solvent model for either CH<sub>2</sub>Cl<sub>2</sub> or water, with UFF radii<sup>S3</sup>) or the B97D/6-311+G(d,p) level of theory<sup>S3</sup> (including the CPCM solvation model for either CH<sub>2</sub>Cl<sub>2</sub> or water<sup>S4,S5</sup>). Frequency calculations were performed to confirm stationary points as minima or first-order saddle points. All  $\Delta E$  and  $\Delta E^\ddagger$  values include zero-point corrections. For previous reports benchmarking the methods utilized, see ref. S6 and S7. Coordinates, total energies, and imaginary frequencies (transition states) are provided in the XYZ files.

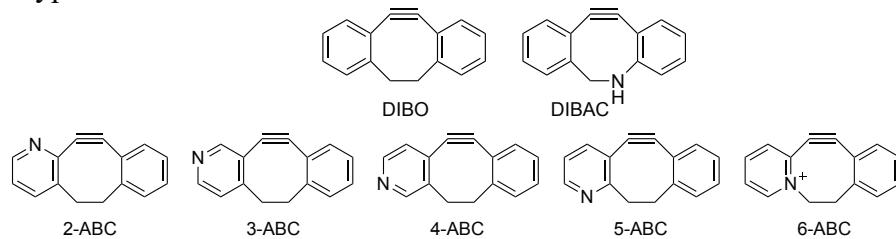
**Conformational Search.** Manual conformational searches were performed at both levels of theory employing the specified solvation models for water. Low energy structures were re-optimized in dichloromethane. For simplicity and brevity, tables contain the activation energies and free energies corresponding to located minima which display the most favorable free energy of activation. Figures S1 and S2 contain representative, low lying transition states located for 2-ABC and ABC.

**Starting Materials.** In all cyclooctynes (other than DIBAC), rotation about the ethylene bridge gives an enantiomer. For DIBAC, nitrogen inversion was considered. For diazoacetamides, the *s-cis* and *s-trans* conformers of the diazo group relative to the carbonyl were optimized. For azidoacetamides, rotation about the  $\psi$  (NC–C<sub>α</sub>N<sub>3</sub> dihedral) and  $\phi$  (CC<sub>α</sub>–NN<sub>2</sub>) dihedral angles were examined via input geometries that varied in 60° increments. These input geometries converged on 2–3 structures, dependent upon the level of theory and solvation model, that were within ~0.5–2.0 kcal/mol.

**$\alpha$ -Azidoacetamide Transition States.** Modes of approach considered the  $\psi$  and  $\phi$  dihedral angles described above and rotation about the dihedral angle of the azido group (NN–NC<sub>α</sub>). The cyclooctyne ring limits the latter to a potential range of ~180° in the TS. For non-symmetric cyclooctynes (DIBAC and ABCs) both the *syn*- and *anti*-approach of the 1,3-dipole substituents relative to the aza-/azabenzene-ring were considered. For DIBAC, all of the above was considered, along with nitrogen inversion. Low energy transition states are included.

**$\alpha$ -Diazoacetamide Transition States.** Modes of approach were chosen as input geometries for each diazoacetamide conformation (*i.e.*, the *s-cis* and *s-trans* conformers). Low energy transition states are included.

**Table S1.** Energies and free energies of activation (kcal/mol) for cycloadditions of 2-azido-*N*-methylacetamide (**1**) and 2-diazo-*N*-methylacetamide (**2**) with constitutional isomers of dibenzoazacyclooctyne (DIBAC). Geometries were optimized at both M06-2X/6-311++G(d,p) employing the IEFPCM solvation model and B97D/6-311+G(d,p) employing the CPCM solvation model for either CH<sub>2</sub>Cl<sub>2</sub> or water.<sup>a</sup> Energies for the preferred regioisomer are in bold typeface.



Method	Solvent	Compound	Azide <b>1</b>				Diazo Compound <b>2</b>			
			<i>syn</i> TS		<i>anti</i> TS		<i>syn</i> TS		<i>anti</i> TS	
			$\Delta E^\ddagger$	$\Delta G^\ddagger$	$\Delta E^\ddagger$	$\Delta G^\ddagger$	$\Delta E^\ddagger$	$\Delta G^\ddagger$	$\Delta E^\ddagger$	$\Delta G^\ddagger$
M06-2X	CH <sub>2</sub> Cl <sub>2</sub>	DIBO	9.6	23.5	—	—	10.6	23.7	—	—
		DIBAC	8.9	<b>22.2</b>	8.9	22.4	10.1	23.2	<b>8.8</b>	<b>21.6</b>
		2-ABC	<b>6.1</b>	<b>19.5</b>	10.4	23.9	<b>5.5</b>	<b>18.8</b>	10.7	23.7
		3-ABC	9.8	<b>23.0</b>	<b>9.5</b>	23.1	9.9	22.9	<b>9.8</b>	<b>22.7</b>
		4-ABC	9.5	<b>22.2</b>	<b>9.5</b>	23.0	10.0	22.9	<b>9.7</b>	<b>22.5</b>
		5-ABC	9.8	<b>22.7</b>	<b>9.6</b>	23.1	10.5	23.2	<b>9.7</b>	<b>21.1</b>
		6-ABC	8.6	22.5	<b>7.5</b>	<b>21.0</b>	7.0	20.7	<b>6.1</b>	<b>19.3</b>
M06-2X	H <sub>2</sub> O	DIBO	10.3	23.7	—	—	10.6	23.7	—	—
		DIBAC	9.6	23.3	<b>9.1</b>	<b>22.9</b>	10.1	23.3	<b>8.9</b>	<b>22.1</b>
		2-ABC	<b>8.3</b>	<b>21.9</b>	10.5	23.8	<b>6.2</b>	<b>19.5</b>	10.6	23.7
		3-ABC	10.0	23.5	<b>9.7</b>	<b>23.2</b>	10.0	22.8	<b>9.8</b>	<b>22.8</b>
		4-ABC	9.8	23.3	<b>9.6</b>	<b>22.9</b>	9.9	<b>22.7</b>	<b>9.8</b>	22.9
		5-ABC	10.1	23.5	<b>9.7</b>	<b>23.1</b>	10.3	23.2	<b>10.0</b>	<b>23.2</b>
		6-ABC	8.6	22.1	<b>7.7</b>	<b>21.3</b>	7.1	20.5	<b>6.8</b>	<b>19.7</b>
B97D	CH <sub>2</sub> Cl <sub>2</sub>	DIBO	5.2	19.6	—	—	7.8	20.9	—	—
		DIBAC	4.7	18.9	5.5	<b>18.5</b>	6.9	20.3	<b>6.0</b>	<b>19.3</b>
		2-ABC	<b>3.9</b>	<b>18.2</b>	6.2	20.4	<b>3.6</b>	<b>17.3</b>	8.3	21.7
		3-ABC	5.0	19.2	<b>4.8</b>	<b>19.2</b>	7.0	<b>20.0</b>	7.0	20.2
		4-ABC	5.2	19.5	<b>4.8</b>	<b>19.2</b>	7.2	<b>20.2</b>	<b>7.1</b>	20.3
		5-ABC	5.2	19.6	<b>4.9</b>	<b>19.4</b>	7.7	20.7	<b>7.2</b>	<b>20.3</b>
		6-ABC	5.3	19.3	<b>4.9</b>	<b>18.7</b>	4.6	17.8	<b>4.0</b>	<b>16.9</b>
B97D	H <sub>2</sub> O	DIBO	5.5	18.9	—	—	7.9	20.9	—	—
		DIBAC	5.2	18.2	<b>5.8</b>	<b>17.9</b>	7.1	20.4	<b>6.2</b>	<b>19.4</b>
		2-ABC	<b>4.3</b>	<b>17.5</b>	6.4	19.8	<b>4.1</b>	<b>17.8</b>	8.4	21.6
		3-ABC	5.4	18.6	<b>5.1</b>	<b>18.4</b>	7.4	20.4	<b>7.1</b>	<b>20.1</b>
		4-ABC	5.5	18.7	<b>5.1</b>	<b>18.4</b>	7.2	<b>20.1</b>	7.2	20.4
		5-ABC	5.6	18.8	<b>5.3</b>	<b>18.6</b>	7.7	<b>20.1</b>	<b>7.4</b>	20.4
		6-ABC	5.3	18.6	<b>5.3</b>	<b>18.0</b>	4.8	17.8	<b>4.6</b>	<b>17.5</b>

<sup>a</sup>Energies given for conformers favored by  $\Delta G^\ddagger$ . See Figure S1 and the computational details.

**Table S2.** Distortion/Interaction (Activation–Strain) analysis of energies (kcal/mol) for cycloadditions of 2-azido-*N*-methylacetamide (**1**) with constitutional isomers of dibenzoazacyclooctyne (DIBAC). Geometries optimized at both M06-2X/6-311++G(d,p) employing the IEFPCM solvation model for either CH<sub>2</sub>Cl<sub>2</sub> or water, and B97D/6-311+G(d,p) employing the CPCM solvation model for either CH<sub>2</sub>Cl<sub>2</sub> or water. Energies for the preferred regioisomer are in bold typeface.

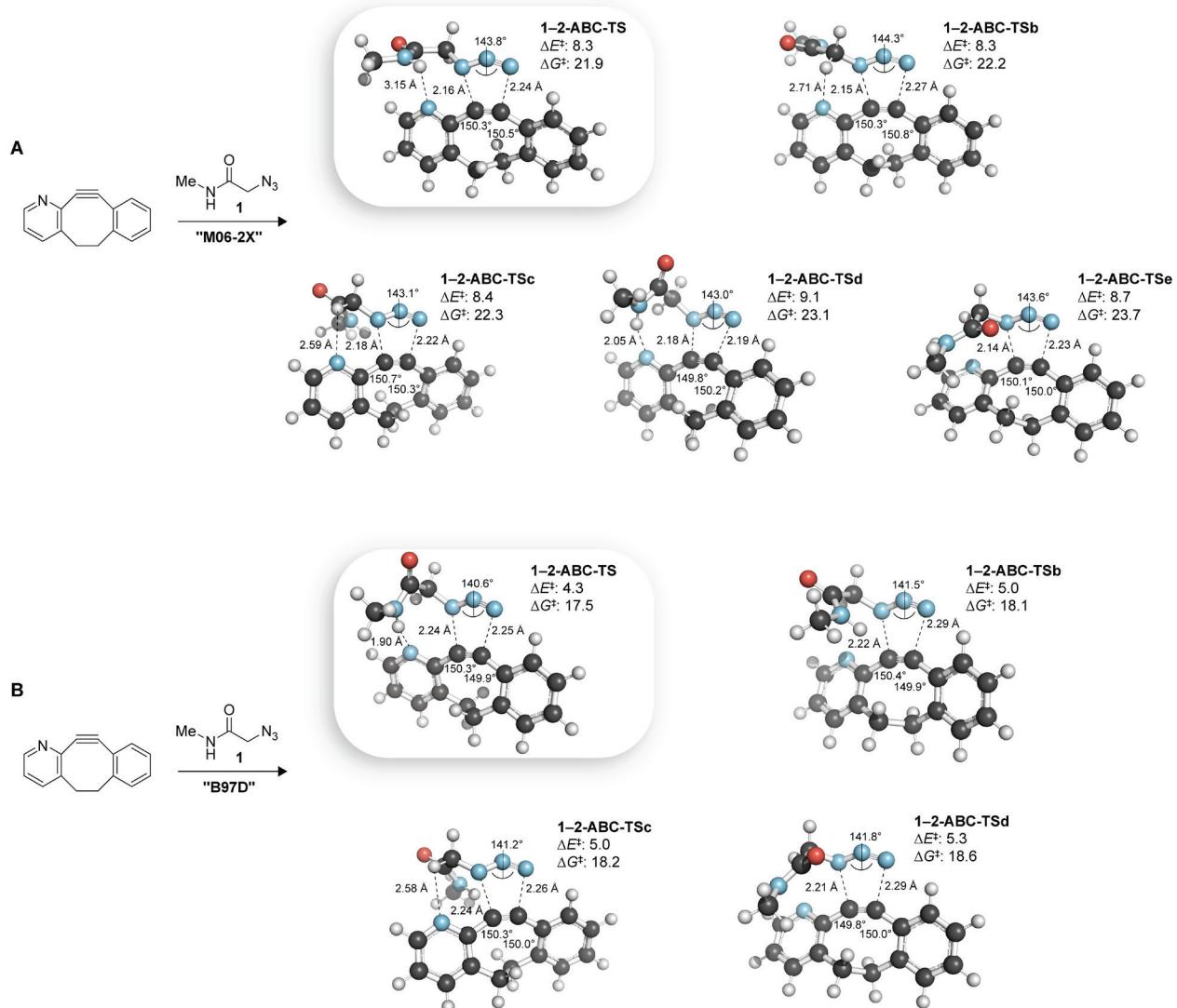
Method	Solvent	Compound	syn TS			anti TS			$\Delta E^\ddagger_{\text{Interaction}}$	
			$\Delta E^\ddagger_{\text{distortion}}$			$\Delta E^\ddagger_{\text{distortion}}$				
			1,3-Dipole	Alkyne	Total	1,3-Dipole	Alkyne	Total		
M06-2X	CH <sub>2</sub> Cl <sub>2</sub>	DIBO	18.6	3.2	21.8	-12.1	—	—	—	
		DIBAC	18.3	3.0	21.3	-12.5	16.1	2.5	18.6	
		2-ABC	<b>16.7</b>	<b>2.6</b>	<b>19.3</b>	<b>-13.2</b>	16.2	2.4	18.7	
		3-ABC	16.7	2.9	19.6	-9.9	<b>16.7</b>	<b>2.9</b>	<b>19.6</b>	
		4-ABC	16.8	3.0	19.8	-10.3	<b>16.7</b>	<b>2.9</b>	<b>19.6</b>	
		5-ABC	17.0	3.0	20.0	-10.2	<b>16.8</b>	<b>2.9</b>	<b>19.7</b>	
		6-ABC	16.7	3.7	20.4	-11.8	<b>16.0</b>	<b>3.8</b>	<b>19.7</b>	
M06-2X	H <sub>2</sub> O	DIBO	17.0	3.0	20.1	-9.8	—	—	—	
		DIBAC	18.3	3.1	22.1	-11.8	<b>16.1</b>	<b>2.6</b>	<b>18.7</b>	
		2-ABC	<b>16.6</b>	<b>2.6</b>	<b>19.2</b>	<b>-10.9</b>	16.8	3.0	19.8	
		3-ABC	16.7	3.0	19.7	-9.7	<b>16.6</b>	<b>3.0</b>	<b>19.6</b>	
		4-ABC	16.7	3.1	19.8	-10.0	<b>16.7</b>	<b>2.9</b>	<b>19.6</b>	
		5-ABC	16.9	3.0	19.9	-9.8	<b>16.8</b>	<b>2.9</b>	<b>19.7</b>	
		6-ABC	16.5	3.6	20.1	-11.5	<b>15.9</b>	<b>3.7</b>	<b>19.6</b>	
B97D	CH <sub>2</sub> Cl <sub>2</sub>	DIBO	16.3	3.0	19.4	-14.2	—	—	—	
		DIBAC	16.2	2.9	19.1	-14.4	<b>13.7</b>	<b>2.2</b>	<b>15.9</b>	
		2-ABC	<b>17.1</b>	<b>3.3</b>	<b>20.5</b>	<b>-16.6</b>	16.0	3.1	19.1	
		3-ABC	16.0	3.0	19.0	-14.0	15.8	3.0	18.8	
		4-ABC	16.0	3.2	19.1	-13.9	<b>15.9</b>	<b>2.9</b>	<b>18.8</b>	
		5-ABC	16.2	3.0	19.2	-14.0	<b>16.1</b>	<b>2.9</b>	<b>19.0</b>	
		6-ABC	15.4	3.9	19.2	-13.5	<b>14.0</b>	<b>3.6</b>	<b>17.5</b>	
B97D	H <sub>2</sub> O	DIBO	16.4	3.0	19.4	-13.9	—	—	—	
		DIBAC	16.3	2.9	19.2	-14.0	<b>13.9</b>	<b>2.2</b>	<b>16.1</b>	
		2-ABC	<b>17.1</b>	<b>3.3</b>	<b>20.4</b>	<b>-16.1</b>	16.1	3.1	19.2	
		3-ABC	16.1	3.0	19.1	-13.7	<b>15.9</b>	<b>2.9</b>	<b>18.8</b>	
		4-ABC	16.1	3.2	19.3	-13.8	<b>16.0</b>	<b>2.9</b>	<b>18.9</b>	
		5-ABC	<b>16.3</b>	<b>3.0</b>	<b>19.3</b>	<b>-13.8</b>	<b>16.2</b>	<b>2.9</b>	<b>19.1</b>	
		6-ABC	<b>15.5</b>	<b>3.8</b>	<b>19.3</b>	<b>-14.0</b>	<b>14.1</b>	<b>3.5</b>	<b>17.6</b>	

**Table S3.** Distortion/Interaction (Activation–Strain) analysis of energies (kcal/mol) for cycloadditions of 2-diazo-*N*-methylacetamide (**2**) with constitutional isomers of dibenzoazacyclooctyne (DIBAC). Geometries optimized at both M06-2X/6-311++G(d,p) employing the IEFPCM solvation model for either CH<sub>2</sub>Cl<sub>2</sub> or water, and B97D/6-311+G(d,p) employing the CPCM solvation model for either CH<sub>2</sub>Cl<sub>2</sub> or water. Energies for the preferred regioisomer are in bold typeface.

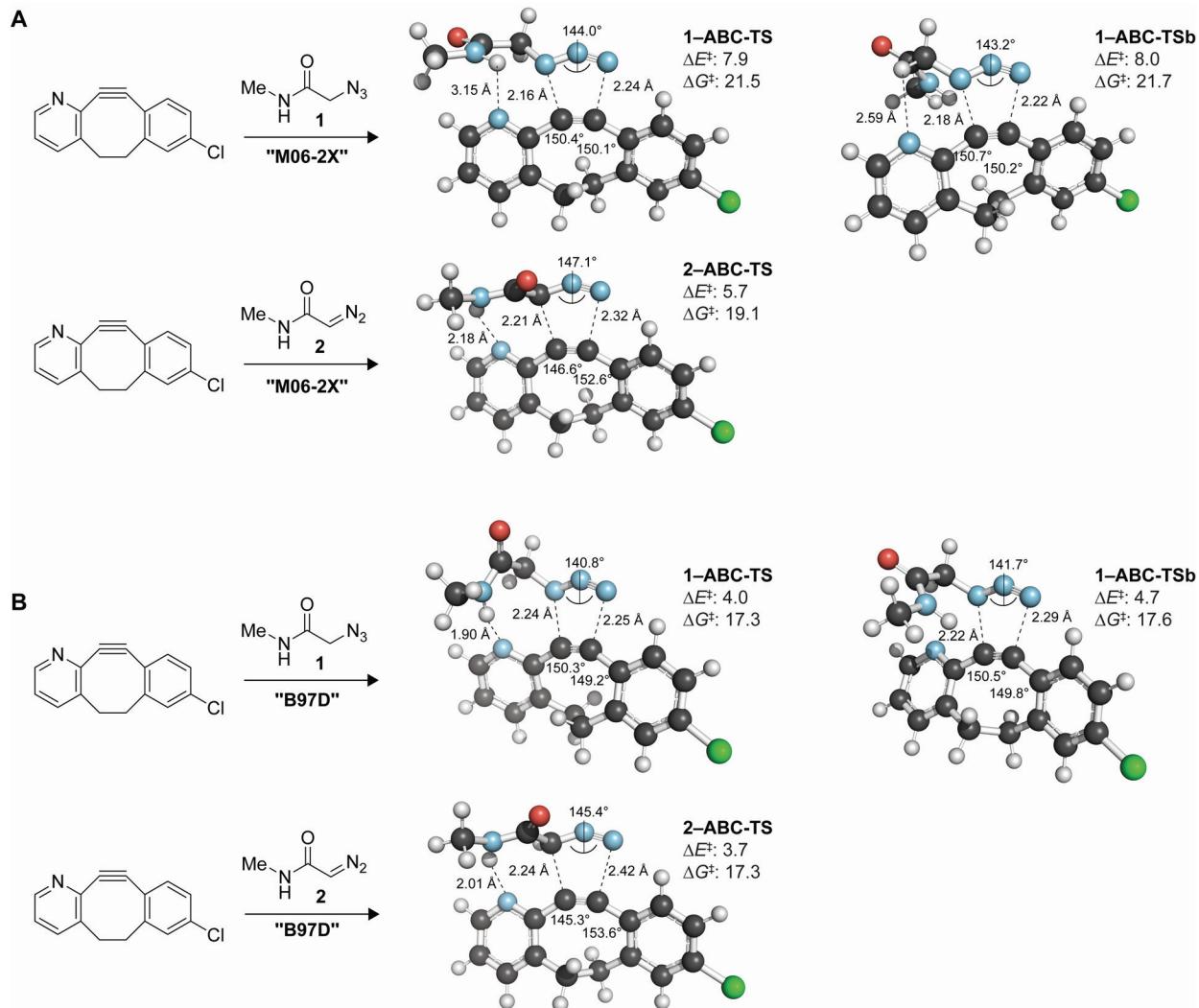
Method	Solvent	Compound	syn TS			anti TS			$\Delta E^\ddagger_{\text{interaction}}$	
			$\Delta E^\ddagger_{\text{distortion}}$			$\Delta E^\ddagger_{\text{distortion}}$				
			1,3-Dipole	Alkyne	Total	1,3-Dipole	Alkyne	Total		
M06-2X	CH <sub>2</sub> Cl <sub>2</sub>	DIBO	18.6	5.0	23.7	-13.0	—	—	—	
		DIBAC	17.5	4.3	21.8	-11.7	<b>17.5</b>	<b>4.4</b>	<b>21.9</b>	
		2-ABC	<b>16.8</b>	<b>3.8</b>	<b>20.6</b>	<b>-15.1</b>	18.5	5.2	23.6	
		3-ABC	18.0	4.8	22.8	-12.9	<b>18.1</b>	<b>5.0</b>	<b>23.1</b>	
		4-ABC	17.9	5.0	22.9	-13.0	<b>18.2</b>	<b>5.0</b>	<b>23.1</b>	
		5-ABC	18.2	4.9	23.1	-12.6	<b>18.3</b>	<b>4.9</b>	<b>23.2</b>	
		6-ABC	17.4	5.6	23.0	-15.9	<b>16.6</b>	<b>5.7</b>	<b>22.3</b>	
M06-2X	H <sub>2</sub> O	DIBO	18.6	5.0	23.6	-13.0	—	—	—	
		DIBAC	17.6	4.2	21.9	-11.7	<b>17.1</b>	<b>4.4</b>	<b>21.5</b>	
		2-ABC	<b>16.8</b>	<b>3.7</b>	<b>20.5</b>	<b>-14.3</b>	18.3	5.1	23.4	
		3-ABC	18.1	4.8	22.9	-12.9	<b>18.0</b>	<b>5.0</b>	<b>23.0</b>	
		4-ABC	18.0	5.0	23.0	-13.1	18.2	4.9	23.1	
		5-ABC	18.3	4.9	23.2	-12.9	<b>18.1</b>	<b>4.8</b>	<b>23.0</b>	
		6-ABC	17.2	5.4	22.6	-15.5	<b>16.7</b>	<b>5.6</b>	<b>22.3</b>	
B97D	CH <sub>2</sub> Cl <sub>2</sub>	DIBO	17.1	4.5	21.6	-13.8	—	—	—	
		DIBAC	15.8	3.7	19.5	-12.6	<b>15.4</b>	<b>3.9</b>	<b>19.3</b>	
		2-ABC	<b>16.0</b>	<b>3.5</b>	<b>19.5</b>	<b>-16.0</b>	16.9	4.6	21.5	
		3-ABC	16.4	4.3	20.7	-13.7	16.4	4.4	20.8	
		4-ABC	16.4	4.4	20.8	-13.7	16.6	4.3	21.0	
		5-ABC	16.7	4.5	21.2	-13.5	<b>16.8</b>	<b>4.3</b>	<b>21.1</b>	
		6-ABC	<b>15.7</b>	<b>5.1</b>	<b>20.8</b>	<b>-16.2</b>	<b>15.0</b>	<b>5.4</b>	<b>20.4</b>	
B97D	H <sub>2</sub> O	DIBO	17.0	4.4	21.5	-13.5	—	—	—	
		DIBAC	15.8	3.6	19.4	-12.3	<b>15.4</b>	<b>3.9</b>	<b>19.3</b>	
		2-ABC	<b>16.0</b>	<b>3.5</b>	<b>19.5</b>	<b>-15.4</b>	16.8	4.6	21.4	
		3-ABC	16.3	4.3	20.6	-13.2	<b>16.3</b>	<b>4.4</b>	<b>20.7</b>	
		4-ABC	16.3	4.4	20.7	-13.5	16.5	4.3	20.8	
		5-ABC	16.6	4.5	21.1	-13.4	16.7	4.2	20.9	
		6-ABC	15.6	5.1	20.7	-15.9	<b>15.0</b>	<b>5.4</b>	<b>20.4</b>	

**Table S4.** Energies and free energies of activation (kcal/mol) for cycloadditions of *N*-substituted 2-diazoacetamides and *N*-substituted 2-azidoacetamides (*R*=H or Me) with ABC. Geometries optimized at both M06-2X/6-311++G(d,p) employing the IEFPCM solvation model for either CH<sub>2</sub>Cl<sub>2</sub> or water, and B97D/6-311+G(d,p) employing the CPCM solvation model for either CH<sub>2</sub>Cl<sub>2</sub> or water. Energies for the preferred regioisomer are in bold typeface.

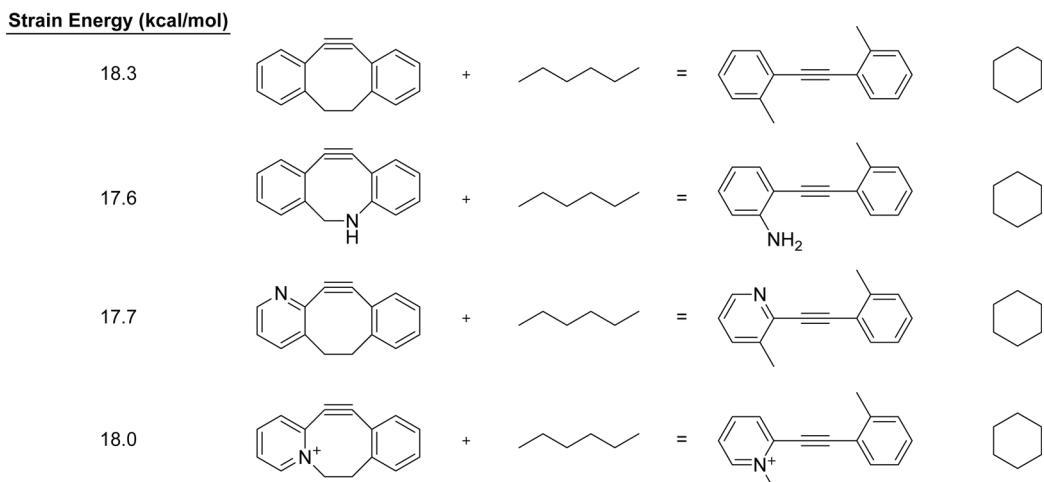
Method	Solvent	<i>R</i>	2-azido- <i>N</i> -R- <i>N</i> -methylacetamide				2-diazo- <i>N</i> -R- <i>N</i> -methylacetamide			
			<i>syn</i> TS		<i>anti</i> TS		<i>syn</i> TS		<i>anti</i> TS	
			$\Delta E^\ddagger$	$\Delta G^\ddagger$	$\Delta E^\ddagger$	$\Delta G^\ddagger$	$\Delta E^\ddagger$	$\Delta G^\ddagger$	$\Delta E^\ddagger$	$\Delta G^\ddagger$
M06-2X	CH <sub>2</sub> Cl <sub>2</sub>	H	<b>7.3</b>	<b>20.6</b>	10.1	23.1	<b>5.0</b>	<b>18.4</b>	10.3	23.2
	H <sub>2</sub> O	H	<b>7.9</b>	<b>21.5</b>	10.2	23.3	<b>5.7</b>	<b>19.1</b>	10.2	23.2
	CH <sub>2</sub> Cl <sub>2</sub>	Me	<b>8.1</b>	<b>20.5</b>	9.8	24.3	<b>7.1</b>	<b>20.8</b>	10.9	24.9
	H <sub>2</sub> O	Me	<b>8.6</b>	<b>21.3</b>	9.8	24.3	<b>7.5</b>	<b>21.1</b>	10.9	25.0
B97D	CH <sub>2</sub> Cl <sub>2</sub>	H	<b>3.6</b>	<b>18.1</b>	6.1	20.5	<b>3.2</b>	<b>16.9</b>	8.0	21.1
	H <sub>2</sub> O	H	<b>4.0</b>	<b>17.3</b>	6.3	19.6	<b>3.7</b>	<b>17.3</b>	8.1	21.2
	CH <sub>2</sub> Cl <sub>2</sub>	Me	<b>5.1</b>	<b>18.6</b>	6.3	21.0	<b>5.1</b>	<b>18.3</b>	7.9	21.4
	H <sub>2</sub> O	Me	<b>5.8</b>	<b>18.5</b>	6.8	21.2	<b>5.4</b>	<b>18.8</b>	8.0	21.6



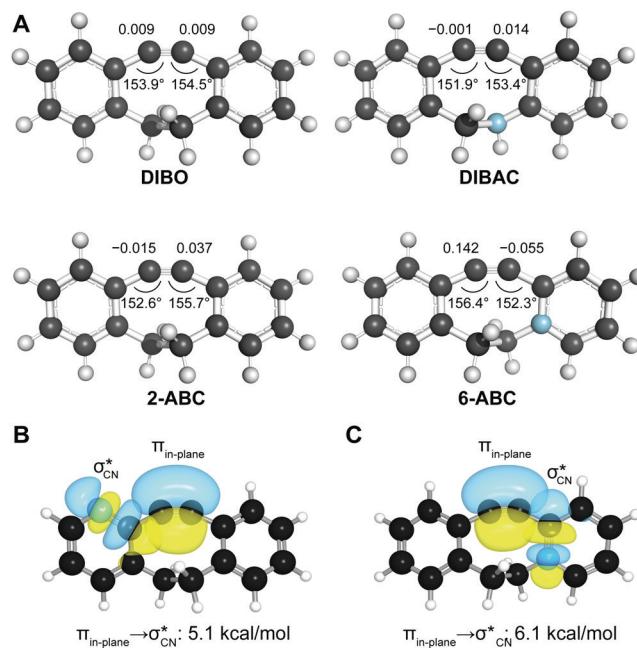
**Figure S1.** Optimized transition state geometries for the *syn* reaction of 2-azido-*N*-methylacetamide (**1**) with 2-ABC at (A) the M06-2X/6-311++G(d,p) employing the IEFPCM solvation model for water, and (B) the B97D/6-311+G(d,p) employing the CPCM solvation model for water.



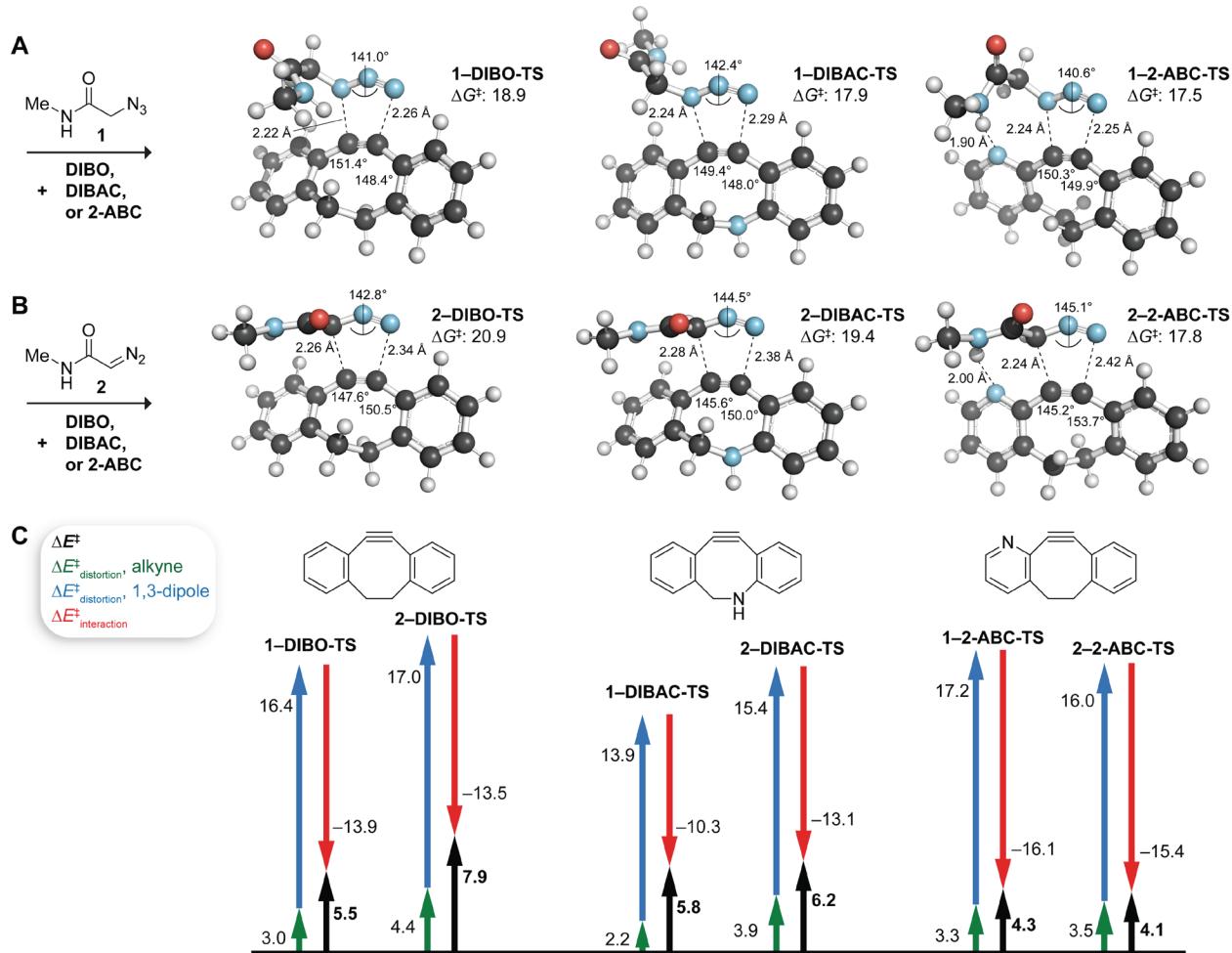
**Figure S2.** Optimized transition state geometries for the reaction of 2-azido-*N*-methylacetamide (**1**) and 2-diazo-*N*-methylacetamide (**2**) with ABC at the M06-2X/6-311++G(d,p) employing the IEFPCM solvation model for water (A), and the B97D/6-311+G(d,p) employing the CPCM solvation model for water (B).



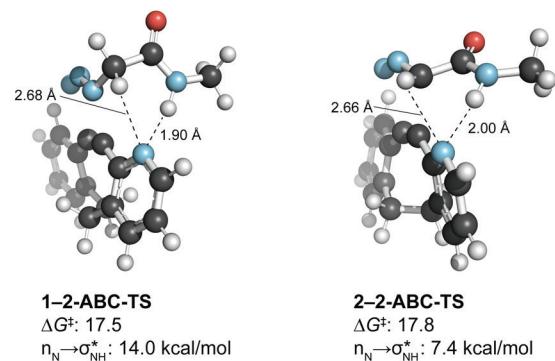
**Figure S3.** Isodesmic equations used to calculate strain energies of dibenzocyclooctyne (DIBO), dibenzoazacyclooctyne (DIBAC), 2-azabeno-benzocyclooctyne (2-ABC), and 6-azabeno-benzocyclooctyne (6-ABC) calculated at the M06-2X/6-311++G(d,p) employing the IEFPCM solvation model (water). A correction of 2.2 kcal/mol was used to account for the nonzero strain energy of cyclohexane.<sup>S9</sup>



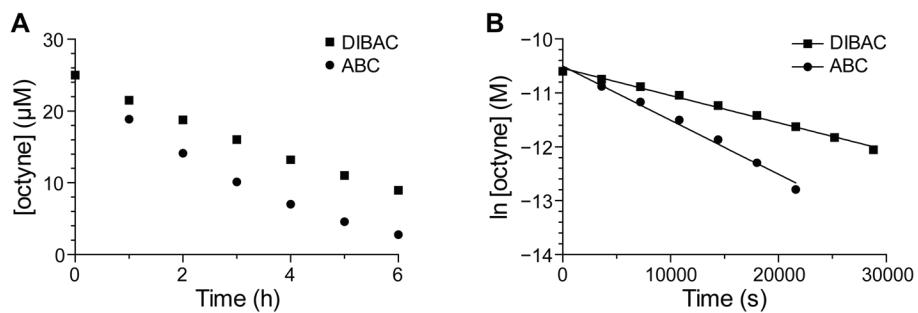
**Figure S4.** Combining increased strain with electronic activation. (A) Optimized geometries and NBO charge on each alkyne carbon of dibenzocyclooctyne (DIBO), dibenzoazacyclooctyne (DIBAC), 2-azabeno-benzocyclooctyne (2-ABC), and 6-azabeno-benzocyclooctyne (6-ABC) calculated at the B97D/6-311+G(d,p) employing the CPCM solvation model (water). (B,C) Natural bonding orbitals depicting  $\pi_{\text{CC}} \rightarrow \sigma_{\text{CN}}^*$  interactions with the the *syn*-periplanar C–N bond in 2-ABC (B) and *anti*-periplanar C–N bond in 6-ABC (C).



**Figure S5.** Computational analysis of cycloadditions with 2-azido-*N*-methylacetamide (**1**) and 2-diazo-*N*-methylacetamide (**2**). (A,B) Optimized transition state geometries and free energies of activation (kcal/mol) calculated at the B97D/6-311+G(d,p) level employing the CPCM solvation model (water). (C) Distortion/Interaction (Strain–Activation) analysis.



**Figure S6.** Comparison of hydrogen bonding interactions in 2-ABC cycloadditions with 2-azido-*N*-methylacetamide (**1**) (left) and 2-diazo-*N*-methylacetamide (**2**) (right). Second-order perturbations obtained from the NBO analysis provide a measure of relative hydrogen bond strengths. Optimized transition state geometries are from Figure S5.



**Figure S8.** Stability of DIBAC and ABC in the presence of 1 mM reduced glutathione and 0.2 mM oxidized glutathione in PBS containing DMSO (2% v/v) at 37 °C. (A) Concentration of remaining DIBAC and ABC as determined by HPLC. (B) Natural logarithm of the concentration of DIBAC and ABC over time in order to determine the first-order half-life of each cyclooctyne. The half-lives were 3.8 h and 1.9 h for DIBAC and ABC, respectively. Values are the mean  $\pm$  SD for triplicate experiments. (Error bars are smaller than the data points.)

## Experimental Procedures

**General.** All chemicals were from commercial sources and were used without further purification. NMR spectra were acquired with an Avance Neo 400 spectrometer or Avance Neo 500 spectrometer from Bruker (Billerica, MA, USA). Mass spectra were acquired by using positive ionization with an AccuTOF-DART 4G instrument from JEOL (Tokyo, Japan). HPLC experiments were carried out on a 1200 series HPLC from Agilent Technologies (Santa Clara, CA, USA) equipped with a Varian Microsorb-MV 100-5 C18 250 × 4.6 mm column. Gradients were run with water containing TFA (0.1% v/v) and ACN containing TFA (0.1% v/v). Absorbance was measured at 280 nm. Column chromatography was performed with an Isolera automated purification system from Biotage (Uppsala, Sweden) using prepacked SNAP KP silica gel columns. Thermostability was assessed with a Stanford Research Systems Optimelt automated melting point system.

The phrase “concentrated under reduced pressure” refers to the removal of solvents and other volatile materials using a rotary evaporator at water aspirator pressure (<20 Torr) while maintaining the water-bath temperature of 40 °C. Residual solvent was removed from samples by the vacuum (<0.1 Torr) achieved by a mechanical belt-drive oil pump.

All procedures were performed in air at ambient temperature (~22 °C) and pressure (1.0 atm) unless indicated otherwise.

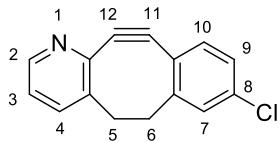
**2-Azido-N-benzylacetamide (5) and 2-diazo-N-benzylacetamide (6).** These compounds were synthesized as reported previously.<sup>S10</sup>

**4-((*N*-Tetrazolyl)methyl)morpholine (8).** To a cold (0 °C), stirred solution of tetrazole (0.70 g, 10.0 mmol, 1.0 equiv) in methanol (10 mL) was added morpholine (0.957 g, 0.95 mL, 11.0 mmol, 1.1 equiv), and the mixture was stirred for 15 min. An aqueous solution of 37% v/v formaldehyde (0.98 mL, 12.0 mmol, 1.2 equiv) was added dropwise, and the mixture was stirred overnight at room temperature. The reaction mixture was then concentrated under reduced pressure, and the residue was recrystallized from a 1:2 v/v mixture of CH<sub>2</sub>Cl<sub>2</sub> and hexanes to provide compound **8** as a mixture of *N* tautomers, as white crystals (1.48 g, 8.74 mmol, 88%). All spectral data matches published data.<sup>11</sup> **1H NMR** (400 MHz, CDCl<sub>3</sub>, δ): 8.67 (s, 0.2H), 8.56 (s, 0.8H), 5.53 (s, 1.6H), 5.31 (s, 0.4H), 3.82–3.61 (m, 4H), 2.69–2.65 (m, 3.2H), 2.62 (t, *J* = 4.7 Hz, 0.8H). **13C NMR** (101 MHz, CDCl<sub>3</sub>, δ): 152.69, 74.00, 66.64, 66.44, 49.85, 49.76. **HRMS** *m/z* calcd for C<sub>6</sub>H<sub>12</sub>N<sub>5</sub>O [M + H]<sup>+</sup>, 170.10364; found, 170.10408.

**8-Chloro-11-(1*H*-tetrazol-5-yl)-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-ol (4).** To a stirred solution of 4-((*N*-tetrazolyl)methyl)morpholine (**8**) (0.70 g, 4.12 mmol, 2.0 equiv) and 8-chloro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11(6*H*)-one (**3**) (0.50 g, 2.06 mmol, 1.0 equiv) in THF (10 mL), under N<sub>2</sub>(g) at -78 °C (acetone/CO<sub>2</sub>), was added 1 M LiHMDS in THF (4.33 mL, 4.33 mmol, 2.1 equiv) dropwise via a syringe. The reaction mixture was stirred for 2 h at -78 °C then allowed to warm to room temperature overnight. The reaction mixture was concentrated under reduced pressure, and the remaining residue was treated with aqueous HCl (1 M, 25 mL) and stirred at room temperature for 1 h. The solution was then extracted with EtOAc (3 × 50 mL), and the combined organic extracts were dried over Na<sub>2</sub>SO<sub>4</sub>(s), filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (hexanes/EtOAc 85:15→25:75) to provide compound **4** (0.336 g, 1.07 mmol, 52%) as a white solid. **1H NMR** (500 MHz, CDCl<sub>3</sub>, δ): 8.78 (s, 1H), 8.46 (s, 1H), 8.09 (dd, *J* = 8.4, 4.0 Hz, 1H), 7.64 (d, *J* = 7.6 Hz, 1H), 7.37 (d, *J* = 6.6 Hz, 1H), 7.25 (d, *J* = 8.9 Hz, 1H), 7.17 (s, 1H), 3.40–3.32 (m, 1H), 3.05–2.93 (m, 3H), 2.74–2.67 (m, 1H). **13C NMR** (126 MHz, CDCl<sub>3</sub>, δ): 161.98, 152.43, 143.57, 142.67, 141.72, 140.09, 138.54, 134.70, 134.38, 129.45,

127.21, 126.36, 124.64, 30.81, 29.95. **HRMS**  $m/z$  calcd for  $C_{15}H_{13}ON_5Cl$  [M + H]<sup>+</sup>, 314.08086; found, 314.08960.

**2-Azabenzo-8-chlorobenzocyclooctyne (ABC).** To a stirred solution of compound **4** (0.514 g, 1.64 mmol, 1.0 equiv) in THF (5.0 mL), was treated with EDC (0.345 g, 1.80 mmol, 1.1 equiv) and allowed to react overnight. The reaction mixture was concentrated under reduced pressure, and the residue was purified by flash column chromatography on silica gel (hexanes/EtOAc 85:15→25:75) to provide ABC (0.157 g, 0.66 mmol, 40%) as a pale yellow solid. mp: decomposition observed at  $\geq 80$  °C. **<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>,  $\delta$ ): 8.44 (dd,  $J$  = 5.0, 1.6 Hz, 1H), 7.51 (dd,  $J$  = 7.6, 1.6 Hz, 1H), 7.30–7.20 (m, 3H), 7.12 (dd,  $J$  = 7.7, 4.9 Hz, 1H), 3.30–3.18 (m, 2H), 2.36 (ddq,  $J$  = 15.1, 8.5, 3.7 Hz, 2H). **<sup>13</sup>C NMR** (126 MHz, CDCl<sub>3</sub>,  $\delta$ ): 155.00, 148.55, 147.75, 144.65, 136.34, 134.52, 129.74, 127.45, 126.88, 121.97, 121.20, 113.31, 109.88, 35.62, 34.79. **HRMS**  $m/z$  calcd for  $C_{15}H_{11}NCl$  [M + H]<sup>+</sup>, 240.05800; found, 240.06485. The atom-numbering and IUPAC name of ABC is as follows:



8-chloro-5,6-dihydro-11,12-didehydrobenzo[5,6]cycloocta[1,2-b]pyridine

**5-(1*H*-Tetrazol-5-yl)-10,11-dihydro-5*H*-dibenzo[a,d][7]annulen-5-ol (9).** To a stirred solution of compound **8** (0.413 g, 2.44 mmol, 2.0 equiv) and dibenzosuberone (0.25 g, 1.22 mmol, 1.0 equiv) in THF (5 mL), under N<sub>2</sub>(g) at  $-78$  °C (acetone/CO<sub>2</sub>), was added 1 M LiHMDS in THF (2.56 mmol, 2.56 mL, 2.1 equiv) dropwise via a syringe. The reaction mixture was stirred for 2 h at  $-78$  °C then allowed to warm to room temperature overnight. The reaction mixture was concentrated under reduced pressure, and the remaining residue was treated with aqueous HCl (1 M, 25 mL) and stirred at room temperature for 1 h. The solution was then extracted with EtOAc (3 × 50 mL), and the combined organic extracts were dried over Na<sub>2</sub>SO<sub>4</sub>(s), filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (2% v/v MeOH in CH<sub>2</sub>Cl<sub>2</sub>) to provide compound **9** (0.3596 g) as a white solid with some impurities but was used in the next step without further purification. **<sup>1</sup>H NMR** (400 MHz, MeOD,  $\delta$ ): 8.09–7.99 (m, 2H), 7.31–7.25 (m, 4H), 7.18–7.12 (m, 2H), 2.83 (s, 4H). **<sup>13</sup>C NMR** (101 MHz, MeOD,  $\delta$ ): 162.16, 141.12, 137.93, 130.25, 128.05, 125.83, 125.22, 71.82, 32.05. **HRMS**  $m/z$  calcd for  $C_{16}H_{15}ON_4$  [M + H]<sup>+</sup>, 279.12458; found, 279.12665.

**Dibenzocyclooctyne (DIBO).** A stirred solution of compound **9** (0.200 g, 0.72 mmol, 1.0 equiv) in CH<sub>2</sub>Cl<sub>2</sub> (4 mL) was treated with DIC (0.109 g, 0.86 mmol, 1.2 equiv) and allowed to react overnight. The reaction mixture was concentrated under reduced pressure and purified by flash column chromatography on silica gel (hexanes) to provide DIBO (0.072 g, 0.373 mmol, 51%) as a white solid. **<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>,  $\delta$ ): 7.38–7.31 (m, 4H), 7.31–7.26 (m, 4aH), 3.38–3.29 (m, 2H), 2.50–2.40 (m, 2H). **<sup>13</sup>C NMR** (126 MHz, CDCl<sub>3</sub>,  $\delta$ ): 153.62, 129.41, 127.69, 126.52, 126.12, 123.95, 111.55, 36.47. **HRMS**  $m/z$  calcd for  $C_{16}H_{13}$  [M + H]<sup>+</sup>, 205.10172; found, 205.10245.

**2-Bromo-N-benzyl-N-methylacetamide (10).** To a stirred solution of *N*-methylbenzylamine (0.606 g, 5 mmol, 1.0 equiv) and triethylamine (0.7 mL, 5 mmol, 1.0 equiv) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added a solution of bromoacetyl bromide (1.06 g, 5.25 mmol, 1.05 equiv) in CH<sub>2</sub>Cl<sub>2</sub> (2 mL) dropwise at 0 °C. The resulting mixture was allowed to react for 4 h at room temperature. The reaction was quenched with saturated NaHCO<sub>3</sub> (10 mL) at 0 °C, extracted with diethyl ether

( $3 \times 25$  mL), washed with brine, dried over  $\text{Na}_2\text{SO}_4(s)$ , filtered and concentrated under reduced pressure. The resultant crude material was used in subsequent steps without further purification.

**2-Azido-N-benzyl-N-methylacetamide (11).** To a stirred solution of compound **10** (1.34 g, 5.53 mmol, 1 equiv) in DMF (25 mL), was added sodium azide (0.719 g, 11.06 mmol, 2.0 equiv) and the resulting mixture was allowed to react overnight at room temperature. A mixture of  $\text{H}_2\text{O}/\text{Et}_2\text{O}$  1:1 was added to the reaction mixture, and the aqueous phase was extracted with  $\text{Et}_2\text{O}$  ( $3 \times 25$  mL), the organic extract was then washed with water ( $8 \times 20$  mL) and brine, and dried over  $\text{Na}_2\text{SO}_4(s)$ . The resulting mixture was concentrated under reduced pressure, and the residue was purified by flash column chromatography on silica gel (hexanes/EtOAc 85:15→25:75) to provide compound **11** (0.351 g, 31%) as a colorless oil.  **$^1\text{H NMR}$**  (500 MHz,  $\text{CDCl}_3$ ,  $\delta$ ): 7.31 (ddd,  $J = 34.5, 19.6, 7.7$  Hz, 5H), 7.15 (d,  $J = 7.5$  Hz, 1H), 4.61 (s, 1H), 4.45 (s, 1H), 3.96 (d,  $J = 11.2$  Hz, 2H), 3.01 (s, 1H), 2.86 (s, 2H).  **$^{13}\text{C NMR}$**  (126 MHz,  $\text{CDCl}_3$ ,  $\delta$ ): 167.29, 136.47, 135.57, 129.15, 128.73, 128.24, 128.00, 127.70, 126.21, 52.76, 51.25, 50.64, 50.51, 34.38, 33.88. **HRMS**  $m/z$  calcd for  $\text{C}_{10}\text{H}_{13}\text{ON}_4$  [ $\text{M} + \text{H}]^+$ , 205.10894; found, 205.11737.

**2-Diazo-N-benzyl-N-methylacetamide (12).** Compound **11** (0.120 g, 0.586 mmol, 1.0 equiv) was dissolved in  $\text{H}_2\text{O}/\text{THF}$  1:9 (20 mL). To this solution was added 2,5-dioxopyrrolidin-1-yl 3-(diphenylphosphanyl)propanoate (0.219 g, 0.615 mmol, 1.05 equiv), and the reaction mixture was stirred for 4 h at room temperature before a saturated aqueous solution of  $\text{NaHCO}_3$  (15 mL) was added. The reaction mixture was then stirred vigorously for 3 h. The reaction mixture was diluted with brine and extracted with  $\text{CH}_2\text{Cl}_2$  (3×). The combined organic extracts were dried over  $\text{Na}_2\text{SO}_4(s)$ , filtered, and concentrated under reduced pressure, and the residue was purified with silica gel chromatography (hexanes/EtOAc 1:1) to provide compound **12** (33.3 mg, 29%).  **$^1\text{H NMR}$**  (400 MHz,  $\text{CDCl}_3$ ,  $\delta$ ): 7.40–7.21 (m, 5H), 5.01 (s, 1H), 4.65–4.38 (m, 2H), 2.89 (s, 3H).  **$^{13}\text{C NMR}$**  (101 MHz,  $\text{CDCl}_3$ ,  $\delta$ ): 166.14, 128.78, 127.55, 46.53, 34.33, 33.98, 25.64, 24.97. **HRMS**  $m/z$  calcd for  $\text{C}_{10}\text{H}_{12}\text{ON}_3$  [ $\text{M} + \text{H}]^+$ , 190.09804; found, 190.10475.

**Cycloaddition General Procedure A.** Azides or diazo compounds were dissolved in anhydrous  $\text{CH}_2\text{Cl}_2$  (0.5 mL) in a scintillation vial at room temperature with stirring. To this solution was added a solution of cyclooctyne in anhydrous  $\text{CH}_2\text{Cl}_2$  (0.5 mL), and the reaction mixture was stirred overnight. The reaction mixture was concentrated under reduced pressure, and the residue was purified by flash column chromatography on silica gel (hexanes/EtOAc 85:15→25:75) to provide the desired product.

**ABC–2-azido-N-benzylacetamide Cycloadduct (13).** Following Cycloaddition General Procedure A, a solution of azide **5** (7.989 mg, 0.042 mmol) dissolved in anhydrous  $\text{CH}_2\text{Cl}_2$  was treated with ABC (10 mg, 0.042 mmol) to provide compound **13**.  **$^1\text{H NMR}$**  (500 MHz,  $\text{CDCl}_3$ ,  $\delta$ ): 8.27 (dd,  $J = 4.7, 1.6$  Hz, 1H), 7.65 (dd,  $J = 7.9, 1.6$  Hz, 1H), 7.53 (d,  $J = 8.2$  Hz, 1H), 7.49 (t,  $J = 5.9$  Hz, 1H), 7.35–7.18 (m, 8H), 5.26 (s, 2H), 4.47 (d,  $J = 5.7$  Hz, 2H), 3.22 (dd,  $J = 8.3, 5.0$  Hz, 2H), 3.13 (dd,  $J = 8.3, 5.0$  Hz, 2H).  **$^{13}\text{C NMR}$**  (126 MHz,  $\text{CDCl}_3$ ,  $\delta$ ): 171.19, 165.82, 147.31, 144.97, 144.36, 140.34, 139.83, 137.80, 136.56, 134.59, 134.46, 132.25, 129.71, 128.70, 128.36, 127.68, 127.56, 126.93, 123.85, 52.64, 43.62, 34.24, 33.31. **HRMS**  $m/z$  calcd for  $\text{C}_{24}\text{H}_{21}\text{ON}_5\text{Cl}$  [ $\text{M} + \text{H}]^+$ , 430.14346; found, 430.16281.

**ABC–N-diazo-N-benzylacetamide Cycloadduct (14).** Following Cycloaddition General Procedure A, a solution of diazo compound **6** (7.358 mg, 0.042 mmol) dissolved in anhydrous  $\text{CH}_2\text{Cl}_2$  was treated with ABC (10 mg, 0.042 mmol) to provide compound **14**.  **$^1\text{H NMR}$**  (500 MHz,  $\text{CDCl}_3$ ,  $\delta$ ): 9.77 (s, 1H), 8.10 (dd,  $J = 4.8, 1.7$  Hz, 1H), 7.66 (dd,  $J = 7.8, 1.7$  Hz, 1H), 7.37–7.12 (m, 3H), 7.25 (m, 3H), 7.19 (d,  $J = 2.2$  Hz, 1H), 7.15 (m, 2H), 4.58 (d,  $J = 5.2$  Hz, 2H), 3.34–3.22 (m, 2H), 3.11 (d,  $J = 7.1$  Hz, 2H).  **$^{13}\text{C NMR}$**  (126 MHz,  $\text{CDCl}_3$ ,  $\delta$ ): 159.67, 146.36, 139.54,

138.91, 137.34, 136.84, 134.22, 134.01, 130.07, 128.67, 127.81, 127.51, 126.46, 123.01, 43.92, 35.45, 32.34. **HRMS**  $m/z$  calcd for  $C_{24}H_{20}ON_4Cl$  [M + H]<sup>+</sup>, 415.13256; found, 415.14917.

**ABC–2-azido-N-benzyl-N-methylacetamide Cycloadduct (15).** Following Cycloaddition General Procedure A, a solution of compound **11** (8.578 mg, 0.042 mmol) dissolved in anhydrous  $CH_2Cl_2$  was treated with 2-ABC (10 mg, 0.042 mmol) to provide compound **15** as regioisomers. **<sup>1</sup>H NMR** (500 MHz,  $CDCl_3$ ,  $\delta$ ): 8.45 (dd,  $J$  = 4.7, 1.7 Hz, 1H), 8.37 (dd,  $J$  = 4.8, 1.6 Hz, 0.5H), 7.66 (ddd,  $J$  = 7.5, 5.6, 1.7 Hz, 2H), 7.55 (d,  $J$  = 8.3 Hz, 1H), 7.53 (d,  $J$  = 8.3 Hz, 1H), 7.41–7.33 (m, 2H), 7.25 (m, 8H), 7.13–7.07 (m, 1H), 6.94–6.86 (m, 2H), 5.73 (s, 3H), 4.58 (s, 1H), 4.46 (s, 2H), 3.35–3.30 (m, 3H), 3.27 (dd,  $J$  = 8.1, 4.7 Hz, 3H), 2.96 (s, 3H), 2.87 (s, 2H). **<sup>13</sup>C NMR** (126 MHz,  $CDCl_3$ ,  $\delta$ ): 165.51, 165.25, 147.08, 146.98, 146.16, 146.03, 144.96, 144.93, 140.61, 139.37, 139.32, 136.84, 136.73, 136.29, 135.41, 134.77, 134.62, 134.16, 134.14, 132.42, 129.81, 129.78, 129.18, 128.88, 128.86, 128.62, 128.04, 127.75, 127.54, 126.68, 126.67, 126.29, 123.37, 123.31, 52.70, 51.28, 50.32, 50.11, 34.49, 34.02, 33.98, 33.88. **HRMS**  $m/z$  calcd for  $C_{25}H_{23}ON_5Cl$  [M + H]<sup>+</sup>, 444.15911; found, 444.18030.

**2-ABC–2-diazo-N-benzyl-N-methylacetamide Cycloadduct (16).** Following Cycloaddition General Procedure A, a solution of compound **12** (8.136 mg, 0.042 mmol) dissolved in anhydrous  $CH_2Cl_2$  was treated with 2-ABC (10 mg, 0.042 mmol) to provide compound **16** as regioisomers. **<sup>1</sup>H NMR** (500 MHz,  $CDCl_3$ ,  $\delta$ ): 8.43 (d,  $J$  = 4.7 Hz, 1H), 8.34–8.27 (m, 0.59H), 7.51 (d,  $J$  = 7.5 Hz, 2H), 7.35–7.20 (m, 12H), 7.16–7.03 (m, 3H), 4.69 (s, 3H), 3.25–3.19 (m, 2H), 3.19–3.09 (m, 5H), 2.89 (s, 2H), 2.87 (s, 3H). **<sup>13</sup>C NMR** (126 MHz,  $CDCl_3$ ,  $\delta$ ): 164.85, 164.36, 149.68, 149.43, 146.90, 143.64, 143.39, 141.45, 139.26, 136.76, 136.51, 134.97, 134.83, 133.86, 131.49, 129.94, 129.81, 128.56, 128.49, 128.23, 128.14, 127.59, 127.39, 127.32, 126.76, 122.05, 121.92, 121.07, 54.84, 50.94, 36.07, 34.66, 34.45, 33.61, 33.53, 32.82. **HRMS**  $m/z$  calcd for  $C_{25}H_{22}ON_4Cl$  [M + H]<sup>+</sup>, 429.14821; found, 429.16635.

**ABC–benzyl 2-diazoacetate Cycloadduct (17).** Following Cycloaddition General Procedure A, a solution of compound **7** (7.399 mg, 0.042 mmol) dissolved in anhydrous  $CH_2Cl_2$  was treated with ABC (10 mg, 0.042 mmol) to provide compound **17**. **<sup>1</sup>H NMR** (400 MHz,  $CDCl_3$ ,  $\delta$ ): 11.31 (s, 1H), 8.39 (dd,  $J$  = 4.7, 1.7 Hz, 1H), 7.57 (dd,  $J$  = 7.8, 1.7 Hz, 1H), 7.34–7.29 (m, 4H), 7.25–7.09 (m, 5H), 5.29 (s, 2H), 3.15 (s, 4H). **<sup>13</sup>C NMR** (101 MHz,  $CDCl_3$ ,  $\delta$ ): 147.28, 137.92, 131.99, 130.18, 128.44, 128.22, 128.08, 126.69, 122.64, 66.92, 34.55, 33.14. **HRMS**  $m/z$  calcd for  $C_{24}H_{19}O_2N_3Cl$  [M + H]<sup>+</sup>, 416.11658; found, 416.11660.

**DIBO–2-azido-N-benzylacetamide Cycloadduct (18).** Following Cycloaddition General Procedure A, a solution of azide **5** (7.989 mg, 0.042 mmol) dissolved in anhydrous  $CH_2Cl_2$  was treated with DIBO (10 mg, 0.042 mmol) to provide compound **18**. **<sup>1</sup>H NMR** (500 MHz,  $CDCl_3$ ,  $\delta$ ): 7.55–7.48 (m, 1H), 7.41–7.17 (m, 12H), 7.14 (dd,  $J$  = 7.4, 1.2 Hz, 1H), 5.16 (d,  $J$  = 16.4 Hz, 1H), 5.03 (d,  $J$  = 16.6 Hz, 1H), 4.53 (dd,  $J$  = 14.7, 6.0 Hz, 1H), 4.43 (dd,  $J$  = 14.9, 5.4 Hz, 1H), 3.35 (td,  $J$  = 12.1, 10.3, 4.5 Hz, 1H), 3.15–3.03 (m, 2H), 2.92–2.81 (m, 1H). **<sup>13</sup>C NMR** (126 MHz,  $CDCl_3$ ,  $\delta$ ): 165.43, 147.01, 141.71, 137.85, 137.27, 135.18, 131.70, 130.92, 130.46, 130.25, 129.23, 129.01, 128.81, 128.41, 127.77, 127.72, 126.80, 126.13, 125.14, 51.32, 43.75, 36.34, 33.02. **HRMS**  $m/z$  calcd for  $C_{25}H_{23}N_4O$  [M + H]<sup>+</sup>, 395.18718; found, 395.19003.

**DIBO–2-diazo-N-benzylacetamide Cycloadduct (19).** Following Cycloaddition General Procedure A, a solution of diazo compound **6** (7.350 mg, 0.042 mmol) dissolved in anhydrous  $CH_2Cl_2$  was treated with DIBO (10 mg, 0.042 mmol) to provide compound **19**. **<sup>1</sup>H NMR** (500 MHz,  $CDCl_3$ ,  $\delta$ ): 11.58 (s, 1H), 7.34–7.08 (m, 13H), 6.84 (s, 1H), 4.73–4.57 (m, 1H), 4.50–4.39 (m, 1H), 3.44–2.87 (m, 4H). **<sup>13</sup>C NMR** (126 MHz,  $CDCl_3$ ,  $\delta$ ): 171.21, 160.59, 140.56, 139.01, 137.82, 131.05, 130.95, 130.91, 130.88, 129.85, 128.68, 128.64, 128.30, 127.69, 127.45, 126.09,

126.07, 120.39, 43.26, 36.30, 33.18. **HRMS**  $m/z$  calcd for  $C_{25}H_{22}ON_3 [M + H]^+$ , 380.17629; found, 380.185730.

**DIBO–benzyl 2-diazoacetate Cycloadduct (20).** Following Cycloaddition General Procedure A, a solution of compound 7 (7.399 mg, 0.042 mmol) dissolved in anhydrous  $CH_2Cl_2$  was treated with DIBO (10 mg, 0.042 mmol) to provide compound 20.  **$^1H$  NMR**  $^1H$  NMR (400 MHz,  $CDCl_3$ ,  $\delta$ ): 9.22 (s, 1H), 7.56–6.91 (m, 13H), 5.48–5.11 (m, 2H), 3.47–2.78 (m, 4H).  **$^{13}C$  NMR** (101 MHz,  $CDCl_3$ ,  $\delta$ ): 139.88, 139.22, 134.98, 131.52, 131.23, 129.81, 129.16, 128.51, 128.36, 128.25, 126.25, 125.66, 67.06, 36.35, 32.84. **HRMS**  $m/z$  calcd for  $C_{25}H_{21}N_2O_2 [M + H]^+$ , 381.16030; found, 381.16040.

## Kinetic Analyses

**Kinetics General Method A.** Stock solutions at the specified concentrations in the specified solvents were prepared for each dipole and dipolarophile. Aliquots (0.5 mL) of dipole and dipolarophile were mixed, and reactions were monitored by HPLC with aliquots injected at the timepoints shown in the kinetic traces below. Each reaction was carried out in triplicate. The concentration of remaining dipolarophile was obtained from its corresponding peak in the chromatogram monitored at 280 nm. Second-order rate constants were calculated from the slope of the plot of  $[dipolarophile]^{-1}$  versus time.

**Kinetics General Method B.** Stock solutions of the specified dipoles and dipolarophiles were prepared in DMSO at 2.5 mM. An aliquot (10  $\mu$ L) of each stock was added to 1 mL of PBS (final concentration: 25  $\mu$ M), and reactions were monitored by HPLC with aliquots injected at the timepoints specified in Figure S3. Each reaction was carried out in triplicate. The concentration of remaining dipolarophile was obtained from its corresponding peak in the chromatogram monitored at 280 nm. Second-order rate constants were calculated from the slope of the plot of  $[dipolarophile]^{-1}$  versus time.

**Reaction of Compound 5 with DIBO in  $CH_2Cl_2$ .** Kinetics General Method A was followed using stock solutions at 2 mM and resulting in final reaction concentrations of 1 mM compound 5 and 1 mM DIBO.

**Reaction of Compound 6 with DIBO in  $CH_2Cl_2$ .** Kinetics General Method A was followed using stock solutions at 2 mM and resulting in final reaction concentrations of 1 mM compound 6 and 1 mM DIBO.

**Reaction of Compound 5 with ABC in  $CH_2Cl_2$ .** Kinetics General Method A was followed using stock solutions at 20  $\mu$ M and resulting in final reaction concentrations of 10  $\mu$ M compound 5 and 10  $\mu$ M ABC.

**Reaction of Compound 6 with ABC in  $CH_2Cl_2$ .** Kinetics General Method A was followed using stock solutions at 80  $\mu$ M and resulting in final reaction concentrations of 40  $\mu$ M compound 6 and 40  $\mu$ M ABC.

**Reaction of Compound 7 with ABC in  $CH_2Cl_2$ .** Kinetics General Method A was followed using stock solutions at 400  $\mu$ M and resulting in final reaction concentrations of 200  $\mu$ M compound 7 and 200  $\mu$ M ABC.

**Reaction of Compound 11 with ABC in  $CH_2Cl_2$ .** Kinetics General Method A was followed using stock solutions at 80  $\mu$ M and resulting in final reaction concentrations of 40  $\mu$ M compound 11 and 40  $\mu$ M ABC.

**Reaction of Compound 12 with ABC in CH<sub>2</sub>Cl<sub>2</sub>.** Kinetics General Method A was followed using stock solutions at 80 μM and resulting in final reaction concentrations of 40 μM compound **12** and 40 μM ABC.

**Reaction of Compound 5 with ABC in MeOH.** Kinetics General Method A was followed using stock solutions at 20 μM and resulting in final reaction concentrations of 10 μM compound **5** and 10 μM ABC.

**Reaction of Compound 6 with ABC in MeOH.** Kinetics General Method A was followed using stock solutions at 80 μM and resulting in final reaction concentrations of 40 μM compound **6** and 40 μM ABC.

**Reaction of Compound 11 with ABC in MeOH.** Kinetics General Method A was followed using stock solutions at 80 μM and resulting in final reaction concentrations of 40 μM compound **11** and 40 μM ABC.

**Reaction of Compound 12 with ABC in MeOH.** Kinetics General Method A was followed using stock solutions at 80 μM and resulting in final reaction concentrations of 40 μM compound **12** and 40 μM ABC.

**Reaction of Compound 5 with ABC in PBS Containing DMSO (2% v/v).** Kinetics General Method B was followed.

**Reaction of Compound 6 with ABC in PBS Containing DMSO (2% v/v).** Kinetics General Method B was followed.

**Reaction of Compound 11 with ABC in PBS Containing DMSO (2% v/v).** Kinetics General Method B was followed.

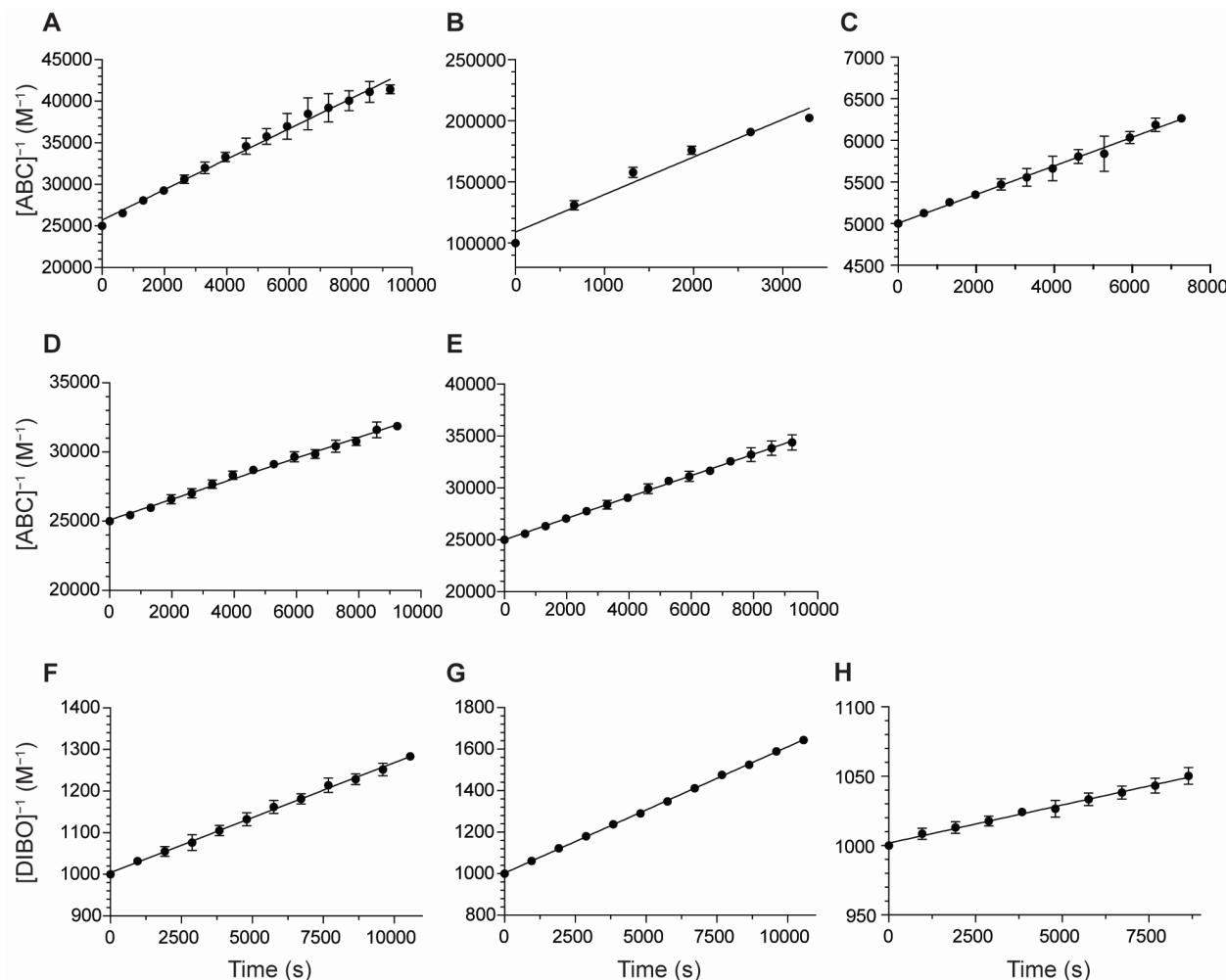
**Reaction of Compound 12 with ABC in PBS Containing DMSO (2% v/v).** Kinetics General Method B was followed.

## Cyclooctyne Stability Experiments

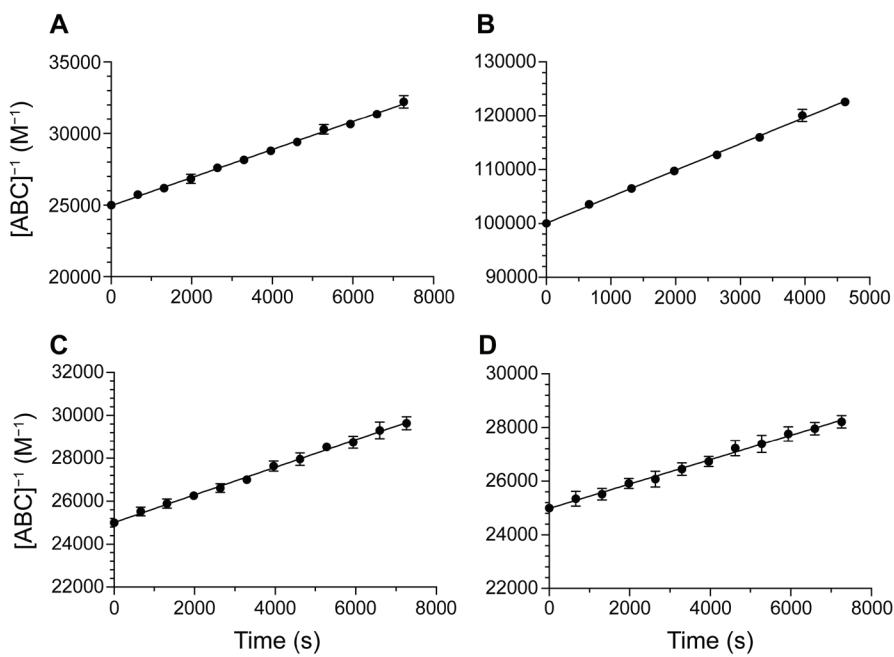
A solution of ABC (25 μM) or DIBAC (25 μM) was prepared in phosphate-buffered saline containing reduced glutathione (1.0 mM), oxidized glutathione (0.2 mM), and DMSO (2% v/v). The solutions were incubated at 37 °C, and HPLC analyses were performed at hourly intervals to determine the remaining concentration of dipolarophile. Subsequently, plots of ln(concentration) versus time were prepared to calculate half-lives of each cyclooctyne. (Note: The DMSO cosolvent was used to solubilize the alkynes, but does oxidize reduced glutathione. Hence, the ratio of the half-lives is more meaningful than the individual values.)

## Kinetic Traces

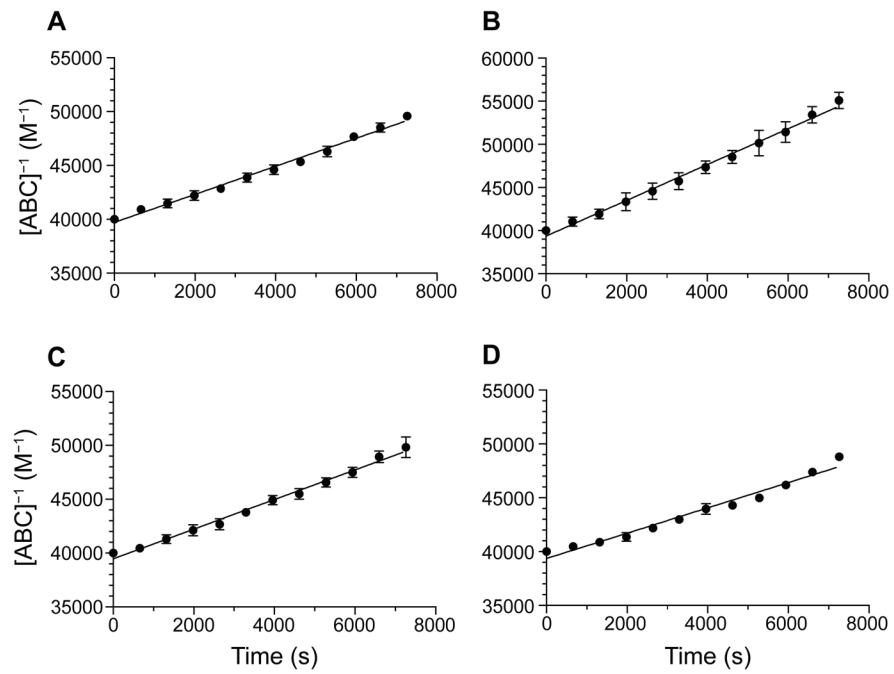
Kinetic traces for the reactions of ABC with (A) azide **5**, (B) diazo compound **6**, (C) diazo compound **7**, (D) azide **11** ( $k = 1.0 \text{ M}^{-1}\text{s}^{-1}$ ), and (E) diazo compound **12** ( $k = 0.75 \text{ M}^{-1}\text{s}^{-1}$ ); and the reactions of DIBO with (F) azide **5**, (G) diazo compound **6**, and (H) diazo compound **7**. All reactions were carried out in  $\text{CH}_2\text{Cl}_2$  at 26 °C and were monitored by HPLC. Values are the mean  $\pm$  SD for triplicate experiments.



Kinetic traces for the reactions of ABC with (A) azide **5**, (B) diazo compound **6**, (C) azide **11**, and (D) diazo compound **12**. All reactions were carried out in MeOH at 26 °C and were monitored by HPLC. Values are the mean ± SD for triplicate experiments.



Kinetic traces for the reactions of ABC with (A) azide **5**, (B) diazo compound **6**, (C) azide **11**, and (D) diazo compound **12**. All reactions were carried out in PBS containing DMSO (2% v/v) at 26 °C and were monitored by HPLC. Values are the mean ± SD for triplicate experiments.

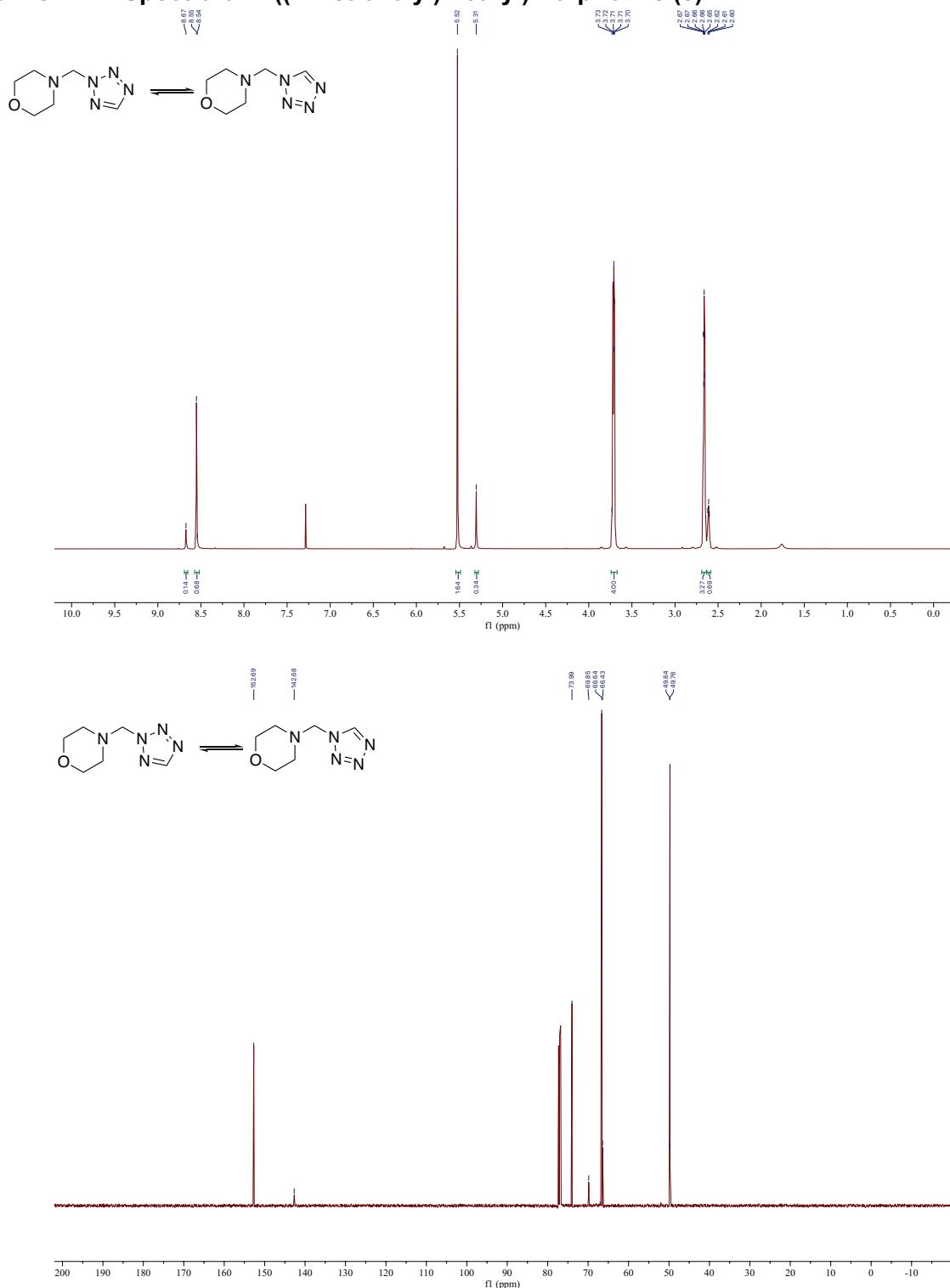


## References

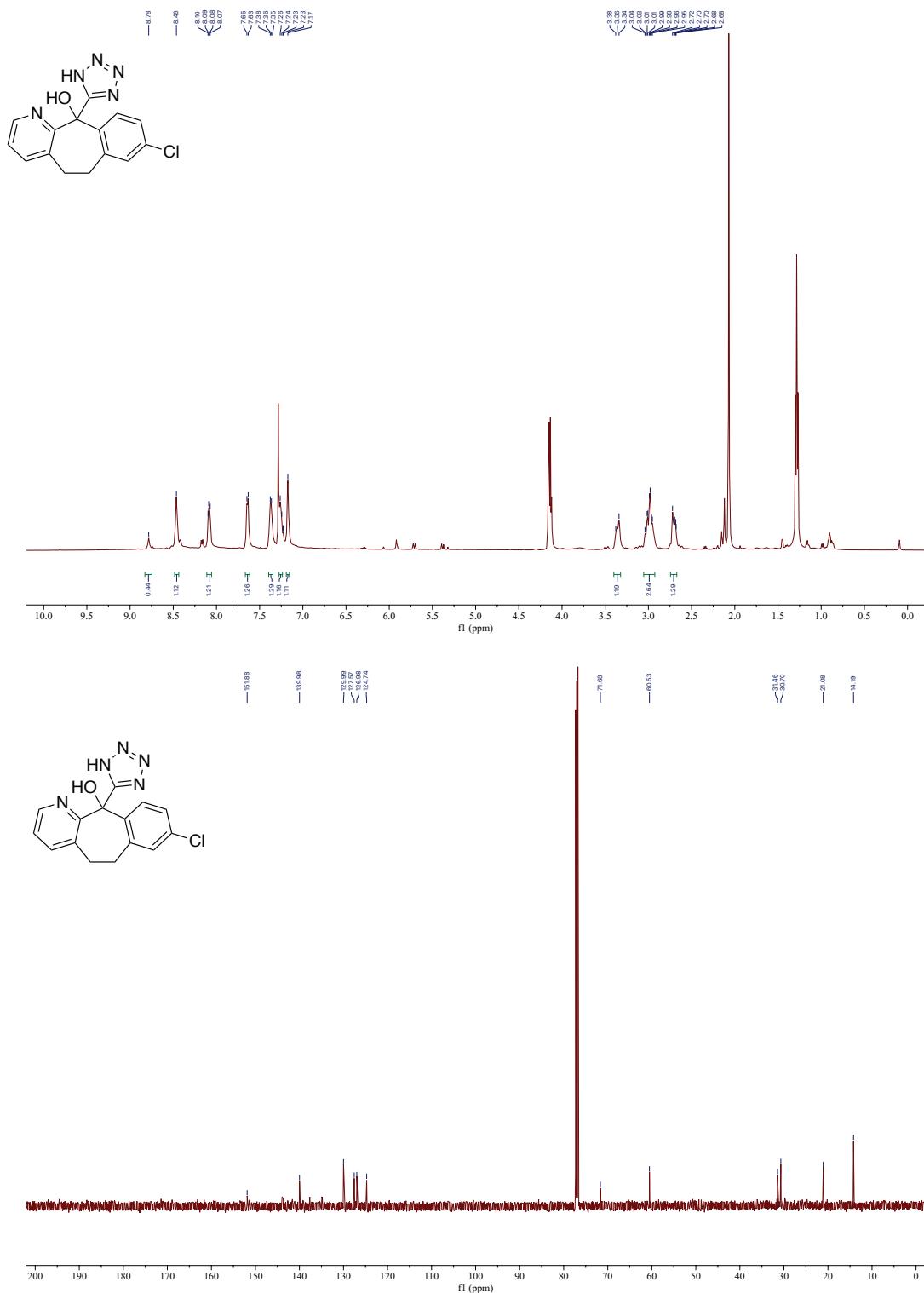
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**NMR Spectra** (All compounds were dissolved in CDCl<sub>3</sub> unless indicated otherwise.)

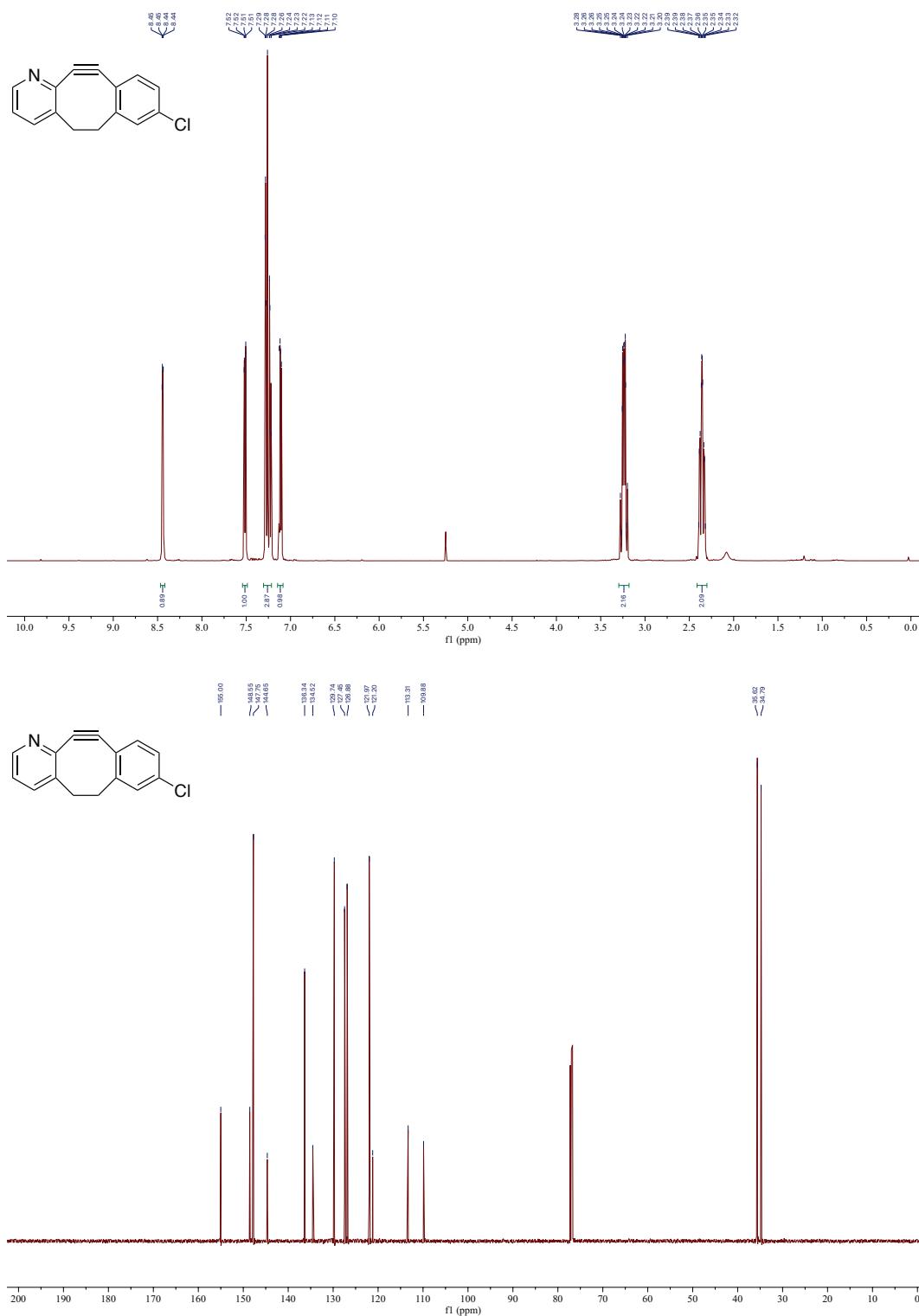
**<sup>1</sup>H and <sup>13</sup>C NMR Spectra of 4-((N-Tetrazolyl)methyl)morpholine (8)**

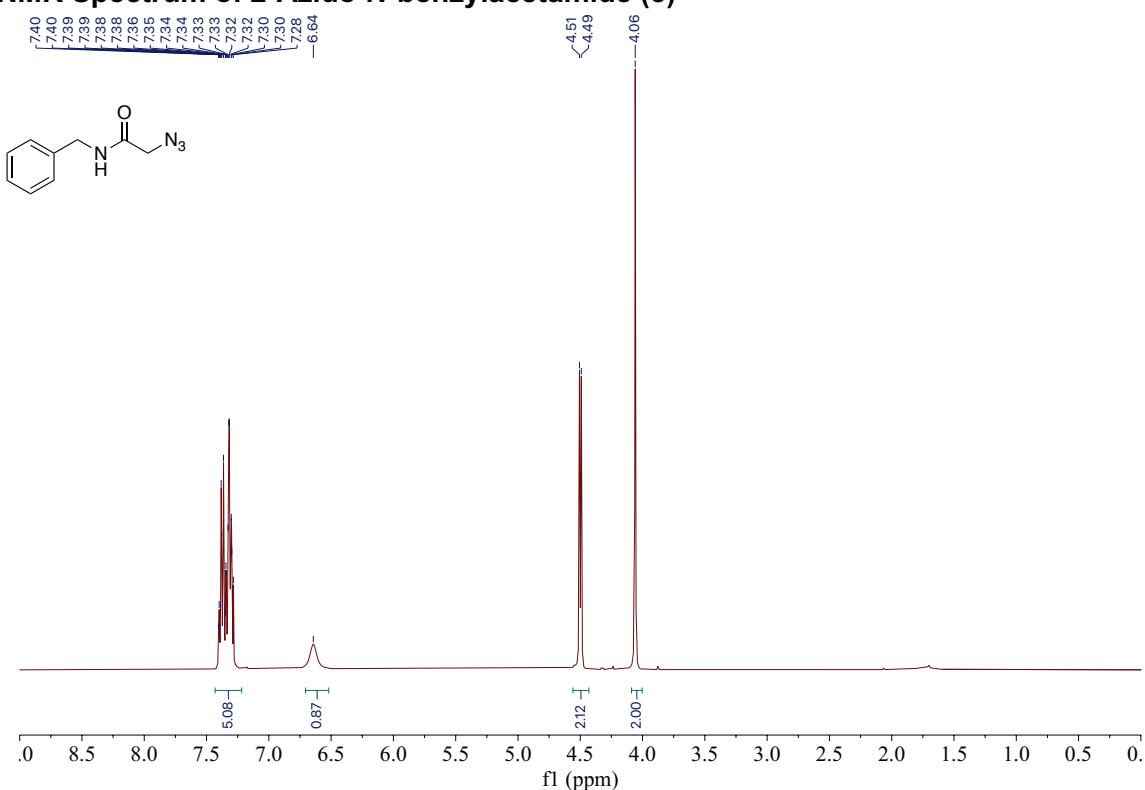
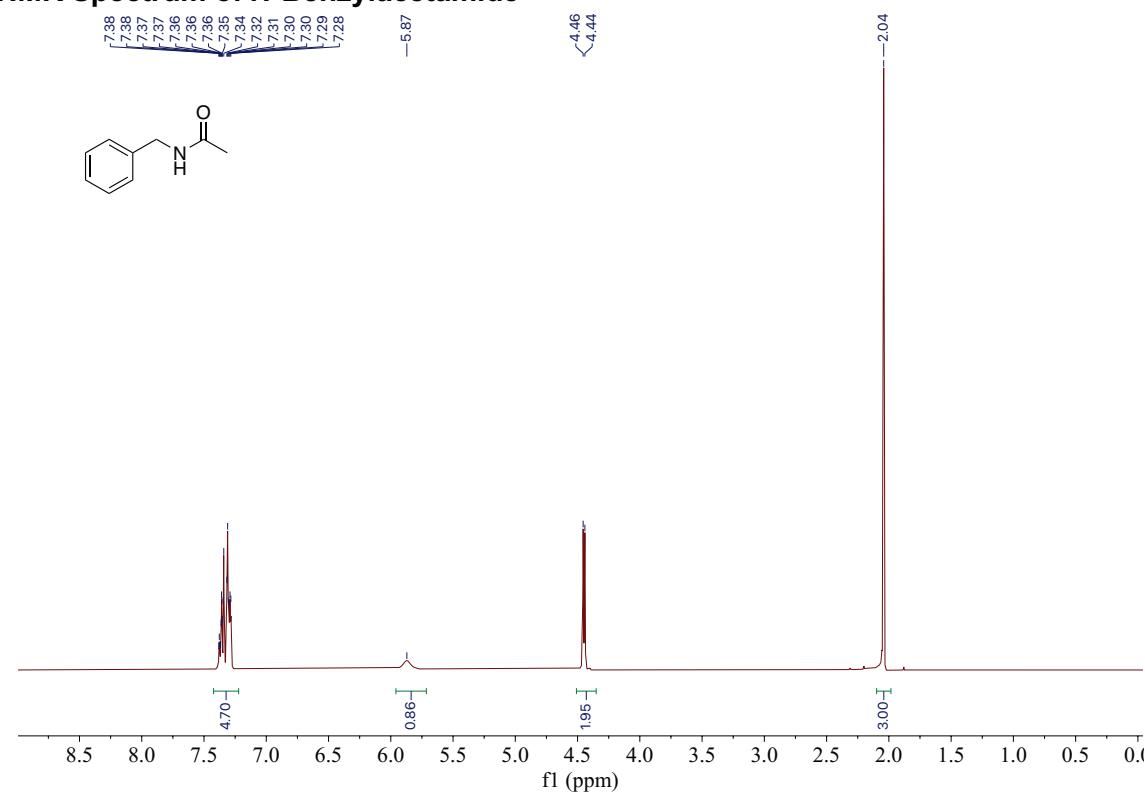


**<sup>1</sup>H and <sup>13</sup>C NMR Spectra of 8-Chloro-11-(1*H*-tetrazol-5-yl)-6,11-dihydro-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-ol (4)**

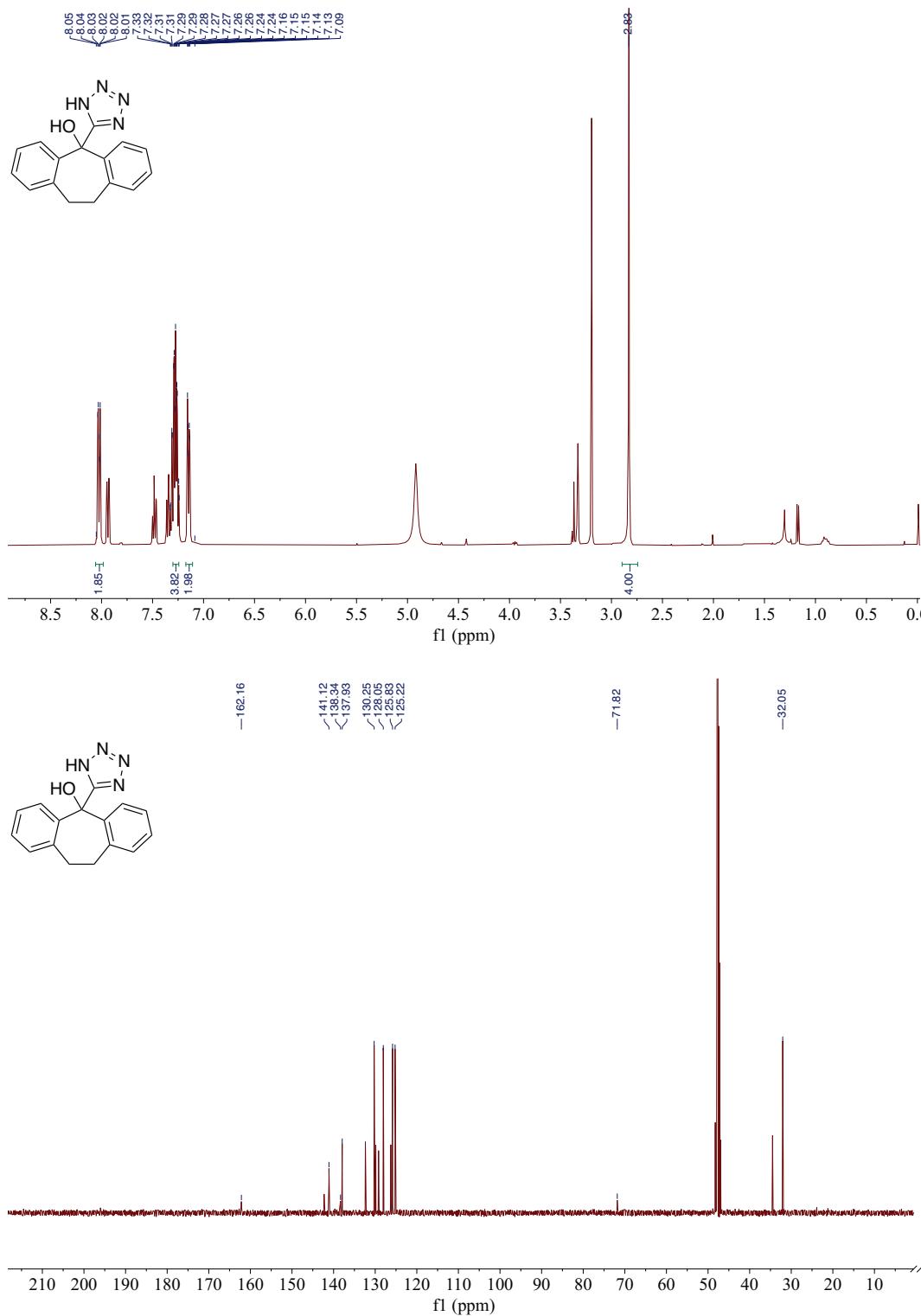


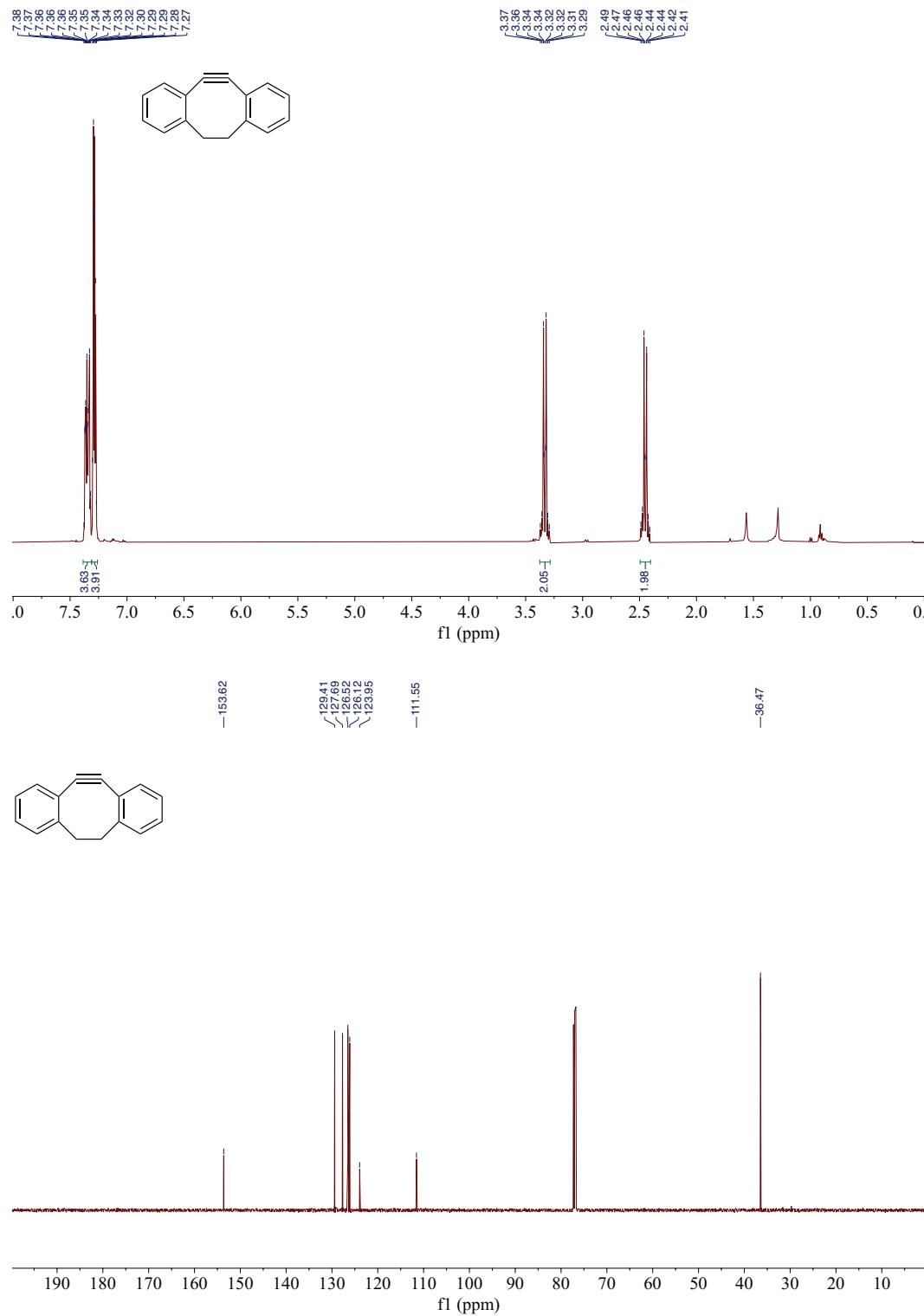
**<sup>1</sup>H and <sup>13</sup>C NMR Spectra of 2-Azabenzo-8-chlorobenzocyclooctyne (ABC)**



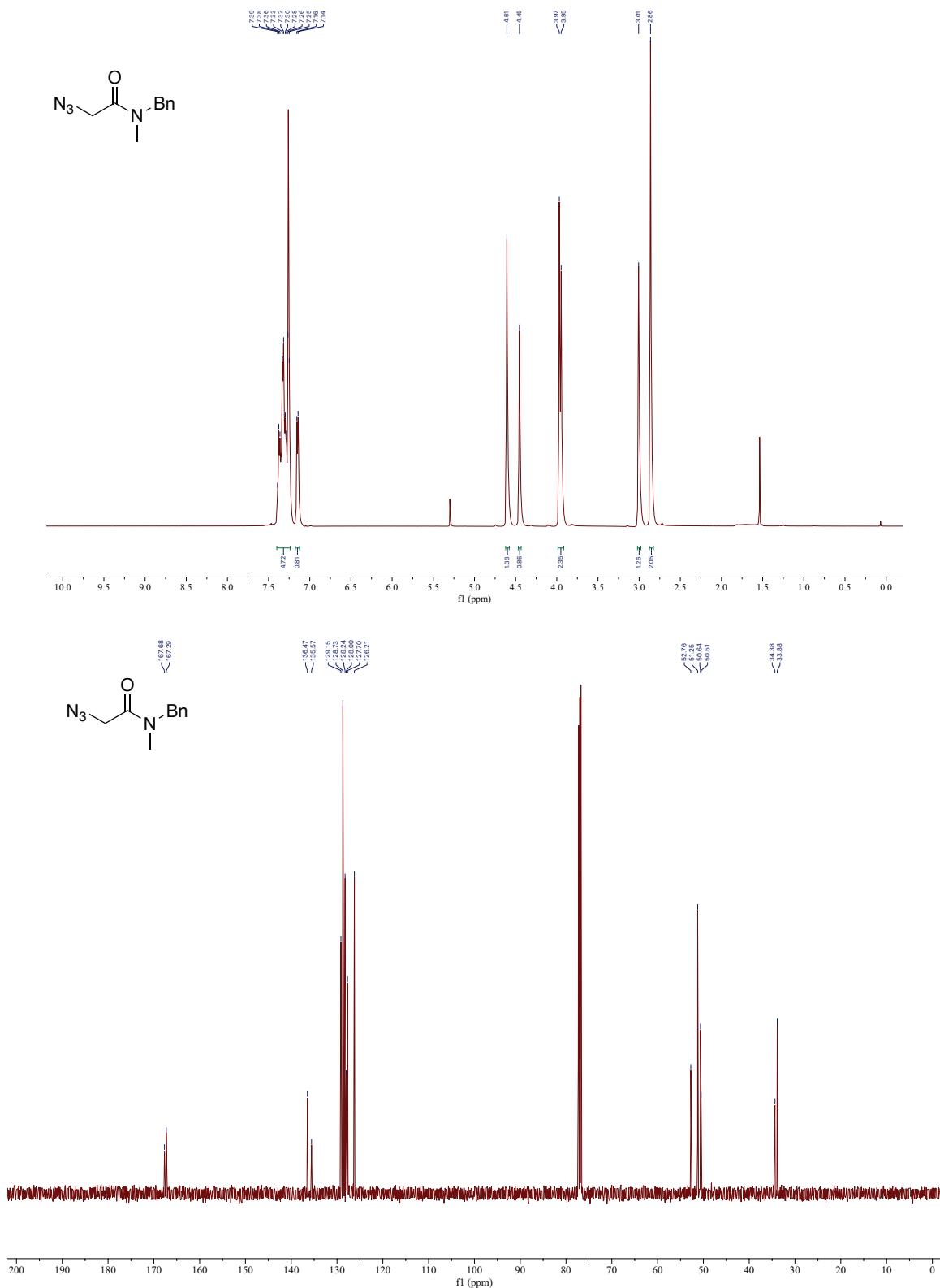
**<sup>1</sup>H NMR Spectrum of 2-Azido-N-benzylacetamide (5)****<sup>1</sup>H NMR Spectrum of *N*-Benzylacetamide**

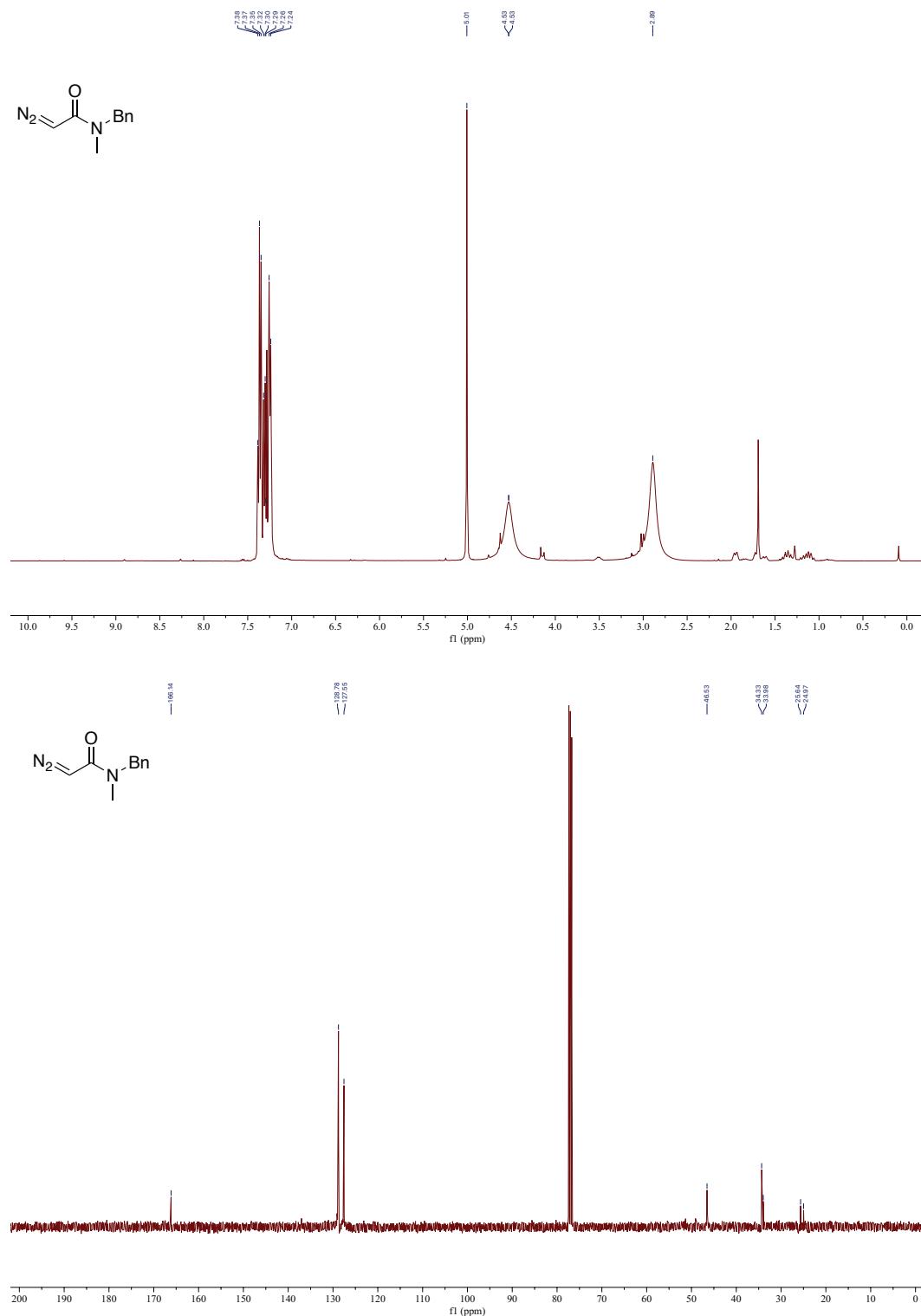
**<sup>1</sup>H and <sup>13</sup>C NMR Spectra of 5-(1H-Tetrazol-5-yl)-10,11-dihydro-5H-dibenzo[a,d][7]annulen-5-ol (9).** Both spectra were obtained in CD<sub>3</sub>OD.



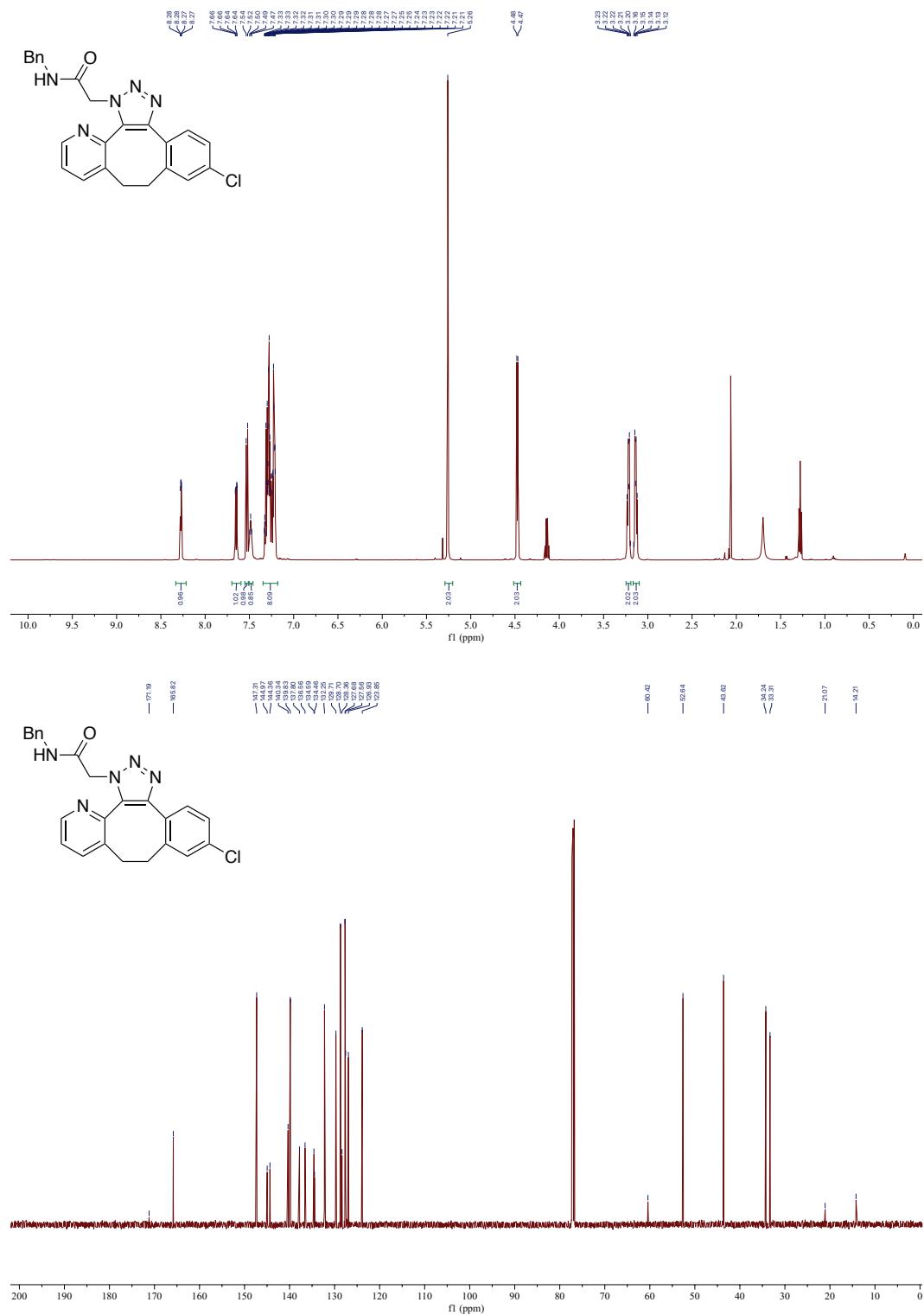
**<sup>1</sup>H and <sup>13</sup>C NMR Spectra of Dibenzocyclooctyne (DIBO)**

**<sup>1</sup>H and <sup>13</sup>C NMR Spectra of 2-Azido-N-methyl-N-(phenylmethyl)acetamide (11)**

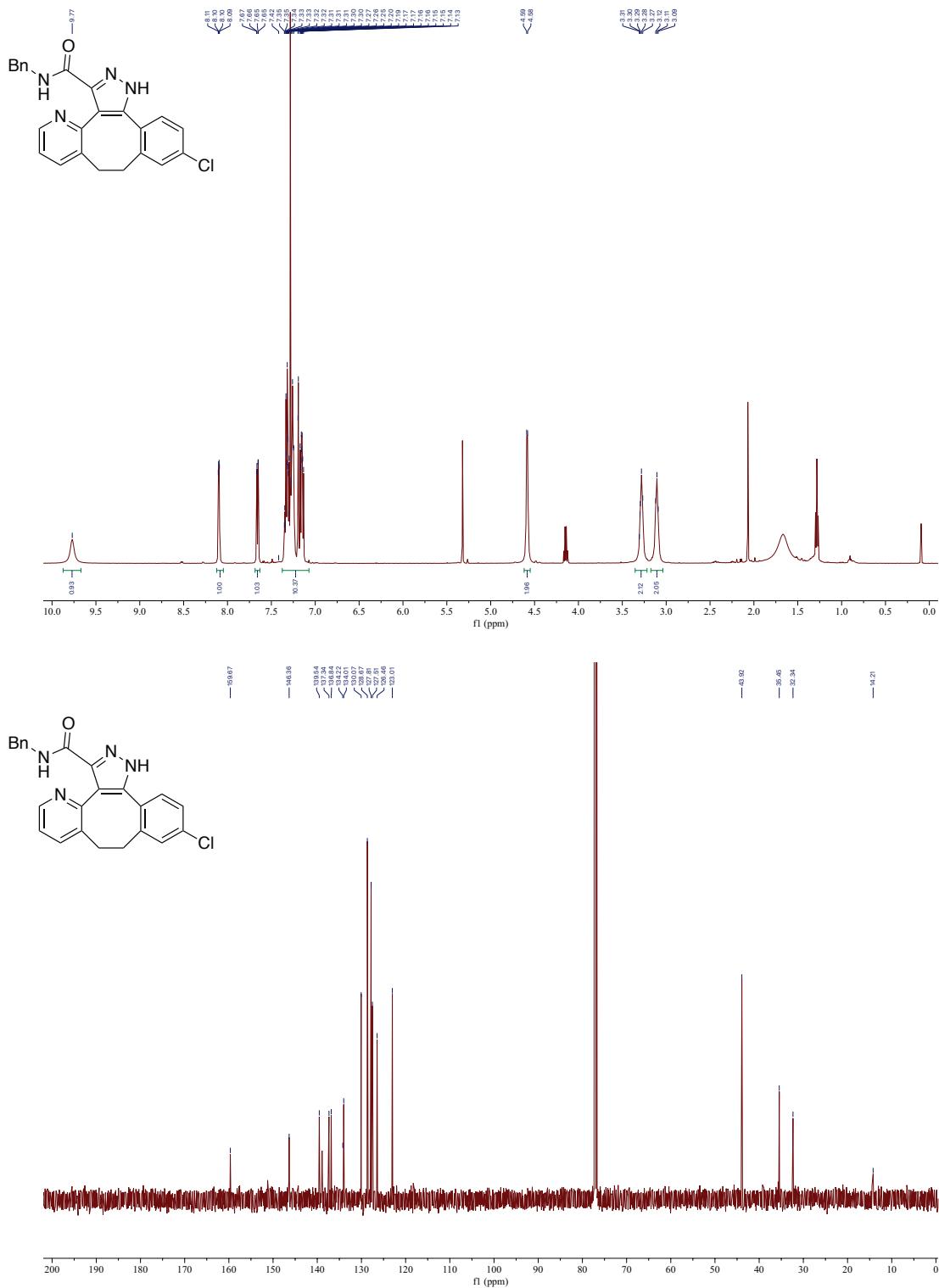


**<sup>1</sup>H and <sup>13</sup>C NMR Spectra of 2-Diazo-N-methyl-N-(phenylmethyl)acetamide (12)**

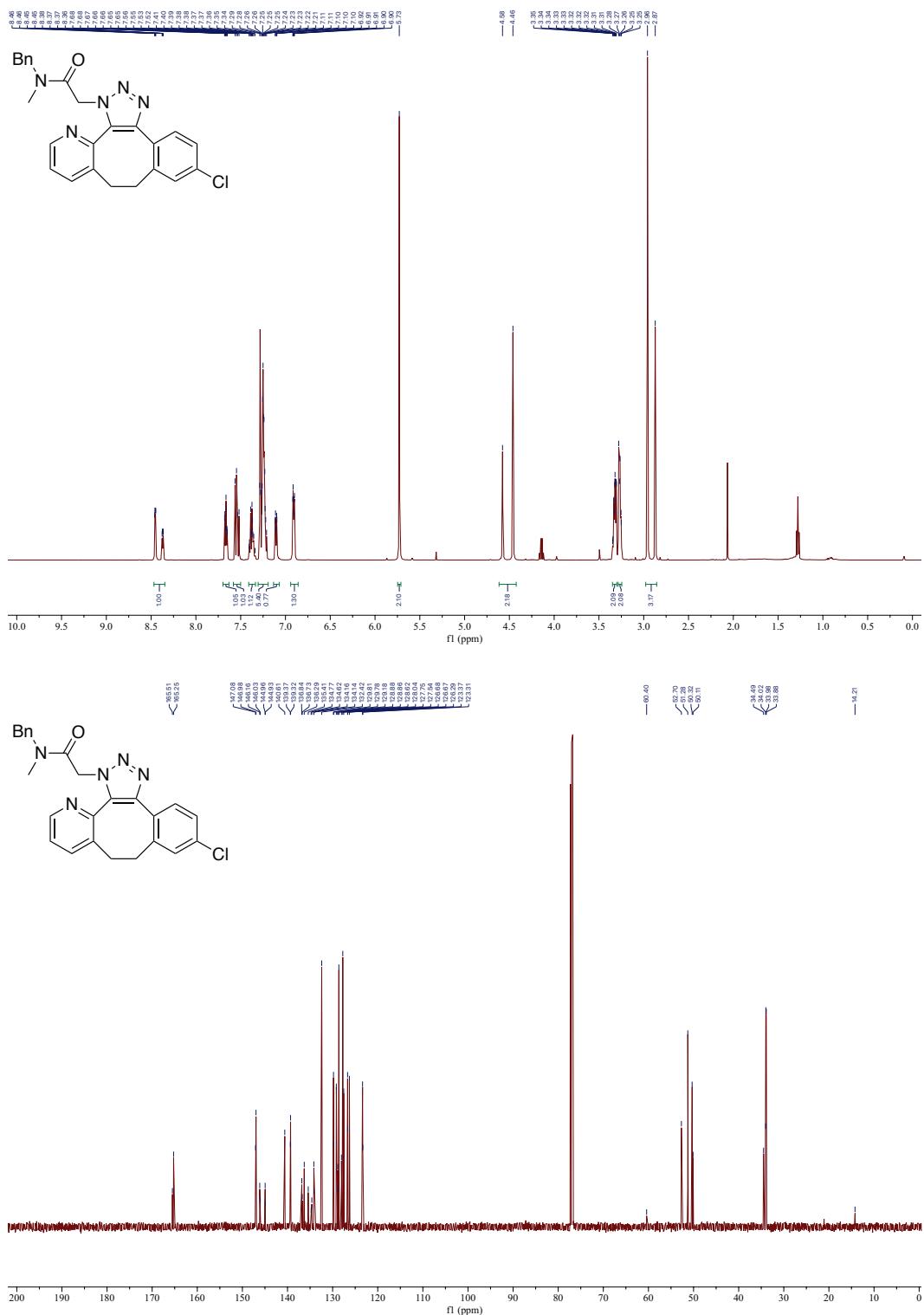
## <sup>1</sup>H and <sup>13</sup>C NMR Spectra of ABC-2-diazo-N-benzylacetamide Cycloadduct (13)



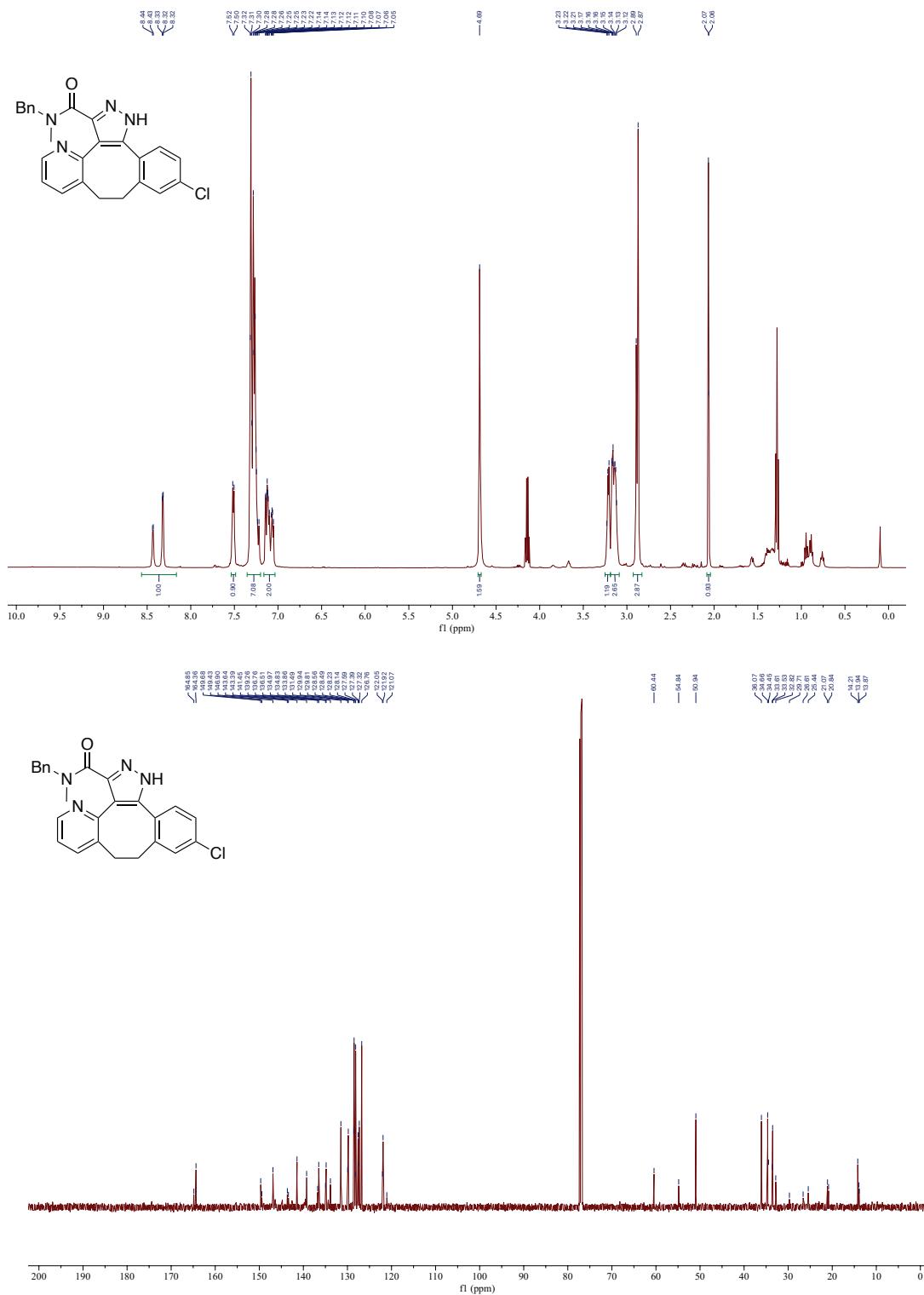
## <sup>1</sup>H and <sup>13</sup>C NMR Spectra of ABC-2-diazo-N-benzylacetamide Cycloadduct (14)



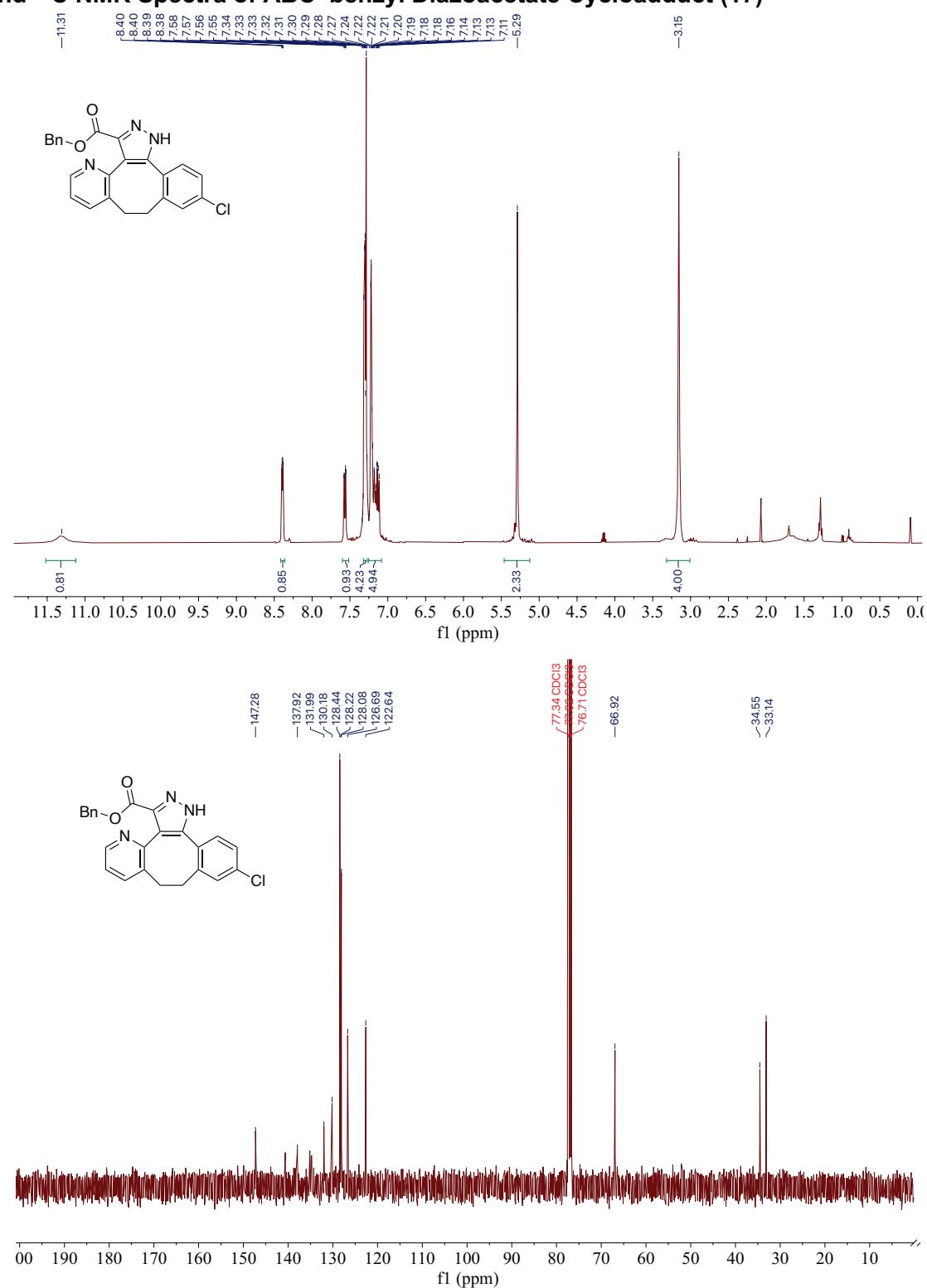
## **<sup>1</sup>H and <sup>13</sup>C NMR Spectra of ABC–2-azido-N-methyl-N-(phenylmethyl)acetamide Cycloadduct (15)**



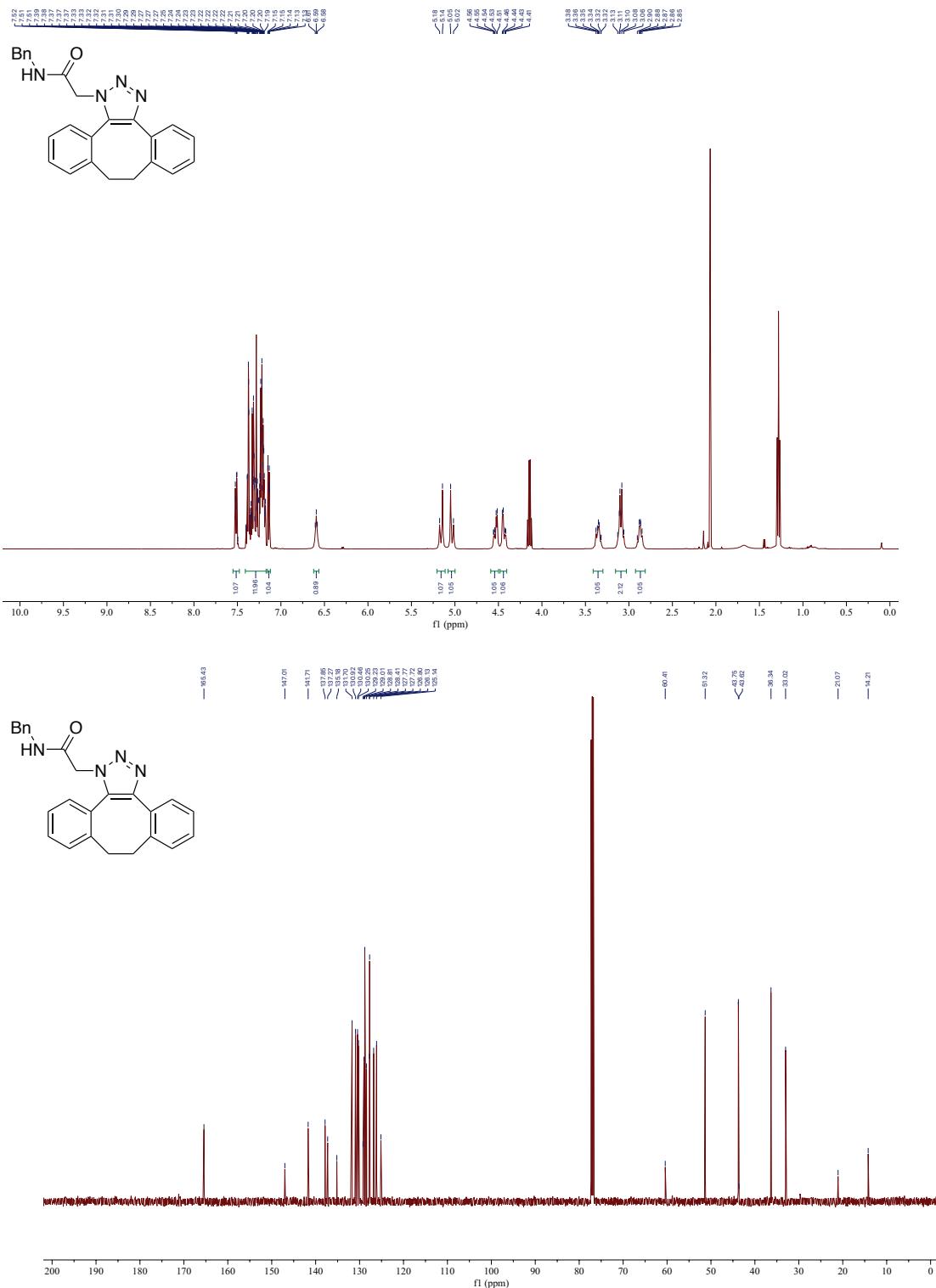
## **<sup>1</sup>H and <sup>13</sup>C NMR Spectra of ABC–2-diazo-N-methyl-N-(phenylmethyl)acetamide Cycloadduct (16)**



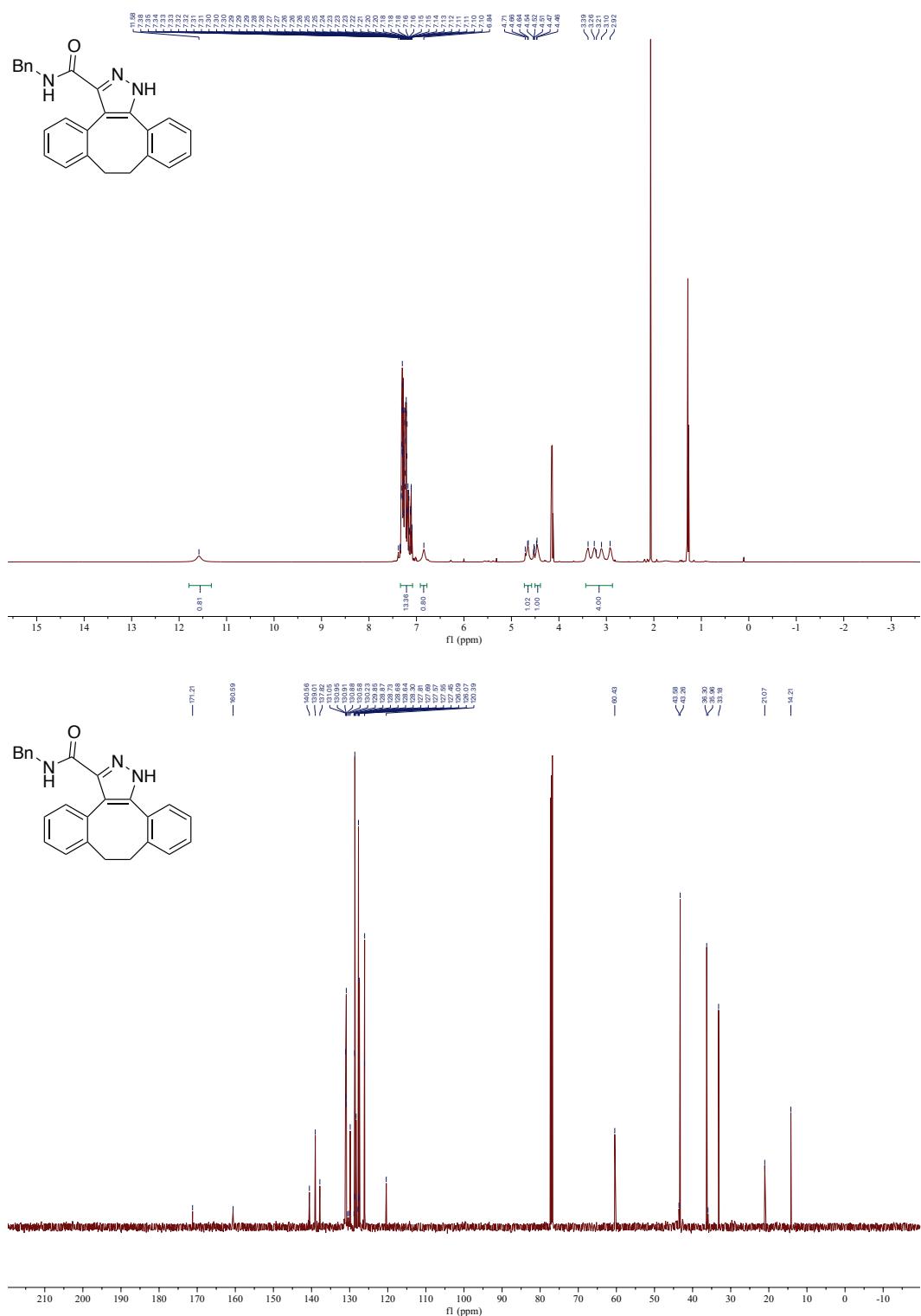
**<sup>1</sup>H and <sup>13</sup>C NMR Spectra of ABC–benzyl Diazoacetate Cycloadduct (17)**



### <sup>1</sup>H and <sup>13</sup>C NMR Spectra of DIBO–2-azido-N-benzylacetamide Cycloadduct (18)



## <sup>1</sup>H and <sup>13</sup>C NMR Spectra of DIBO–2-diazo-N-benzylacetamide Cycloadduct (19)



**<sup>1</sup>H and <sup>13</sup>C NMR Spectra of DIBO–benzyl Diazoacetate Cycloadduct (20)**