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**Bergmann et al.**

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(54) **COMPOUND FOR SEQUENCING BY SYNTHESIS**

(71) Applicant: **Roche Diagnostics Operations, Inc.**, Indianapolis, IN (US)

(72) Inventors: **Frank Bergmann**, Iffeldorf (DE); **Dieter Heindl**, Paehl (DE); **Jessica Steger-Domandl**, Munich (DE)

(73) Assignee: **ROCHE MOLECULAR SYSTEMS INC.**, Pleasanton, CA (US)

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(51) **Int. Cl.**

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| ***C12P 19/34*** | (2006.01) |
| ***C07H 19/10*** | (2006.01) |
| ***C07H 19/20*** | (2006.01) |
| *C07H 19/00* | (2006.01) |

(52) **U.S. Cl.**

CPC ..... ***C12Q 1/6869*** (2013.01); ***C07H 19/10*** (2013.01); ***C07H 19/20*** (2013.01)

(58) **Field of Classification Search**

CPC ..... C12Q 1/6869; C12Q 2525/186
USPC ..... 536/4.1, 23.1; 435/6.1, 91.1
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

| | | | | |
|---|---|---|---|---|
| 5,990,300 | A | 11/1999 | Hiatt et al. | |
| 7,057,026 | B2 * | 6/2006 | Barnes et al. | 536/23.1 |
| 2007/0117104 | A1 | 5/2007 | Buzby | |
| 2008/0138804 | A1 | 6/2008 | Buzby | |

FOREIGN PATENT DOCUMENTS

| | | | |
|---|---|---|---|
| DE | 2160104 | A1 | 12/1971 |
| WO | 2006/073451 | A3 | 7/2006 |

OTHER PUBLICATIONS

Search Report issued Jan. 9, 2014, in European Application No. 13193588.4, 10 pages.

Canard, Bruno and Sarfati, Robert S. "DNA polymerase fluorescent substrates with reversible 3'-tags," Gene, 1994, pp. 1-6, vol. 148.

Duschinsky, R. and Eppenberger, Ursula, "Nitric Acid Esters of Pyrimidine Nucleosides," Tetrahedron Letters, 1967, pp. 5103-1508, No. 50.

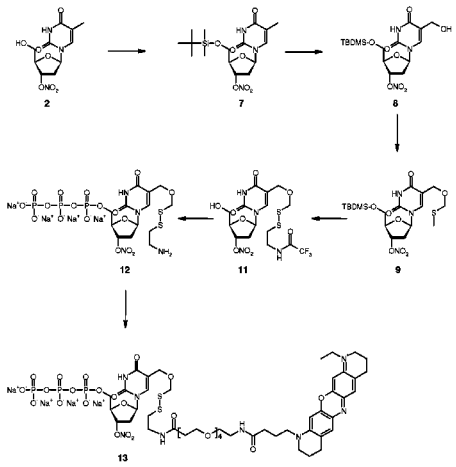
Naimi, Ebrahim et al., "Synthesis of 3'- and 5'-Nitrooxy Pyrimidine Nucleoside Nitrate Esters: 'Nitric Oxide Donor' Agents for Evaluation of Anticancer and Antiviral Agents," Journal of Medicinal Chemistry, 2003, pp. 995-1004, vol. 46.

(Continued)

*Primary Examiner* — Jezia Riley
(74) *Attorney, Agent, or Firm* — Olga Kay

(57) **ABSTRACT**

The present invention provides deoxynucleoside tri- or tetraphosphate comprising a 3' nitrate and a detectable label covalently bound to the oxygen atom of an oxymethyl or oxyallyl or oxypropargyl substitution of a nucleobase. Such compounds provide new possibilities for future Sequencing by Synthesis technologies.

**3 Claims, 3 Drawing Sheets**

Synthesis of MR121-labelled 3'-*O*-nitro-2'-deoxy-*β*-D-uridine 5'-triphosphate (13)