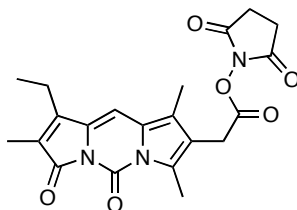


## Diazaindacene NHS Ester (FD 13001)

### Product Information



C<sub>21</sub>H<sub>21</sub>N<sub>3</sub>O<sub>6</sub>  
MW 411.41

3a,4a-Diaza-4,5-dioxo-7-ethyl-3a,4,4a,5-tetrahydro-1,3,6-trimethyl-s-indacen-2-yl-acetic acid, N-hydroxysuccinimide ester

Lightner and co-workers<sup>1-3</sup> reported the synthesis and spectroscopic properties of fluorophores based on the 3a,4a-diazaindacene ring system ("xanthglows"). Key properties include a high quantum yield (>0.9) and a large Stokes shift (>100 nm). Berry & Associates offers an amine-reactive version, Diazaindacene NHS Ester, for incorporation of the diazaindacene fluorophore into biomolecules.

**Spectroscopic properties:** The absorption maximum of the corresponding carboxylic acid is reported to be 425 nm in methanol, extinction coefficient = 15,300. The emission maximum of the carboxylic acid is 535 nm in methanol with a quantum yield of 0.91.<sup>1</sup>

When installed onto a 5'-amino-modified oligonucleotide,<sup>4</sup> the absorption and emission maxima appear at 430 nm and 530 nm, respectively, in aqueous MgCl<sub>2</sub>/KCl/Tris-HCl (pH 8.0). For comparison, an analogous TET-labeled oligonucleotide showed absorption and emission maxima at 522 nm and 538 nm, respectively. A comparison of equimolar amounts of these two oligonucleotides indicated that the diazaindacene-labeled material exhibited a fluorescence intensity of 6% of the TET-labeled material, despite the known high quantum yield of fluorescence for the diazaindacene.

### Coupling with an amino-modified oligonucleotide:

Please see the attached protocol.

### References:

- (1) Brower, J. O.; Lightner, D. A. Synthesis and spectroscopic properties of a new class of strongly fluorescent dipyrinones. *J. Org. Chem.* **2002**, *67*, 2713-2716.
- (2) Boiadjiev, S. E.; Lightner, D. A. Intensely fluorescent dipyrinones. *J. Phys. Org. Chem.* **2004**, *17*, 675-679.
- (3) Woydziak, Z. R.; Boiadjiev, S. E.; Norona, W. S.; McDohagh, A. F.; Lightner, D. A. Synthesis and hepatic transport of strongly fluorescent cholephilic dipyrinones. *J. Org. Chem.* **2005**, *70*, 8417-8423.
- (4) We thank Professor Salvatore A. E. Marras for these data.

# Conjugation of Diazaindacene NHS Ester to a amino-modified oligodeoxyribonucleotides

Courtesy of Professor Salvatore A. E. Marras

## Reagents and Equipment:

- Primary amino-modified oligodeoxyribonucleotide
- Diazaindacene NHS Ester (Berry & Associates, FD 13001)
- 0.1 M Sodium bicarbonate, pH 8.5
- *N,N*-Dimethylformamide (DMF)
- HPLC Buffer A: 0.1 M Triethylammonium acetate, pH 6.5, filtered and degassed
- HPLC Buffer B: 0.1 M Triethylammonium acetate in 75% acetonitrile, pH 6.5, filtered and degassed
- 3M Sodium acetate, pH 5.2
- 100 % Ethanol
- 70 % Ethanol
- TE buffer: 1 mM EDTA, 10 mM Tris-HCl, pH 8.0
  
- Microcentrifuge
- Sephadex G-25 column
- 0.2  $\mu$ m Centrex MF-0.4 filter
- High-pressure liquid chromatograph (HPLC) with a C-18 reverse-phase column

## Procedure:

The starting material for the synthesis of a diazaindacene-labeled oligodeoxyribonucleotide is an oligodeoxyribonucleotide that contains a primary amino group at its 5' terminal end or its 3' terminal end. Alternatively, diazaindacene can be incorporated at various positions in oligodeoxyribonucleotides that contain amino modified deoxyadenosines, deoxycytidines, deoxyguanosines, or thymidines. The trifluoroacetyl protecting group on the primary amine is removed during the standard post-synthesis ammonium hydroxide deprotection step.

In order to conjugate diazaindacene to the oligodeoxyribonucleotide, dissolve 50 - 250 nanomoles of dry oligodeoxyribonucleotide in 500  $\mu$ L of 0.1 M sodium bicarbonate, pH 8.5. Dissolve about 1 - 5 mg of Diazaindacene NHS Ester in 10  $\mu$ L of *N,N*-dimethylformamide and add this solution to the oligodeoxyribonucleotide solution. Incubate the dye - oligodeoxyribonucleotide solution overnight, protected from light, on a slowly rocking platform at room temperature.

After the overnight incubation, remove particulate material by spinning the solution in a microcentrifuge for one minute at 10,000 rpm. In order to remove the unreacted Diazaindacene NHS Ester, pass the supernatant through a gel-exclusion column. Thus,

equilibrate a Sephadex G-25 column with three column volumes of HPLC Buffer A, load the supernatant and elute with 1 mL HPLC Buffer A. Filter the eluate through a 0.2  $\mu$ m Centrex MF-0.4 filter.

Purify the oligonucleotide by high-pressure liquid chromatography (HPLC) on a C-18 reverse-phase column, using a linear elution gradient of 20 to 70 % HPLC Buffer B in HPLC Buffer A and run for 25 minutes at a flow rate of 1 mL / min. Monitor the absorption of the elution stream at 260 nm, which is the absorption maximum for oligodeoxyribonucleotides, and 430 nm, which is the absorption maximum for diazaindacene. Collect the HPLC fractions that show both absorption at 260 nm and 430 nm.

Precipitate the labeled oligodeoxyribonucleotide by adding 1 / 10 volume 3 M sodium acetate, pH 5.2, and 1.5 volumes 100 % ethanol to each collected HPLC fraction and incubate the solutions for 2 hours at -20 °C. After the incubation step, spin the solutions in a microcentrifuge for 15 minute at 15,000 rpm. Discard the supernatant and wash the oligodeoxyribonucleotide pellets with 70 % ethanol. Dry the pellets by air for 15 minutes.

The diazaindacene-labeled oligodeoxyribonucleotide is dissolved in TE buffer and stored, protected from light, at -20 °C.