(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
3 April 2008 (03.04.2008)

PCT

(10) International Publication Number
WO 2008/039367 A1

(51) International Patent Classification:
C07C 271/24 (2006.01) *C12Q 1/68* (2006.01)
C07C 303/40 (2006.01) *G01N 33/68* (2006.01)
C07K 14/00 (2006.01)

(21) International Application Number:
PCT/US2007/020466

(22) International Filing Date:
21 September 2007 (21.09.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/846,354 22 September 2006 (22.09.2006) US
60/896,667 23 March 2007 (23.03.2007) US

(71) Applicant *(for all designated States except US)*: **THE GOVERNMENT OF THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES** [US/US]; Office of Technology Transfer, National Institutes of Health, 6011 Executive Boulevard, Suite 325, Rockville, MD 20852-3804 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **APPELLA, Daniel, H.** [US/US]; 13718 Willow Tree Drive, Rockville, MD 20850 (US). **XU, Qun** [CN/US]; 709 Monroe Street, Apartment 202, Rockville, MD 20850 (US). **ZHANG, Ning** [CN/US]; 906 Hooper Avenue, Apartment D, Baltimore, MD 21229 (US).

(74) Agents: **CALDWELL, John, W.** et al.; Woodcock Washburn LLP, 2929 Arch Street, Suite 1200, Philadelphia, PA 19104 (US).

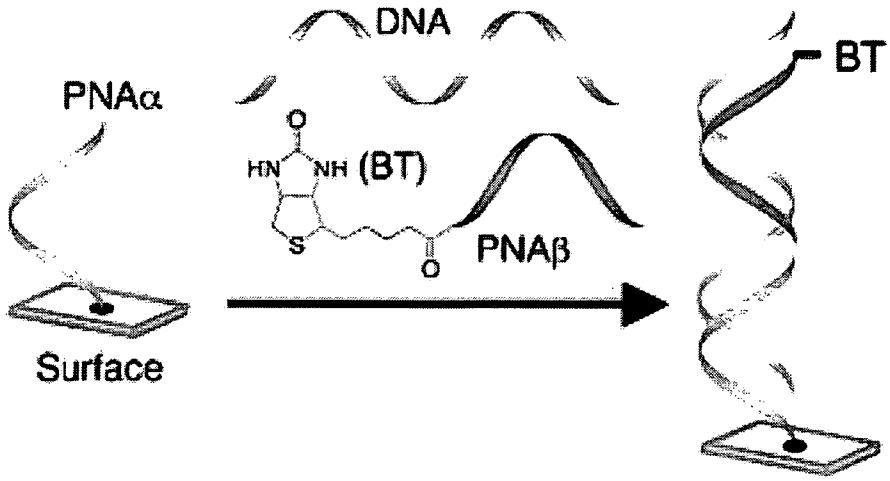
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

[Continued on next page]

(54) Title: SYNTHESIS OF *TRANS-TERT*-BUTYL-2-AMINOCYCLOPENTYLCARBAMATE



(57) **Abstract:** The present invention concerns methods of synthesis of *trans-tert*-butyl-2- aminocylcopentylcarbamate comprising contacting 6-tosyl-6-azabicyclo[3.1.0]hexane with $TMSN_3$ and TBAF to produce 2-azido-N-tosylcyclopentananiine; reducing the 2-azido-N-tosylcyclopentanamine to produce 2-amino-N-tosylcyclopentanamine; contacting the 2-amino-N-tosylcyclopentanamine with di-*tert*/-butyl dicarbonate to produce *tert*-butyl- 2-(tosylamino)cyclopentylcarbamate; and detosylation of *tert*-butyl (tosylamino) cyclopentylcarbamate to produce *trans-tert*-butyl-2-aminocyclopentylcarbamate. The invention also concerns PNAs comprising residues of the monomers of the invention in the backbone and uses of such PNAs. The PNAs of the invention can be used to detect DNAs of infectious agents or to suppress expression of protein associated with cancer.

WO 2008/039367 A1

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

— *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

SYNTHESIS OF *TRANS-TERT*-BUTYL-2-AMINOCYCLOPENTYLCARBAMATE

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims benefit of U.S. Provisional Application Nos. 60/846,354, filed September 22, 2006 and 60/896,667, filed March 23, 2007, each of which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0002] The instant invention concerns methods for the synthesis of entantiomerically pure *trans-tert*-butyl-2-aminocylcopentylcarbamate and its use as a precursor to 1,2-*trans*-cylcopentane diamine.

BACKGROUND OF THE INVENTION

[0003] Optically active 1,2-diamines are important components of many biologically active natural products and medicinal agents. *See* Lucet, *et al.*, Angew. *Chem., Int. Ed.* **1998**, 37, 2580-2627 and Kotti, *et al.*, *Chem. Bio. Drug Des.* **2006**, 67, 101-114. Bidentate C_2-symmetric ligands based on 1,2-diamine functionality have also found widespread applications in asymmetric catalysis. Yoon and Jacobsen, *Science* **2003**, 299, 1691-1693. For example, chiral salen ligands derived from *trans*-1,2-diaminocyclohexane effect remarkable enantioselectivity for a broad range of transformations. Larrow and Jacobson, *Organomet. Chem.* **2004**, 6, 123-152. In contrast, the use of *trans*-1,2-diaminocyclopentane as a chiral scaffold has received less attention

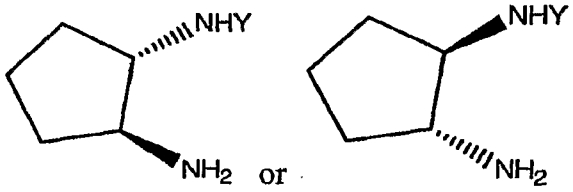
due to the limited availability of both enantiomers. *See*, Luna, *et al.*, *Org. Lett.* **2002**, 4, 3627-3629; Toftlund and Pedersen, *Acta Chem. Scand.* **1972**, 26, 4019-4030; Ongeri, *et al.*, *Synth. Commun.* **2000**, 30, 2593-2597; Gouin, *et al.*, *Tetrahedron* **2002**, 58, 1131-1136; and Daly and Gilheany, *Tetrahedron: Asymmetry* **2003**, 14, 127-137.

[0004] Recently, it was reported that incorporating *trans*-1,2-diaminocyclopentane into aminoethylglycine peptide nucleic acids (aegPNAs) significantly increases binding affinity and sequence specificity to complementary DNA. See, Pokorski, *et al*, *J. Am. Chem. Soc.* **2004**, 126, 15067-15073 and Myers, *et al*, *Org. Lett.* **2003**, 5, 2695-2698. Despite the promise of PNAs with 1,2-diaminocyclopentane residues in the backbone, a commercially viable synthetic method that can provide multigram quantities of both enantiomers of 1,2-diaminocyclopentane or a suitable precursor is not available.

[0005] Technologies for genomic detection most commonly use DNA probes to hybridize to target sequences. To achieve required sensitivity, the use of PCR to amplify target sequences has remained standard practice in many labs. Direct detection methods that eliminate the requirement for a PCR step could afford faster and simpler devices that can be used outside of a laboratory. Devices based on nanotechnology have yielded impressive results, yet the use of PCR is still predominant in most applications. There is a need in the art for probes with improved selectivity and lower detection limits to avoid the need for PCR.

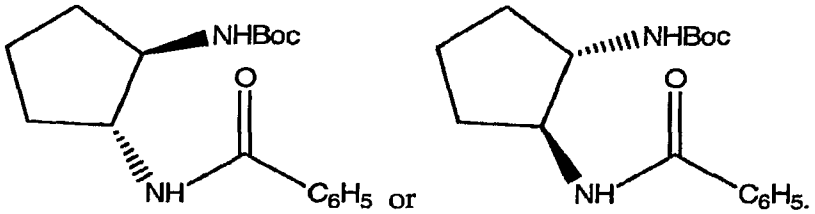
SUMMARY OF THE INVENTION

[0006] In some embodiments, the invention concerns compounds of the formula



having at least 99% enantiomeric purity. In these compounds, Y is a protecting group. In some preferred embodiments, Y is t-butyl carbamate (Boc).

[0007] Additionally, the invention concerns a compound of the formula:



[0008] The invention also concerns a method of synthesis of 2-azido-N-tosylcyclopentanamine comprising the step of contacting 6-tosyl-6-azabicyclo[3.1.0]hexane with trimethylsilyl azide ($TMSN_3$) and tetra-n-butylammonium fluoride (TBAF). In some embodiments, the step is performed at 25-50 °C. In certain embodiments, the molar ratio of $TMSN_3$ to TBAF is 20:1 to 1:1. Some embodiments additionally comprise sodium azide.

[0009] In yet other embodiments, the invention concerns a method of synthesis of *trans-tert*-butyl-2-diaminocyclopentylcarbamate comprising contacting 6-tosyl-6-azabicyclo[3.1.0]hexane with $TMSN_3$ and TBAF to produce 2-azido-N-tosylcyclopentanamine. In some embodiments, the method further comprising converting 2-azido-N-tosylcyclopentanamine to *tert*-butyl-2-(tosylamino)cyclopentylcarbamate. In certain embodiments, the converting of 2-azido-N-tosylcyclopentanamine to *tert*-butyl-2-(tosylamino)cyclopentylcarbamate comprises:

reducing the 2-azido-N-tosylcyclopentanamine to produce 2-amino-N-tosylcyclopentanamine; and

contacting the 2-amino-N-tosylcyclopentanamine with di-*tert*-butyl dicarbonate to produce *tert*-butyl-2-(tosylamino)cyclopentylcarbamate.

[0010] In still other embodiments, the method further comprises detosylation of *tert*-butyl-(tosylamino)cyclopentylcarbamate to produce *trans-tert*-butyl-2-aminocyclopentylcarbamate.

The invention also concerns a method comprising:

reducing the 2-azido-N-tosylcyclopentanamine to produce 2-amino-N-tosylcyclopentanamine;

contacting the 2-amino-N-tosylcyclopentanamine with di-*tert*-butyl dicarbonate to produce *tert*-butyl-2-(tosylamino)cyclopentylcarbamate; and

detosylation of *tert*-butyl-(tosylamino)cyclopentylcarbamate to produce *trans-tert*-butyl-2-aminocyclopentylcarbamate.

[0011] In some embodiments, the reduction of 2-azido-N-tosylcyclopentanamine is obtained by Pd-catalyzed hydrogenation or Staudinger reduction.

[0012] The detosylation of *tert*-butyl-(tosylamino) cyclopentylcarbamate can be accomplished by contacting *tert*-butyl-(tosylamino)cyclopentylcarbamate with lithium and naphthalene in an aprotic solvent in some methods of the invention. In some embodiments, the aprotic solvent is tetrahydrofuran (THF) or 1,2-dimethoxyethane (DME). In certain embodiments, the detosylation of *tert*-butyl-(tosylamino) cyclopentylcarbamate is performed at a temperature between -20 °C and 10 °C.

[0013] The invention further comprises a method which contacts *trans-tert*-butyl-2-aminocyclopentylcarbamate with a resolving agent to produce a precipitate comprising S,S-*tert*-butyl-2-aminocyclopentylcarbamate. One preferred resolving agent is 10-camphorsulfonic acid. Some preferred embodiments further comprise isolating the S,S-*tert*-butyl-2-aminocyclopentylcarbamate. The R,R-enantiomer can be likewise be resolved and isolated using appropriate resolving agents or by recovering the unresolved enantiomers from the solution from which the S,S-enantiomer was precipitated.

[0014] Yet another embodiment of the invention concerns a method for producing a peptide nucleic acid containing at least one (S,S)-*trans*-cyclopentane or (R,R)-*trans*-cyclopentane unit in the PNA backbone comprising:

contacting 6-tosyl-6-azabicyclo[3.1.0]hexane with trimethylsilyl azide ($TMSN_3$) and tetra-n-butylammonium fluoride (TBAF) to produce 2-azido-N-tosylcyclopentanamine;

converting the 2-azido-N-tosylcyclopentanamine to *trans-tert*-butyl-2-aminocylcopentylcarbamate; and

linking a heterocyclic base moiety to the *trans-tert*-butyl-2-aminocylcopentylcarbamate, and

incorporating the resulting compound into the PNA backbone. In some embodiments, a plurality of (S,S)-*trans*-cyclopentane and/or (R,R)-*trans*-cyclopentane units are incorporated in the PNA backbone.

[0015] The PNAs of the instant invention can be used in a method for detecting a nucleic acid comprising contacting a sample suspected of containing the nucleic acid with a peptide nucleic acid prepared in accordance with the invention. These PNAs can be used in a kit for detecting a nucleic acid comprising at least one peptide nucleic acid prepared in accordance with the invention. The kits can be adapted for detecting an infectious agent such as anthrax, avian flu, severe acute respiratory syndrome (SARS),

tuberculosis (TB), human papilloma virus (HPV), or human immunodeficiency virus (HIV).

[0016] It should also be noted that in some preferred embodiments, this method is applicable to kits that do not require using a machine to read to test results but rather, allow an observer to visually (by eye) determine if the infectious agent is present via the appearance of color.

[0017] In some embodiments, the detection is performed by a method comprising:

contacting a solution having a first cyclopentane-containing PNA with a substrate having a second cyclopentane-containing PNA affixed thereto, the first cyclopentane-containing PNA having a reporter molecule attached thereto and the first and second cyclopentane PNAs being complementary to different portions of a target DNA;

contacting a sample DNA with the first and second cyclopentane-containing PNAs;

visually observing the substrate to detect the presence of color from the reporter molecule. This PNA arrangement allows formation of a "sandwich hybridization" complex and detection of DNA without use of DNA amplification approaches. In certain embodiments, the solution is removed prior to observing the substrate.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] **Figure 1** shows a PNA-based sandwich-hybridization assay. PNAα is the capture probe, and PNAβ is the detection probe.

[0019] **Figure 2** illustrates signal amplification from PNA-based sandwich-hybridization using PNAα(2) and PNAβ(2) with 103 fmol DNA and HRP-avidin. Four wells of a 96-well plate are shown, and each column represents identical conditions. Blue color results from initial oxidation of 1-Step TurboTMB, and yellow color is produced once the enzymatic reaction is stopped.

[0020] **Figure 3** shows colorimetric detection of protective antigen DNA (PA) from *Bacillus anthracis* Ames 35 strain (+PA) and Ames 33 strain (-PA). The signal is obtained from PNA-based sandwich-hybridization using PNAα(2) and (2) with poly-HRP-avidin.

DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0021] *Trans*-1,2-diaminocyclopentane can potentially impact a broad range of scientific disciplines. Because the synthesis of the instant invention is easy to perform, and is scalable to large quantities, we envision that *trans*-1,2-diaminocyclopentane produced using the method of the invention will become an important building block in many areas of chemistry. For instance, this method allows each nitrogen atom of cyclopentanediamine to be easily derivatized with identical or dissimilar groups. Such flexibility should allow the development of new chiral ligands for metals that can be applied in asymmetric catalysis, an area in which catalysts are developed to generate molecules that are highly enriched in one enantiomer. Such catalysts are important in the area of pharmaceutical research where selective production of single-enantiomer compounds is important. In addition, *trans*-1,2-diaminocyclopentane could be used as a component of a chiral stationary phase that is important for chromatographic separations of enantiomers by HPLC. Furthermore, *trans*-1,2-diaminocyclopentane could be incorporated into polymeric materials that are being explored for their selective molecules recognition properties. In general, everywhere that *trans*-1,2-diaminocyclohexane has been explored as a chiral unit, *trans*-1,2-diaminocyclopentane could be explored as well, and could convey better properties for a particular application.

[0022] The development of chiral catalysts for asymmetric reactions is one of the most active areas of research both in academia and industry. In the pharmaceutical industry, asymmetric reactions that generate single enantiomer compounds can be highly important to both discovery of new drugs and to the large-scale synthesis of chiral drugs. Interactions of molecules with biological systems can depend on the molecule's chirality, with each enantiomer of a molecule displaying a different biological activity. In some cases, one enantiomer of a molecule may elicit a beneficial therapeutic effect, while the opposite enantiomer could be deadly. The most notorious example of this effect was seen with the drug thalidomide, where one enantiomer prevents morning sickness in pregnant women, while the other enantiomer causes birth defects in newborn children. Current FDA regulations require that pharmaceutical companies determine the biological activities of each enantiomer of a chiral molecule, and in cases where one enantiomer clearly shows the desirable therapeutic effect, the final drug should be marketed as a single enantiomer. Therefore, synthetic access to chiral molecules in one enantiomer form is crucial in the development of new drugs.

[0023] In the course of developing new chiral catalysts that can promote the formation of one enantiomer over another in a chemical reaction, certain chemical units of catalysts have been found to be successful across numerous types of catalysts and substrates. (R,R) and (S,S) trans-1,2-diaminocyclohexane, for example, have emerged as an essential unit for several of the most powerful chiral catalysts used today. Salen ligands, made from a single enantiomer of trans-1,2-diaminocyclohexane, show remarkable abilities as catalysts when complexed to metals. The success of these chiral catalysts would not be possible without the commercial availability of each enantiomer. In contrast, there is no commercial sources of the enantiomers of trans-1,2-diaminocyclopentane, nor is there an attractive synthetic route to make either enantiomer. Application of the synthesis of instant invention will allow the exploitation of trans-1,2-diaminocyclopentane enantiomers.

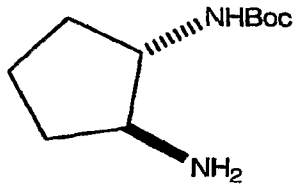
[0024] Incorporation of trans-1,2-diaminocyclopentane into a class of molecules called Peptide Nucleic Acids (PNAs) has a beneficial effect on the recognition of DNA and RNA sequences. As shown herein, this compound can be used in the development of nucleic acid detection kits for various pathogens. Additionally, the synthesis allows each nitrogen atom of cyclopentanediamine to be easily derivatized with identical or dissimilar groups. Such flexibility allows the development of new chiral ligands for metals that can be applied in asymmetric catalysis, an area in which catalysts are developed to generate molecules that are highly enriched in one enantiomer. Such catalysts, for example, are important in the area of pharmaceutical medicaments where selective production of single-enantiomer compounds is important.

[0025] In addition, *trans*-1,2-diaminocyclopentane can be used as a component of a chiral stationary phase that is important for chromatographic separations of enantiomers by HPLC. *Trans*-1,2-diaminocyclopentane can also be incorporated into polymeric materials that are being used for their selective molecular recognition properties. In general, use of *trans*-1,2-diaminocyclohexane as a chiral unit may portend that *trans*-1,2-diaminocyclopentane will convey better properties for a particular application. For instance, in cases where 1,2-diaminocyclohexane is used as part of a chiral ligand in a catalyst that shows only low levels of enantioselectivity in a reaction, replacing the 1,2-diaminocyclohexane unit with 1,2-diaminocyclopentane could provide a catalyst with better enantioselectivity.

[0026] The instant invention provides an improved synthesis of precursors for *trans*-cyclopentane-constrained PNA. In one embodiment, the invention concerns a more

efficient and practical process, which can provide multigram quantities of both enantiomers of **1**. Because the synthesis described herein is easy to perform and is scalable to large quantities, *trans*-1,2-diaminocyclopentane produced using the synthesis can become an important building block in many areas of chemistry.

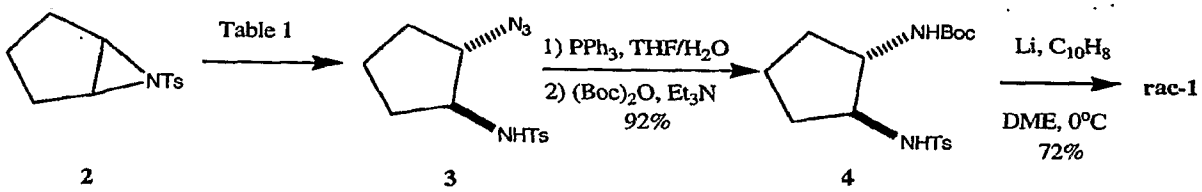
[0027] We report a straightforward approach to preparation of such diamines by ring-opening of an appropriate aziridine with an azide nucleophile in the presence of a promoter. In this way, two amine groups or its equivalents are installed in one step, thus circumventing the tedious functional group transformations. One such compound is *trans-tert*-butyl-2-aminocyclopentylcarbamate (1).



rac-1

[0028] A scheme for producing **rac-1** is illustrated by Scheme I.

Scheme I



[0029] In one embodiment, the synthesis begins with ring opening of tosyl-activated aziridine **2** (Scheme 1, **Table I**), which is readily accessible in one step from commercially available cyclopentene.

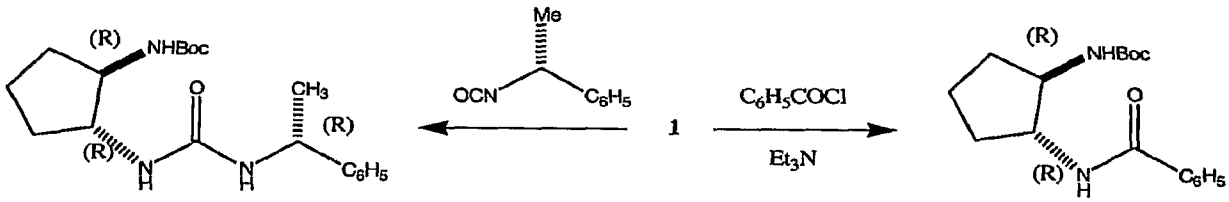
[0030] Without further purification, **3** can be reduced to the corresponding amine by Pd-catalyzed hydrogenation or Staudinger reduction (PPh_3, THF/H_2O). Those skilled in the art appreciate that other reactions may be used to convert **3** to the corresponding amine. Subsequent Boc protection of the resulting amine yielded **4** in 92 % yield for two steps. Each of the aforementioned reactions are well known to those skilled in the art.

[0031] While this method is illustrated with the Boc protecting group, it is understood by those skilled in the art that other suitable protecting groups can be

substituted. Additional protecting groups include any carbamate-based nitrogen protecting group. Examples of suitable protecting groups include fluorenyl-methoxy-carbonyl (Fmoc), carbobenzyloxy (Cbz), and allyloxycarbonyl (Alloc).

[0032] Generally, the major drawback of tosyl-activated aziridine chemistry is that harsh conditions are required for the cleavage of the sulfonamide bond at a later stage of the synthesis. Recently, milder conditions have been developed in this context, and magnesium in methanol under ultrasonic conditions has been successfully applied to a variety of substrates. Under these conditions, **4** underwent clean but very sluggish conversion. After considerable experimentation, the detosylation was achieved with lithium and naphthalene in dimethoxyethane (DME) or tetrahydorfuran (THF). The reaction was temperature-dependent: at low (-78 °C) or room temperature, either very slow conversion (10%) was observed or low yield (40%) resulted. In one embodiment, the reaction was best performed at 0 °C for 5 h to afford **1** in 72% yield.

Scheme II



[0033] The resolution of primary amines with similar structures to **1** has been typically performed with tartaric acid or mandelic acid. Our initial attempts to resolve **1** with these two acids did not give precipitate under various conditions. Therefore, twenty other chiral resolving acids were screened. The resolution results were rapidly examined by ^{1}H NMR method as follows (Scheme II): the precipitated salts were converted to amine **1** and subsequently treated with optically pure R-(+)-1-phenylethylisocyanate in $CDCl_3$, to give corresponding urea disastereomers **5**. The Boc groups of the two disastereomers **5** showed separated peaks at 1.30 and 1.44 ppm in the ^{1}H NMR. Among the different chiral acids that were screened, di-p-toluoyl-tartaric acid, 2-phenylpropionic acid, and menthyloxyacetic acid showed partial resolution. Fortunately, optimal results were obtained when 10-camphorsulfonic acid (CSA) was used as a resolving agent. The precipitate from rac-**1** and CSA (1 : 1 or 1 : 0.5) in acetone showed approximately 60% ee.

[0034] After crystallizations from acetonitrile, the optical purity of **1** was enhanced to over 99 % enantiomeric excess (ee), as determined by HPLC analysis (on a chiral stationary phase) of the benzoylated derivative **6**. The configuration of **1** obtained from the resolution was assigned based on the comparison of HPLC data of **6** (obtained on a chiral stationary phase) to material obtained from previous syntheses performed in our laboratory.

[0035] In conclusion, we have developed a short synthetic route to optically enriched **1**, a versatile building block for chiral ligands and modified PNAs. The synthesis does not require column chromatography and is amenable to large-scale preparation (10 g scale, for example, for rac-1), which can provide gram quantities of both enantiomers.

[0036] Among the end uses of compound **1** is incorporation into the backbones of PNAs. The PNAs of the instant invention can be used in methods for detecting a nucleic acid comprising contacting a sample suspected of containing the nucleic acid with a peptide nucleic acid prepared in accordance with the invention. These PNAs can be used in a kit for detecting a nucleic acid comprising at least one peptide nucleic acid prepared in accordance with the invention. The kits can be adapted for detecting an infectious agent, said infectious agent such as anthrax, avian flu, tuberculosis, severe acute respiratory syndrome (SARS), human papilloma virus (HPV), or human immunodeficiency virus (HIV). This list of agents is illustrative only and those skilled in the art are aware of other infectious agents that can be detected with the use of PNAs comprising 1,2-diaminopentane residues in the PNA backbone.

[0037] Chemical modification in the backbone of a peptide nucleic acid (PNA) lowers the detection limit of anthrax DNA by six orders of magnitude compared to the regular, unmodified PNA. Furthermore, the modified-PNA has improved sequence specificity compared to regular PNA, and is a key component of a colorimetric detection system for anthrax DNA. These findings make PNA a highly desirable probe for incorporation into DNA detection devices, and should stimulate the replacement of traditional DNA probes.

[0038] Technologies for genomic detection most commonly use DNA probes to hybridize to target sequences. To achieve required sensitivity, the use of PCR to amplify target sequences has remained standard practice in many labs. Direct detection methods that eliminate the requirement for a PCR step could afford faster and simpler devices that can be used outside of a laboratory. Devices based on nanotechnology have yielded impressive results, yet the use of PCR is still predominant in most applications. However,

replacing DNA probes with a class of synthetic nucleic acids, such as peptide nucleic acids (PNAs), can significantly improve detection devices. There are numerous advantages to using PNA instead of DNA probes in hybridization assays, including: complete resistance to degradation by enzymes, increased sequence specificity to complementary DNA, and higher stability when bound with complementary DNA. Despite these advantages, the use of PNA in DNA detection systems has received sparse attention, and has not replaced the use of DNA probes. We believe that one reason PNA does not dominate in this area is due to the lack of backbone modifications that can be used to adjust the properties of a probe sequence. Without the ability to improve and fine-tune the basic properties of PNA, it is likely not worth the effort and/or funds for researchers to switch from DNA to PNA probes. We have developed a system of chemical modifications for PNAs, using cyclopentane groups, to predictably improve the melting temperature and sequence specificity of PNA-DNA duplexes. To demonstrate the utility of our chemical strategy and to highlight the importance of PNA in detection, we developed a simple, colorimetric sandwich-hybridization assay to detect anthrax lethal factor DNA using PNA as capture and detection probes (**Figures 1 and 2**). Our system has been developed into a convenient 96-well plate format in which the capture probe (PNAα) is covalently attached to a DNA-Bind® plate. A biotin-labeled PNA detection probe (PNAβ), in combination with commercially available avidin-horseradish peroxidase conjugate (avidin-HRP) and tetramethylbenzidine (TMB), is used to generate a signal if the target DNA is present. If a sandwich complex forms on the plate, the strong interaction between biotin and avidin will retain avidin-HRP on the plate. The HRP will then catalyze oxidation of TMB, and after stopping the reaction with sulfuric acid a colored product that absorbs at 450 nm is generated. These items are accessible to most biomedical research facilities. Incorporation of a cyclopentane-modified PNA residue (tcyp) into the capture probe (PNAα(1)) affords a system with a detection limit of 50 zeptomoles for lethal factor DNA, which is 6 orders of magnitude lower compared to the same system that uses regular, unmodified PNA (PNAα(2)) (**Figure 3** and **Table 5**). Furthermore, the sequence specificity is improved in the tcyp-modified PNA compared to the regular PNA.

[0039] Finally, we have demonstrated that our detection system effectively identifies lethal factor DNA from a whole cell extract of *B. anthracis* DNA, giving a colored signal visible to the naked eye (**Figures 2**). The stability of PNAs, and the ability to synthesize PNAs of different sequences should allow the development of effective detection systems for numerous bacterial and viral pathogens (such as HIV and Avian flu),

as well as single nucleotide polymorphisms associated with several diseases (such as cancer and Alzheimer's disease). Furthermore, the ability to introduce chemical modifications with predictable effects into the PNA should allow researchers to fine-tune the properties of PNA for a specific application, and this flexibility could provide robust genomic detection devices available to healthcare workers in the field and to the general public.

EXPERIMENTAL SECTION

[0040] 2-Azido-N-tosyleyclopentanamine(3). To a mixture of 6-tosyl-6-aza-bicyclo[3,1,0]hexane 2 (20.0 g, 84.4 mmol) and NaN_3 (5.5 g, 84.4 mmol) in dry THF (300 mL) was added TMSN, (2.9 g, 3.0 mL, 25.3 mmol), followed by the addition of TBAF (25.3 mL, 1M in THF, 25.3 mmol). The solution was stirred at 40 °C for 20 h. The reaction solution was cooled to room temperature, saturated $NaHCO_3$ aqueous solution (200 mL) was added. The aqueous layer was extracted with diethyl ether (100 mL x 3). Combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, filtered, and concentrated under vacuum. The oil residue was filtered through a pad of silica gel and washed with a mixture of ethyl acetate/hexanes (1 : 2, 2000 mL). Solvents were removed under vacuum to afford **3** (22.4 g, 95%) as a colorless oil. Spectroscopic data of **3** were consistent with the literature data for this compound.

[0041] The reaction to produce **3** was repeated with a variety of conditions. These are summarized in the table below.

Table 1. Ring Opening Of Aziridine 2 with Azides.

Entry	Reagents	Solvent	Temp (°C)	Time (h)	Conversion (%)[b]	Yield (%)[c]
1	NaN_3, ozone	CH_3CN/H_2O	23	5	0	0
2	NaN_3, 10% CAN	CH_3CN/H_2O	23	20	15	13
3	$TMSN_3$	DMF	40	16	34	30
4	$TMSN_3$, 5% TBAF	THF	40	16	81	76
5	$TMSN_3$, 20% TBAF	THF	40	16	100	93

6	$TMSN_3$, 00% TBAF	THF	40	4	100	96
7	30% $TMSN_3$, 30% TBAF, 100% NaN_3	THF	40	20	100	95

a. All reactions were conducted at 4mmol scale, entries 5-7 were also conducted at ~85 mmol scale.
b. Determined by ^1H NMR.
c. Isolated yield.

[0042] A literature search revealed four examples of ring-opening of **2** with azides. However, our examination of these methods revealed that none of them gave satisfactory results, especially for large-scale (4 mmol scale) synthesis. For instance, attempted opening of **2** with NaN_3 using Oxone in aqueous acetonitrile failed to provide any ring-opening products. The use of ceric ammonium nitrate (CAN) instead of Oxone led to 15% conversion and 13% yield of **3**. Similar results were observed when $TMSN_3$ in DMF was used. Fortunately, adding 5% TBAF to $TMSN_3$ significantly promoted the transformation (entry 4, 80% conversion, 76% yield). However, the operation requires laborious column chromatography to separate azido amine product **3** from unreacted **2**, which has an R*f* value close to that of **3**. Complete conversion was achieved by increasing the amount of TBAF to 20% (entry 5). If 1 equivalent TBAF is used, the reaction time can be shortened to 4 h and **3** can be obtained in 96% yield (entry 6). Similar results can be obtained, although with longer reaction times, by using 30% $TMSN_3$, 30% TBAF, and 100% NaN_3 (entry 7). In some embodiments, these final conditions prove to be the most cost-effective and reliable for preparation of **3**.

[0043] ***tert*-Butyl-2-(tosylamino)cyclopentylcarbamate (4).** Triphenylphosphine (40.3 g, 153.8 mmol) was added to a solution of **3** (21.5 g, 76.9 mmol) in THF/H_2O (600/50 mL). The mixture was stirred at room temperature for 16 h. 1M HCl solution (200 mL) was added. The aqueous layer was separated, extracted with diethyl ether (200 mL x 3), and basified with 2 N NaOH solution to pH 12-14. The aqueous solution was extracted with ethyl acetate (200 mL x 5). Combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, filtered, and concentrated under vacuum to afford a light yellow oil (18.9 g, 97%). Without further purification the resulting oil was dissolved in dry methylene chloride (360 mL), di-*tert*-hutyldicarhonate (15.6 g, 71.6 mmol) and triethylamine (10 mL) were added. The solution was stirred at room temperature for 16 h. Most of the solvent was removed under vacuum, and diethyl

ether (300 ml,) was added. The mixture was with 1 N HCl solution (50 mL x 3), dried over Na_2SO_4, and concentrated to afford a white solid which crystallized from diethyl ether to give white needles (24.9 g, 92% for two steps). R*f*= 0.36 (hexanes/EtOAc 2:1). Mp: 129-130 °C. IR (film) 3680, 2973, 2844, 2866, 1685, 1346, 1160, 1055, 1012 cm^{-1}. ^1H NMR (300 MHz: $CDC1_3$) δ 7.75 (d, ZH), 7.26 (d, 2H), 6.19 (s, br, 1H), 4.55 (s, hr, lH), 3.70 (m, 1H), 3.03 (m, 1H), 2.42 (s, 3H), 2.02 (m, 2H), 1.65 (m, 3H), 1.42 (s, 9H), 1.30 (m, 1H). ^{13}C NMR (75 MHz, $CDC1_3$) δ 156.9, 143.1, 137.4, 129.6, 127.2, 80.1, 61.7, 57.1, 31.3, 29.4, 28.4, 21.6, 20.2. HRMS (EI) m/z calcd for $C_{17}H_{27}N_2O_4S$ $[M+1]^+$ 353.1535, found 353.1554.

[0044] ***tert*-Butyl-2-aminocyclopentylcarbamate (1).** A mixture of lithium granules (1.52 g, 226.6 mmol) and naphthalene (10.9 g, 85.0 mmol) in dry dimethoxyethane (350 mL) were stirred at room temperature for 2 h. The deep blue solution was then cooled to 0 °C, a solution of **4** (10.0 g, 28.33 mmol) in dry dimethoxyethane (40 mL) was added dropwise over 20 min. The mixture was stirred at 0 °C for 5 h. The undissolved lithium was filtered off, and 1 N HCl solution (60 mL) was added to the filtrate. The organic layer was separated and extracted with 1 N HCl (50 mL x 2). The aqueous layers were combined, extracted with diethyl ether (50 mL x 3), and then basified with 2 N NaOH solution to pH 12-14. The aqueous solution was extracted with ethyl acetate (50 mL x 5). Organic layers were combined, dried over Na_2SO_4, solvent was removed under vacuum to afford rac-**1** as a colorless oil, which solidified under vacuum to a white solid (4.08 g, 72%). R*f* = 0.31 (hexanes/EtOAc 2:l). Mp 60-62 °C. IR (film) 3301, 2967, 1689, 1526, 1453, 1390, 1365, 1250, 1170, 1045, 1020, 870,781 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 4.48 (s, hr, IH), 3.48 (m, IH), 2.99 (m, lH), 2.14 (m, lH), 1.98 (m, lH), 1.70 (m, 2H), 1.45 (s, 9H), 1.38(m,2H). ^{13}C NMR (75MHz, $CDCl_3$) δ 156.1, 78.9, 60.5, 59.4, 33.0, 30.9, 28.4,2 0.7. HRMS (EI) m/z calcd for $C_{10}H_{21}N_2O_2$ $[M+1]^+$ 201.1603, found 201.1632.

[0045] Optical Resolution of *tert*-Butyl-2-aminocyclopen+arbamate (1) using (S)-(+)-l0-Camphorsulfonic acid. (S)-(7)-10-Camphorsulfonic acid (8.24 g, 35.5 mmol) in acetone (HPLC grade, 20 mL) was added to a solution of rac-**1** (7.1 g, 35.5 mmol) in acetone (HPLC grade, 20 mL). The mixture was stirred for 6 h and the resulting white precipitate was collected by filtration. The precipitate was recrystallized in acetonitrile twice. The white precipitate was taken up in a mixture of ethyl acetate and 2 N NaOH solution. The aqueous layer was extracted with ethyl acetate (30 mL x 3). The organic layers were combined, dried over Na_2SO_4. The solvent was removed under vacuum to give

a colorless oil (2.34 g, 11.7 mmol), which was dissolved in acetone and treated with a solution of (S)-(+)-10-camphorsulfonic acid (2.71 g, 11.7 mmol) in acetone (HPLC grade, 20 mL). After stirring for 2 h, solvent was evaporated and solid was crystallized from acetonitrile. The same workup procedure described above to make the free base gave a colorless oil which solidified under vacuum to give a white solid (S,S-**1**: 1.90 g, 51% based on one enantiomer). $[\alpha]^{23}_{D}$ +15.8 (c 1.0, EtOH); Mother liquid was basified and extracted with ethyl acetate, resolved with (R)-(-)-10-camphorsulfonic acid following the procedure described above to afford R,R-**1** (1.58 g, 46% based on one enantiomer): $[\alpha]^{23}_{D}$ -16.0 (c 1.0, EtOH).

[0046] Resolution Experiments with Other Chiral Acids. Rac-1 was contacted with various chiral acids as shown in **Table 2**. Conditions were as described for (S)-(+)-10-camphorsulfonic acid. Preferably, conditions where the combination of the compound and a chiral acid dissolves with some heat, and then precipitates when cooled. Ideally, only one enantiomer would precipitate out of solution with the chiral acid. In **Table 2**, a few conditions (including use of ethanol, acetone with some methanol, and isopropanol) are reported. The enantiomeric ratio (er) indicates whether the chiral acid showed potential as a resolving agent. For a number of the chiral acids tested, under most conditions, the salt did not dissolve well, or dissolved too well and never precipitated (NP). In some cases a precipitate forms but the er is 1:1, which showed non-specific precipitation. The best result was with entry number 5 of **Table 2**. We believe, however, that there are many other chiral acids and many other conditions which can produce favorable results.

Scheme III

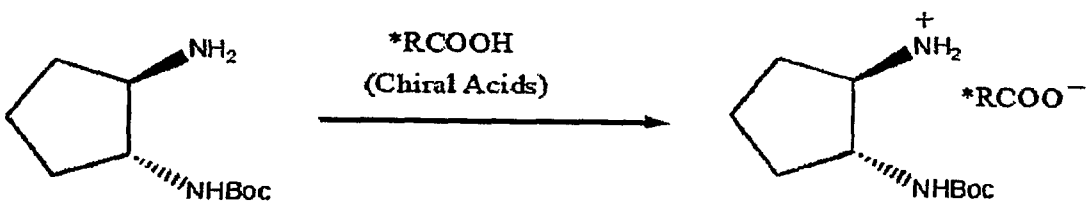


Table 2. Use of Chiral Acids as Resolving Agents

	Chiral Acid	Ethanol	Acetone (+ MeOH)	Isoporpanol
1	Dibenzoyl-L-tartaric acid monohydrate	Waxes	Dissolved, NP	
2	(-)-O,O'-Di-p-toluyl-L-tartaric acid	Waxes	Not dissolved well, er: 2:1	Not completely dissolved
3	(1S)-(-)-Camphanic acid	Waxes	Not dissolved well, er: 1:1	
4	(1R, 3S)- (+)-Camphoric acid	Insoluble	Not dissolved well, er:1:1	
5	(1R)-(-)-10-Camphorsulfonic acid	Waxes	Dissolved (heated), er: 4:1	
6	(R)-(-)-4-Bromomandelic acid	Waxes	Dissolved, NP	
7	(R)-(-)-4-Methylmandelic acid	Waxes	Dissolved, NP	
8	(R)-(-)-3-Chloromandelic acid	Waxes	Dissolved, NP	
9	(R)-(-)O-Acetylmandelic acid	Waxes	Dissolved, NP	
10	(R)-(-)-2-Chloromandelic acid	Waxes	Dissolved, NP	
11	L-Malic acid	Waxes	Dissolved, NP	
12	(S)-(+)-2-Phenylpropionic acid	Waxes	Dissolved (heated), er: 3:1	
13	(S)-(+)-α-Methoxyphenylacetic acid	Waxes	Dissolved (heated), er: 1:1	
14	L-Menthoxyacetic acid	Waxes	Dissolved, er: 5:4	
15	(R)-(+)-Tetrahydro-2-furoic acid	Waxes	Dissolved, NP	
16	(R)-(-)-2,2'-(1,1'-Binaphthyl)phosphoric acid	Waxes	Dissolved, NP	

17	(-)-O,O'-Dibenzoyl-L-tartaric acid mono(dimethylamide)	Waxes	Dissolved, NP	
18	(-)-2'-Bromotartranilic acid	Waxes	Dissolved, NP	
19	(+)-2'-Methyltartranilic acid	Waxes	Dissolved, NP	
20	(+)-2'-Nitrotartarnilic acid	Waxes	Dissolved (heated), NP	

NP: No Precipitate, er: enantiomeric ratio

[0047] HPLC Analysis of *tert*-Butyl-2-(benzamido)cyclopentylcarbamate (6). Benzoyl chloride (20 mg, 0.016 mL, 0.14 mmol) was added to a solution of nonracemic **1** (28 mg, 0.14 mmol) and triethylamine (28 mg, 0.038 mL, 0.28 mmol) in dry methylene chloride (2 mL). The solution was stirred for 16 h, and then washed with 1 M HCl solution (1 mL x 3). Organic layer was dried over Na_2SO_4, solvent was removed under vacuum, and the residue was purified by preparative TLC (solvent: hexanes/EtOAc 2 : 1) to afford **5** (38 mg, 85%) as a white solid. $Rf = 0.29$ (hexanes/EtOAc 2:1). Mp 190-192 °C. IR (film) 3314, 2974, 1638, 1621, 1541, 1302, 1 170, 1033 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 7.75 (m, 2H), 7.34 (m, 3H), 4.75 (s, br, 1H), 3.85 (s, br, 2H), 2.33 (m, 1H), 2.04 (m, 1H), 1.72 (m, 2H), 1.39 (m, 2H), 1.34 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.0, 157.1, 134.3, 131.4, 128.5, 127.1, 80.0, 59.2, 56.5, 30.1, 29.8, 28.8, 28.4, 19.6. HRMS (EI) m/z calcd for $C_{10}H_{21}N_2O_2$ $[M+1]^+$ 327.1685, found 327.1689.

[0048] Compound **6** was dissolved in 2-propanol/hexanes (1: 1) for HPLC analysis. HPLC conditions: column: (S,S)-Whelk-O1, 250 mm x 4.6 mm, 10 micron supplied by Regis Technologies; mobile phase: hexanes/2-propanol (95 : 5); flow rate: 1.5 mL/min; absorbance 0.04; sample concentration: 1 mg/mL; injection volume: 20 μL; retention time: S,S-**1**, 7.07 min; R,R-**1**, 9.53 min.

[0049] ^1H and ^{13}C NMR data was obtained for compounds **1**, **4**, and **6**. HPLC data was obtained on a chiral stationary phase for compound **6**.

[0050] PNA Synthesis and Detection of Infectious Agents. Methods for PNA and cyclopentane-modified PNA synthesis and acquisition of melting temperature data can be found in Pokorski, *et al., J. Am. Chem. Soc.* **2004**, 126, 15067. Addition of one or more cyclopentane groups into a PNA sequence improves the melting temperature to complementary DNA by ~5 °C per cyclopentane, regardless of which base is used.

[0051] To demonstrate the utility of our chemical strategy and to highlight the importance of PNA in detection, we report a simple, colorimetric sandwich-hybridization assay to detect anthrax protective antigen DNA using PNA. In this system, a key component to improving the detection limit and sequence specificity is the incorporation of a cyclopentane-modified PNA into the surface-bound probe. In the sandwich-hybridization strategy, one PNA is used as capture probe (PNAα) to recruit complementary DNA to a surface, and another PNA is used as a detection probe (PNAβ) to generate a signal (Figure 1). This assay has been developed into a convenient 96-well plate format in which PNAα is covalently attached to a DNA-Bind plate. A biotin-labeled PNAβ, in combination with commercially available avidin-horseradish peroxidase conjugate (HRP-avidin) and tetramethylbenzidine (TMB), is used to generate a signal if the target DNA is present. If a sandwich complex forms on the surface, the strong interaction between biotin and avidin will retain HRP-avidin. The HRP will then catalyze oxidation of TMB, and after stopping the reaction with sulfuric acid, a colored product that absorbs at 450 nm is generated. These items are accessible to most biomedical research facilities.

[0052] In the construction of PNA probes, all PNAs were made with a single sequence that corresponds to the protective antigen (PA) portion of the anthrax genome, which is highly conserved. *See*, Edwards, K. A.; Clancy, H. A.; Baeumner, A. *J. Anal. Bioanal. Chem.* **2006**, 384, 73-84. Next, PNAR probes were designed with extended linkers on the N-terminal for covalent attachment to the DNA-Bind plate. The PNAβ probes were outfitted with additional lysine groups so that biotins could be attached. To ensure that there was enough room for the HRP-avidin complex to bind, additional linkers were added onto the lysine side chains (**Table 3**).

Table 3. PNA Capture Probes (α) and PNA Detection Probes (β)

entry	PNA sequence[a]	T_m (°C)[b]
α(1)	H_2N-$(egl)_5$-ATCCTTATCAATATT-$CONH_2$	50.5
α(2)	H_2N-$(egl)_5$-ATCCTTAT$_{tcyp}$CAATATT-$CONH_2$	55.6
β(1)	H_2N-TAACAATAATCC-Lys-Lys-$CONH_2$ $(egl)_2BT$	54.1
β(2)	H_2N-TAACAATAATCC-Lys-Lys-Lys-$CONH_2$ $BT(egl)_2$ $(egl)_{10}BT$	55.1

[a] tcyp = PNA residue derived from (S,S)-*trans*-1,2-cyclopentane diamine, BT = biotin, egl = 8-amino-3,6-dioxaoctanoic acid. [b]Tm represents the melting temperature for the duplex formed between the indicated PNA and antiparallel DNA. Conditions for Tm measurement: 150 mM NaCl, 10 mM sodium phosphate buffer, pH 7.0, 0.1 mM EDTA, UV measured at 260 nm from 90 to 25 °C, in 1 °C increments. All values are averages from two or more experiments. α (1) = SEQ ID NO. 5; α (2) = SEQ ID NO. 6; β (1) = SEQ ID NO. 7; β (2) = SEQ ID NO. 8.

[0053] PNAα probes were attached to the surface of each well of the DNA-Bind plate, and free sites were blocked using a buffer consisting of BSA and lysine. Detection conditions were optimized using synthetic DNA. Ultimately, a set of conditions were developed that involve incubating a solution of target DNA and PNAβ in the well of a DNA-Bind plate that contains PNAα, followed by washing, incubation with HRP-avidin, another washing, and then detection with 1-Step Turbo TMB, a commercially available TMB and peroxide solution. The enzymatic reaction was stopped by the addition of sulfuric acid, and then absorbance at 450 nm was measured. Using unmodified PNA under these conditions, only picomole quantities of DNA could be detected (**Table 4**, entry 4).

Table 4. Absorbance at 450 nm for DNA Detection with Different PNA Probes and Using HRP-Avidin (entries 1-4) versus Poly-HRP-avidin (entries 5-8)

		Femtomoles of Anthrax DNA			
entry	PNAs[a]	10^3	10^1	10^{-1}	10^{-6}
1	α(2)+β(2)	0.23	0.02	0.01	0.01[b]
2	α(2)+β(1)	0.16	0.01	–	–
3	α(1)+β(2)	0.19	0.01	–	–
4	α(1)+β(1)	0.12	–	–	–
5	α(2)+β(2)	0.99	0.15	0.15	0.09[c]
6	α(2)+β(1)	1.05	0.03	0.01	0.01[c]
7	α(1)+β(2)	0.44	0.01	–	–
8	α(1)+β(1)	0.20	–	–	–

[a]*See* **Table 3** for structures of PNAs. Standard deviation values for absorbances range from 0.01 to 0.03. All experiments in **Table 4** were performed with synthetic anthrax DNA sequences. [b]Data for 50 zmol DNA. [c]Data for 10 zmol DNA.

[0054] Several strategies were explored to boost the detection signal and lower the DNA detection limit. A tcyp-modified PNA residue was incorporated into PNAα (PNAα(2)), an additional biotin was attached to PNAβ (PNAβ(2)), and a commercially available polymer of HRP-avidin (poly-HRP-avidin) was used in which the ratio of HRP to avidin is approximately 40:1. **Table 4** represents the absorbance values at 450 nm (over background) obtained when using different combinations of PNAα and PNAβ at several different concentrations of synthetic DNA and when using HRP-avidin (entries 1-4) and poly-HRP-avidin (entries 5-8). The results of **Table 4** demonstrate that all these strategies help improve the signal associated with DNA detection and lower the overall DNA detection limit. In the best combination (entry 5), 10 zmol of DNA can be detected.

[0055] Compared to unmodified PNA, incorporation of tcyp into PNA can improve the discrimination of single base mismatches in DNA. *See*, Pokorski, *et al.*, *J. Am. Chem. Soc.* **2004**, 126, 15067-15073. In the context of the detection assay, we examined the changes in absorbance at 450 nm associated with single base mismatches for tcyp PNA versus regular PNA. The results in **Table 5** show that, under the same conditions, the tcyp PNA (PNAα(2)) shows much clearer differences in signal between matched and mismatched DNA sequences than the regular PNA (PNAα(1)).

Table 5. Absorbance at 450 nm for Detection of Mismatches Located Directly Across from the tcyp in PNAR(2) and Comparison with Unmodified PNAα(1)[a]

PNAs[a]	Mismatch Comparison at 10^3 fmol Anthrax DNA			
	none	TG	TC	TT
α(2)+β(2)	0.31	0.05	0.04	0.05
α(1)+β(2)	0.12	0.04	0.01	0.04

[a] See above for DNA sequences used in detection. Standard deviation values for absorbances range from 0.01 to 0.05. All experiments in **Table 5** were performed with synthetic anthrax.

[0056] Using the most sensitive detection system from our research, we examined the ability to detect protective antigen (PA) DNA obtained from a whole cell extract of *B. anthracis*. In this study, DNA was extracted from two cell lines of anthrax, one that has PA-DNA (Ames 35) (*see*, Green, *et al.*, *Infect. Immun.* **1985**, 49, 291-297. and one that lacks PA-DNA (Ames 33) (*see*, Pomerantsev, *et al.*, *Infect. Immun.* **2006**, 74, 682-693). Our detection system was clearly able to distinguish the two cell lines, giving a colored signal visible to the naked eye (Figure 3). The high thermal stability of PNA-DNA duplexes allows shorter PNA probes to be used compared to DNA. A DNA-based system with equivalent thermal stability of the capture and probe sequences would require DNAs ~30-45 bases long, which could reduce sequence specificity. The ability to introduce chemical modifications with predictable effects into the PNA allows us to equalize the Tm's of the two PNA probes for their DNA targets, which likely promotes uniform hybridization of all probes.

[0057] All PNA oligomers were purified by reverse-phase HPLC with UV detection at 260 nm using VYDEK C18 (d=10 mm, l=250 mm, 5 microns) semi-prep column, eluting with 0.05% TFA in water (Solution A) and 0.05% TFA in acetonitrile (Solution B). An elution gradient of 100% A to 100% B over ~30 minutes at flow rate of 5 mL/min. PNAs were characterized by electrospray mass spectroscopy. All PNA oligomers gave molecular ions consistent with the final product (**Table 6**).

Table 6. Mass Characterization Data for PNAs

Entry[a]	PNA Sequence	*Calculated MW*	*Observed MW*
α(1)	H_2N-$(egl)_5$-ATCCTTATCAATATT-$CONH_2$	4736.6	4736.8
α(2)	H_2N-$(egl)_5$-ATCCTTAT$_{fcyp}$CAATATT-$CONH_2$	4776.7	4776.5
β(1)	H_2N-TAACAATAATCC-Lys-Lys-$CONH_2$ $(egl)_2BT$	3994.1	3994.0
β(2)	H_2N-TAACAATAATCC-Lys-Lys-Lys-$CONH_2$ $BT(egl)_2$ $(egl)_{10}BT$	5800.1	5799.8

[a] The accurate mass Electrospary ionization (ESI) mass spectra were obtained on a Waters LCT Premier time-of-flight(TOF) mass spectrometer. The instrument was operated in W mode at a nominal resolution of 10000. The electrospary capillary voltage was 2KV and the sample cone voltage was 60 volts. The desolvation temperature was 275 (°C) and the desolvation gas was Nitrogen with a flow rate of 300L/hr. Accurate masses were obtained using the internal reference standard method. The sample was introduced into the mass spectrometer via the direct loop injection method. Both positive and negative ion accurate mass data were achieved simply by reversing the instrument's operating polarity. Deconvolution of multiply charged ions was performed with MaxEnt I.

[0058] HRP-avidin, poly HRP-avidin, and 1-Step Turbo TMB were purchased from Pierce. DNA-BIND® 96-well plates were purchased from Corning Life Sciences. Absorbance values for the DNABIND® 96-well plates were determined on a Victor2 1420 microplate reader (Perkin Elmer Life Sciences).

The oligonucleotides listed in **Table 7** were purchased from IDT and used in the research as indicated in **Tables 4 and 5.**

Table 7. DNA Sequences

Anthrax PA: 5'- GGA TTA TTG TTA AAT ATT GAT AAG GAT -3'; SEQ ID NO. 1
TG mismatch: 5'- GGA TTA TTG TTA AAT ATT GGT AAG GAT -3'; SEQ ID NO. 2
TC mismatch: 5'- GGA TTA TTG TTA AAT ATT GCT AAG GAT -3'; SEQ ID NO. 3
TT mismatch: 5'- GGA TTA TTG TTA AAT ATT GTT AAG GAT -3'; SEQ ID NO. 4

[0059] The following abbreviations are used herein: PBS (phosphate saline buffer: 137 mM NaCl, 10 mM sodium phosphate, 2.7 mM KCl, pH 7.0), 0.1 X SSC (15 mM NaCl, 1.5 mM Sodium Citrate), SDS (Sodium Dodecyl Sulfate), TMB (3,3',5,5'-tetramethylbenzidine), OBB (oligo binding buffer: 50 mM Na_2HPO_4/NaH_2PO_4, 1.0 mM EDTA), BB (blocking buffer: 3% BSA and 25 mM lysine in OBB, pH 7.0).

[0060] Attaching PNAα to Surface. PNAα solution (1.0 μM) in OBB (110 μL, pH 7.5) was added to each well of a DNA-BIND® 96-well plate (Corning Life Sciences), incubated at 37 °C for 1 hr, then washed three times with PBS buffer.

[0061] DNA Detection Protocol. Blank absorbance at 450 nm of the DNA-BIND® plate was obtained on the microplate reader. Each well was treated with 200 μL BB for 30 minutes at 37 °C. Target DNA (10 nM to 5 aM) and PNAβ (15 nM) were premixed in 100 μL of 0.15 M aqueous NaCl, then loaded into each well. The plate was sealed with an adhesive film, incubated at 45 °C for 3 hours, then washed twice with 0.1% SDS in 0.1 X SSC and treated with BB (200 μL) for 30 minutes at 33 °C. Next, 100 μL of 1.0 μg/mL avidin-horseradish peroxidase conjugate in BB was added. After 30 minutes at 37 °C, the plate was washed three times with PBS buffer. Next, 100 μL of 1-step turbo TMB was added and incubated at 37 °C for 20 minutes. Then, 2 M H_2SO_4 (50 μL) was added to stop the oxidation. The plate was scanned again at 450 nm to give experimental absorbance readings from which background readings were subtracted. In some cases, very similar absorbance readings were obtained for different concentrations (see **Table 2**, entry 5). Most of these situations arise when using the polymer of HRP-avidin (poly HRP-avidin). This polymeric construct is highly active and difficult to control at higher concentrations. It is certainly ideal for detection of low concentrations of DNA, but can become less active at higher concentrations. Therefore, we suspect that aggregation at higher concentrations could lower enzymatic activity and, in some cases, give similar absorbance readings for different DNA concentrations.

[0062] Bacterial strains and genomic DNA purification. *Bacillus anthracis* Ames 35 ($pXO1^+$ $pXO2^-$) strain. and a plasmid-free Ames 33 strain ($pXO1^-$ $pXO2^-$) were used for genomic DNA purification. Ames 35 harbors the *pag* gene that encodes PA, while Ames 33 does not ((*see*, Green, *et al.*, *Infect. Immun.* **1985**, 49, 291 and Pomerantsev, *et al.*, *Infect. Immun.* **2006**, 74, 682.). For genomic DNA purification, *B. anthracis* strains were grown at 37°C in LB broth overnight. Genomic DNA purification was performed using the Wizard genomic DNA purification kit (Promega) following the protocol provided by the manufacturer and DNA was resuspended in water. DNA concentration was then assessed using a Nanodrop ND-1000 spectrophotometer (Coleman Technologies Inc).

[0063] PNAs targeted to the DNA region of a *P-gp*. PNA has been previously examined for antisense activity (molecules that bind to complementary RNA) and more recently antigene activity (molecules that directly bind to DNA). PNA displays excellent

activity as both an antisense and antigene molecule. Incorporation of cyclopentane units into PNA increases binding to complementary DNA and RNA, so cyclopentane-modified PNA should further improve the activity of PNA in either capacity. PNAs targeted to the DNA region of a protein called *P-gp*, a protein known to promote efflux of chemotherapeutic drugs in cancer cells. This protein is often found in multi-drug resistant cancers. Elimination of this protein can restore chemical sensitivity of these cancers, making chemotherapy a viable option to treat these cancers. Regular and cyclopentane-modified PNA that target a site of DNA on the *P-gp* gene were prepared and the initial results show that cyclopentane-modified PNA is more successful at repressing the expression of the protein than the regular, unmodified PNA.

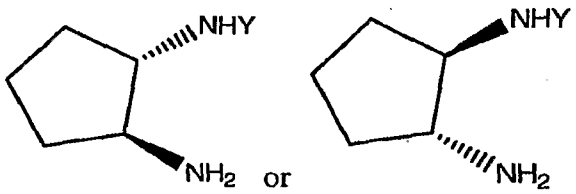
[0064] As used herein, the terms "a", "an", "the" and the like refer to both the singular and plural unless the context clearly indicates otherwise.

[0065] Also as used herein, the description of one or more method steps does not preclude the presence of additional method steps before or after the combined recited steps. Additional steps may also be intervening steps to those described. In addition, it is understood that the lettering or order of process steps or ingredients is a convenient means for identifying discrete activities or ingredients and the recited lettering can be arranged in any reasonable sequence.

[0066] Where a range of numbers is presented in the application, it is understood that the range includes all integers and fractions thereof between the stated range limits. A range of numbers expressly includes numbers less than the stated endpoints and those in-between the stated range. A range of from 1-3, for example, includes the integers one, two, and three as well as any fractions that reside between these integers.

What is Claimed:

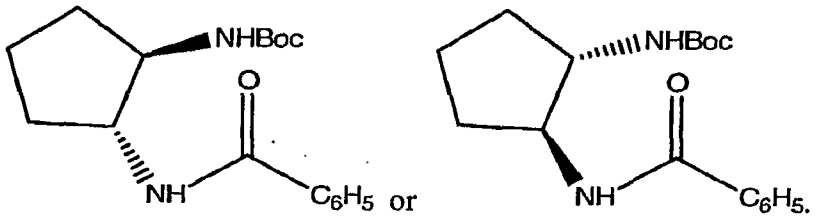
1. A compound of the formula



having at least 99% enantiomeric purity where Y is a protecting group.

2. The compound of claim 1, wherein Y is t-butyl carbamate.

3. A compound of the formula:



4. A method of synthesis of 2-azido-N-tosylcyclopentanamine comprising contacting 6-tosyl-6-azabicyclo[3.1.0]hexane with trimethylsilyl azide ($TMSN_3$) and tetra-n-butylammonium fluoride (TBAF).

5. The method of claim 4, wherein the method is performed at 25-50 °C.

6. The method of claim 4, wherein the molar ratio of $TMSN_3$ to TBAF is 20:1 to 1:1.

7. The method of claim 4, additionally comprising sodium azide.

8. A method of synthesis of *trans-tert*-butyl-2-diaminocyclopentylcarbamate comprising contacting 6-tosyl-6-azabicyclo[3.1.0]hexane with $TMSN_3$ and TBAF to produce 2-azido-N-tosylcyclopentanamine.

9. The method of claim 8, further comprising converting 2-azido-N-tosylcyclopentanamine.
to *tert*-butyl-2-(tosylamino)cyclopentylcarbamate.

10. The method of claim 8, wherein the converting 2-azido-N-tosylcyclopentanamine. to *tert*-butyl-2-(tosylamino)cyclopentylcarbamate comprises:

reducing the 2-azido-N-tosylcyclopentanamine to produce 2-amino-N-tosylcyclopentanamine; and

contacting the 2-amino-N-tosylcyclopentanamine with di-*tert*-butyl dicarbonate to produce *tert*-butyl-2-(tosylamino)cyclopentylcarbamate.

11. The method of claim 9, further comprising detosylation of *tert*-butyl-(tosylamino)cyclopentylcarbamate to produce *trans-tert*-butyl-2-aminocyclopentylcarbamate.

12. The method of claim 8, further comprising:

reducing the 2-azido-N-tosylcyclopentanamine to produce 2-amino-N-tosylcyclopentanamine;

contacting the 2-amino-N-tosylcyclopentanamine with di-*tert*-butyl dicarbonate to produce *tert*-butyl-2-(tosylamino)cyclopentylcarbamate; and

detosylation of *tert*-butyl-(tosylamino)cyclopentylcarbamate to produce *trans-tert*-butyl-2-aminocyclopentylcarbamate.

13. The method of claim 8, wherein the molar ratio of $TMSN_3$ to TBAF is 20:1 to 1:1.

14. The method of claim 8, further comprising sodium azide.

15. The method of claim 12, wherein the reduction of 2-azido-N-tosylcyclopentanamine is obtained by Pd-catalyzed hydrogenation or Staudinger reduction.

16. The method of claim 12, wherein the detosylation of *tert*-butyl-(tosylamino) cyclopentylcarbamate comprises contacting *tert*-butyl-(tosylamino)cyclopentylcarbamate with lithium and naphthalene in an aprotic solvent.

17. The method of claim 16, wherein the aprotic solvent is tetrahydrofuran or 1,2-dimethoxyethane.

18. The method of claim 16, wherein the detosylation of *tert*-butyl-(tosylamino) cyclopentylcarbamate is performed at a temperature between -20 °C and 10 °C.

19. The method of claim 12, further comprising contacting the *trans-tert*-butyl-2-aminocyclopentylcarbamate with a resolving agent to produce a precipitate comprising S,S-*tert*-butyl-2-aminocyclopentylcarbamate.

20. The method of claim 19, wherein the resolving agent is 10-camphorsulfonic acid.

21. The method of claim 19, further comprising isolating the S,S-*tert*-butyl-2-aminocyclopentylcarbamate.

22. A method for producing a peptide nucleic acid containing at least one (S,S,)-*trans*-cyclopentane or (R,R)-*trans*-cyclopentane unit in the PNA backbone comprising:

contacting 6-tosyl-6-azabicyclo[3.1.0]hexane with trimethylsilyl azide ($TMSN_3$) and tetra-n-butylammonium fluoride (TBAF) to produce 2-azido-N-tosylcyclopentanamine;

converting the 2-azido-N-tosylcyclopentanamine to *trans-tert*-butyl-2-aminocylcopentylcarbamate; and

linking a heterocyclic base moiety to the *trans-tert*-butyl-2-aminocylcopentylcarbamate and incorporating the resulting compound into the PNA backbone.

23. The method of claim 22, wherein the 2-azido-N-tosylcyclopentanamine to *trans-tert*-butyl-2-aminocylcopentylcarbamate by a process comprising:

reducing the 2-azido-N-tosylcyclopentanamine to produce 2-amino-N-tosylcyclopentanamine;

contacting the 2-amino-N-tosylcyclopentanamine with di-*tert*-butyl dicarbonate to produce *tert*-butyl-2-(tosylamino)cyclopentylcarbamate; and

detosylation of *tert*-butyl-(tosylamino)cyclopentylcarbamate to produce *trans-tert*-butyl-2-aminocyclopentylcarbamate.

24. A method for detecting a nucleic acid comprising contacting a sample suspected of containing the nucleic acid with a peptide nucleic acid prepared in accordance with claim 22.

25. The method of claim 24, wherein the detection is performed visually by an observer.

26. The method of claim 25, wherein the detection is performed by a method comprising:

contacting a solution comprising a first *trans*-cyclopentane-containing PNA with a substrate having a second *trans*-cyclopentane-containing PNA affixed thereto, wherein the first *trans*-cyclopentane-containing PNA has a reporter molecule attached thereto and the first and second *trans*-cyclopentane PNAs being complementary to different portions of a target DNA;

contacting DNA with the first and second cyclopentane-containing PNAs;

visually observing the substrate to detect the appearance of color from the reporter molecule.

27. A kit for detecting a nucleic acid comprising at least one peptide nucleic acid prepared in accordance with claim 22.

28. The kit of claim 25, wherein the kit is adapted for detecting an infectious agent, said infectious agent being anthrax, avian flu, severe acute respiratory syndrome (SARS), tuberculosis (TB), human papilloma virus (HPV), or human immunodeficiency virus (HIV).

29. The kit of claim 25, further comprising a biotin-labeled PNA detection probe an avidin-horseradish peroxidase conjugate.

Figure 1

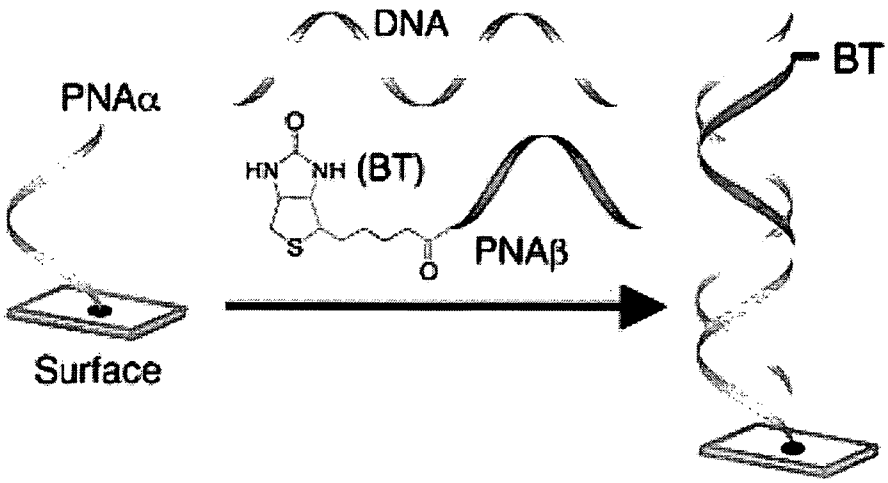


Figure 2

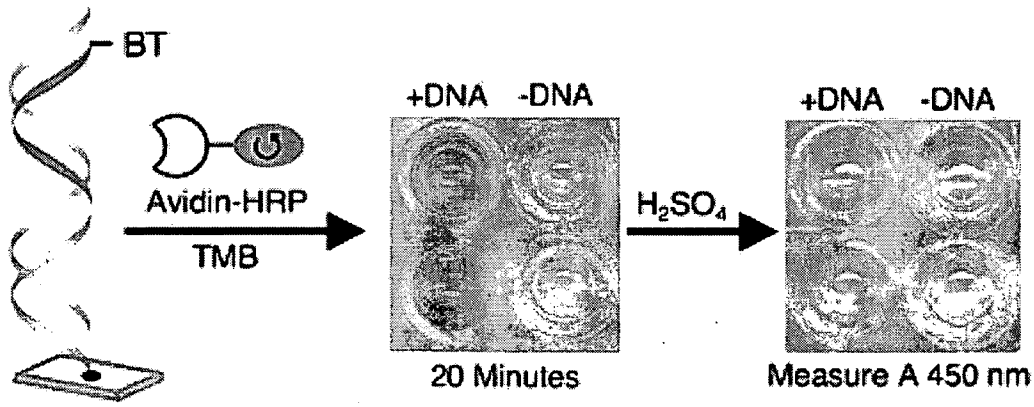
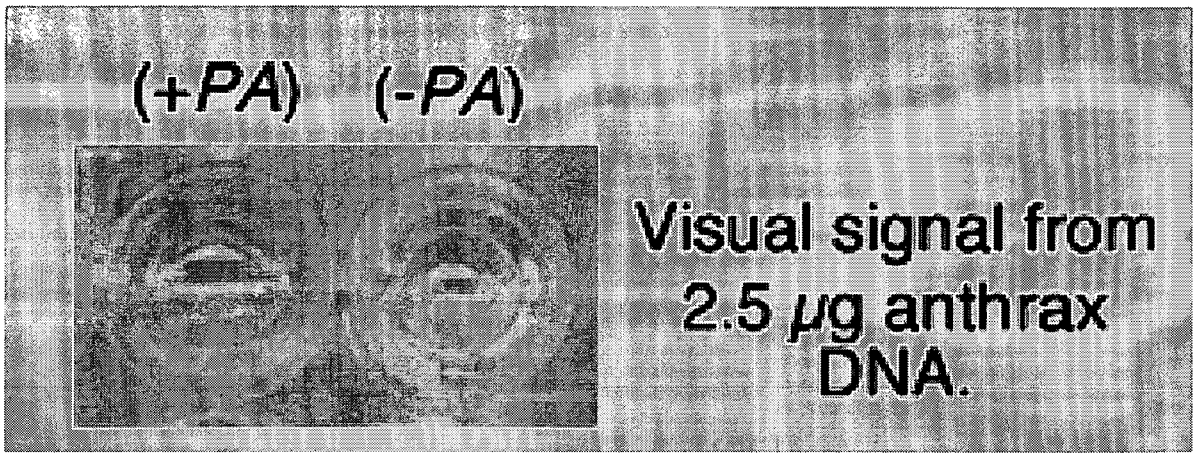


FIGURE 3



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2007/020466

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07C271/24 C07C303/40 C07K14/00 C12Q1/68 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07C C07K C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, CHEM ABS Data, BEILSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	QUN XU ET AL.: "Practical Synthesis of trans-tert-Butyl-2-aminocyclopentylcarbamate and Resolution of Enantiomers" JOURNAL OF ORGANIC CHEMISTRY., vol. 71, 6 October 2006 (2006-10-06), pages 8655-8657, XP002466651 USAMERICAN CHEMICAL SOCIETY. EASTON. the whole document	1-29
X	MICHAEL C. MYERS ET AL.: "A Cyclopentane Conformational Restraint for a Peptide Nucleic Acid: Design, Asymmetric Synthesis, and Improved Binding Affinity to DNA and RNA" ORGANIC LETTERS, vol. 5, 2003, pages 2695-2698, XP002466653 USAMEICAN CHEMICAL SOCIETY. EASTON page 2697, column 1, scheme 2, compound 5	1,2

-/--

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 January 2008	11/02/2008

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Zervas, Brigitte

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2007/020466

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JIE WU ET AL.: "Effective Ring-Opening Reaction of Aziridines with Trimethylsilyl Compounds: A Facile Access to beta-Amino Acids and 1,2-Diamine Derivatives" JOURNAL OF ORGANIC CHEMISTRY., vol. 65, 8 February 2000 (2000-02-08), pages 1344-1348, XP002466652 USAMERICAN CHEMICAL SOCIETY. EASTON.	4-6,8
Y	page 1345, scheme 1, compound 2b; page 1346, column 2, last paragraph - page 1347, column 1, line 20	3,7,9-23
X	POKORSKI JONATHAN K ET AL: "Cyclopentane-modified PNA improves the sensitivity of nanoparticle-based scanometric DNA detection." 28 April 2005 (2005-04-28), CHEMICAL COMMUNICATIONS (CAMBRIDGE, ENGLAND) 28 APR 2005, NR. 16, PAGE(S) 2101 - 2103 , XP002466654 ISSN: 1359-7345	24-29
Y	the whole document	3,7,9-23