Pergamon

Tetrahedron Letters 41 (2000) 441–445

TETRAHEDRON
LETTERS

Combinatorial synthesis using nucleic acid-based (NAB™) scaffold: parallel solid-phase synthesis of nucleotide libraries

Wenqiang Zhou, Arlène Roland, Yi Jin and Radhakrishnan P. Iyer *

Origenix Technologies Inc., 230 Bernard Belleau, Laval, Quebec H7V 4A9, Canada

Received 27 September 1999; revised 3 November 1999; accepted 4 November 1999

Abstract

The solid-phase synthesis of a NAB™ library is described.

Keywords: combinatorial chemistry; nucleic acid analogues; nucleotides; phosphoramidites.

Modern drug discovery approach entails the synthesis and screening of libraries of compounds.[1] The design and synthesis of such libraries is often based on a unique molecular skeleton or a scaffold. By incorporating a variety of structural elements into a scaffold, local as well as global molecular diversity can be achieved, which facilitate specific interactions between a ligand and its receptor. Stated simply, the structural elements contribute to molecular diversity by variable spatial displays of ionic, hydrogen-bonding, charge-transfer, and van der Waals interactions, thus allowing for the selection of the best 'fit' between the ligand and its receptor. In this regard, a NAB™ scaffold is unique in that one could incorporate into it the diversity elements that provide both 'sequence-specific' interactions (hydrogen-bonding interactions between nucleobases), as well as, 'shape-specific' motifs (e.g. bulges, and stem loop structures) that would allow specific recognition of other nucleic acids and proteins.[2] Thus, libraries based on NAB™ scaffold can potentially mimic the molecular recognition that exists between cellular macromolecules. Clearly, such diversity attributes cannot be readily incorporated into a typically small molecule library. Thus, a NAB™ library is potentially a unique source of *biologically relevant chemical diversity* for rapidly identifying 'hits' against novel targets.

As an application of this concept in our drug discovery program in antiinfective therapeutics, we have embarked upon the use of NAB™ small molecule combinatorial libraries. From the perspective of library synthesis, a number of advantages were anticipated with the assembly of libraries based upon a NAB™ scaffold: (a) a NAB™ scaffold could be rigid or non-rigid on which a variety of structural elements can be incorporated; (b) a number of building blocks can be used in the synthesis; (c) a library can be built using established solid-phase synthesis; and (d) compounds with defined structures can be synthesized

* Corresponding author. Tel: (450) 688-8588; fax: (450) 688-8018; e-mail: kiyer@origenix.com (R. P. Iyer)

0040-4039/00/$ - see front matter
PII: S0040-4039(99)02089-4

by parallel synthesis using efficient, rapid, and high yield reactions. Herein, we report a solid-phase synthesis approach to our library.

Fig. 1 shows the general structure of our NAB™ library. Our choice of *R* groups was dictated by the need to incorporate hydrophobic groups in the binding domain at the 5′-position.

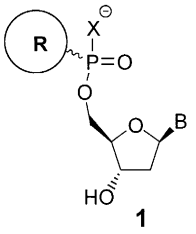
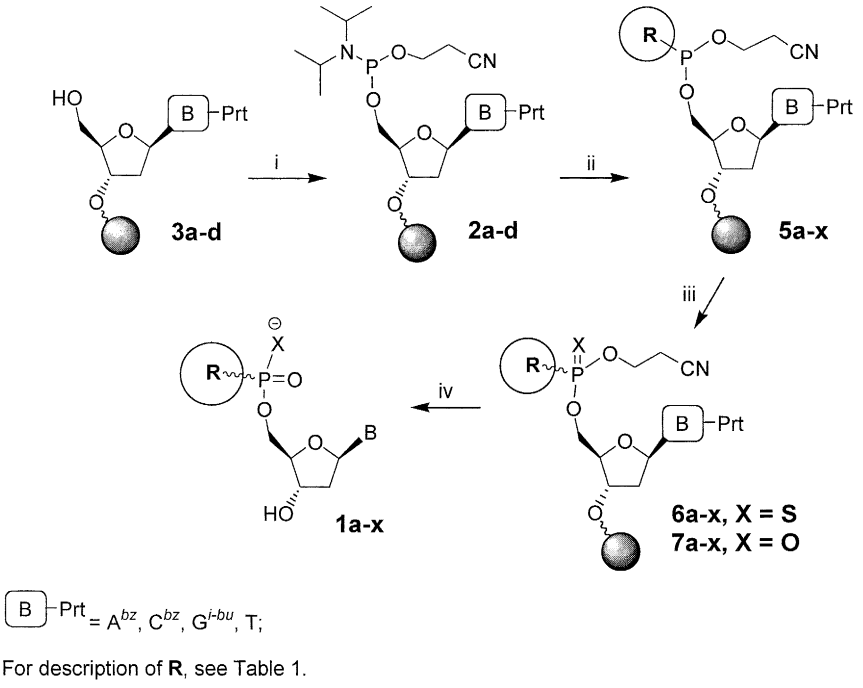


Fig. 1. General structure of a mixed nucleoside-nonnucleoside NAB™ library. X=O, S; R=hydrophobic group (see Table 1)

Our plan for library synthesis uses phosphoramidite chemistry,[3,4] and is shown in Scheme 1. The key synthon was the solid-support-bound phosphoramidite 2. We approached the preparation of 2 by 5′-phosphitylation of controlled-pore-glass (CPG)-bound nucleoside **3**. However, when a methylene chloride solution of β-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (CNDPC) and *N*,*N*-disopropylethylamine was contacted with **3**, the byproduct *N*,*N*-diisopropylethylamine hydrochloride precipitated on the CPG matrix. It was not possible to separate **2** free of the byproduct hydrochloride salt.



Scheme 1. (i) Bis-(*N*,*N*-diisopropylamino)-2-cyanoethylphosphine, diisopropylammonium tetrazolide in CH_2Cl_2; (ii) ROH (**4a**–**f**), 1*H*-tetrazole in CH_3CN; (iii) 3*H*-1,2-benzodithiole-3-one-1,1-dioxide in CH_3CN (**6a**–**x**), or I_2 in Pyr/H_2O/THF (**7a**–**x**); (iv) 28% NH_4OH

To circumvent this problem, β-cyanoethyl bis-(*N*,*N*-diisopropylamino)phosphine (CNP)[5] in the presence of *N*,*N*-diisopropylammonium tetrazolide was used as the phosphitylating reagent. In this case, the byproducts were soluble in methylene chloride. Thus, following the filtration of the reaction mixture, a

quantitative yield of **2** (see experimental for evaluation of **2**) was obtained. Each CPG-bound nucleoside phosphoramidite **2** was stored at −20°C for subsequent use in library synthesis.

Typically for the synthesis of the library (Scheme 1, Fig. 2), each CPG-bound nucleoside phosphoramidite 2 was transferred to a series of spin columns. The alcohols **4a**–**f** were added to the spin columns along with tetrazole in acetonitrile. After 5 min, centrifugation allowed the separation of the unreacted materials and byproducts to give the support-bound coupled product **5a**–**x** as a mixture of R_p-, and S_p-diastereomers. The oxidative sulfurization[6] or, alternatively, the oxidation of **5a**–**x**, was performed using either 3*H*-1,2-benzodithiole-3-one-1,1-dioxide (0.1 M in acetonitrile) or I_2 solution (0.02 M in pyridine/H_2O/tetrahydrofuran), to give the support-bound phosphotriesters **6a**–**x**, **7a**–**x**, respectively. The support-bound library was heated with NH_4OH (28%, 55°C, 5 h) to liberate the crude diesters. Each crude product was passed through a Sep-Pak cartridge® (Waters) to give **1a**–**x**, that was 90 to 95% pure as determined by reversed-phase HPLC. Table 1 shows a representative 24-member library. The isolated yields of each member in the library were in the range of 50–70% as determined by the A_{260} measurements. Representative members (including those that carry guanosine nucleobase) were additionally characterized by ^{31}P NMR and MS to ascertain their structural integrity.

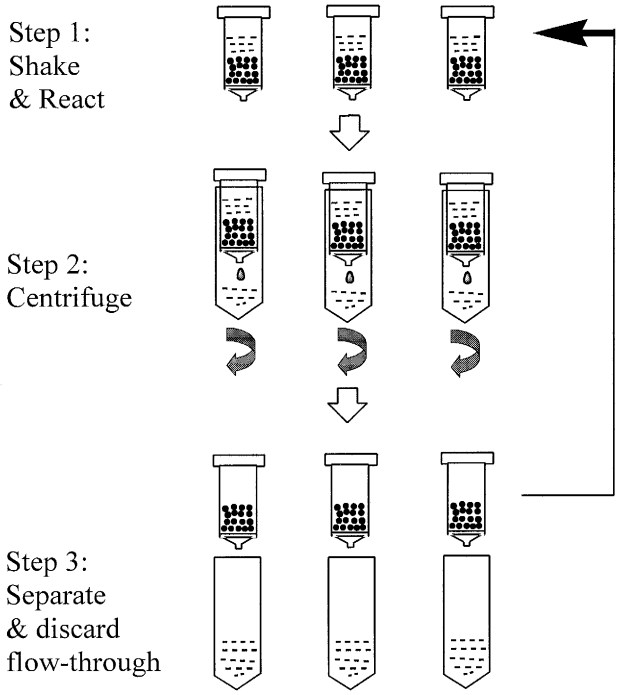
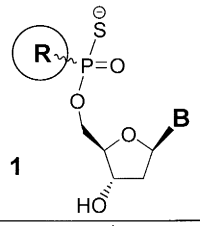


Fig. 2. Flow diagram for library assembly. The library synthesis was performed in a parallel format using QIAquick® spin columns (Qiagen) each fitted with a filter at the bottom. The reactants were placed in the spin columns and contents shaken (Step 1). Following the reaction, each column was placed in a receptacle vial and centrifuged (Step 2). This allowed facile filtration of the mixture into the receptacle. The spin column was separated (Step 3), the next reagent introduced, and the process continued until the completion of the library synthesis

In summary, we have developed a practical synthetic protocol for nucleotide libraries carrying diversity elements at multiple sites on a nucleoside framework. In a complementary approach, we have also developed a solution-phase approach to the synthesis of these libraries. The evaluation of the library against certain molecular targets is under way.

Typical experimental procedures. (a) Preparation of the CPG-bound phosphoramidite **2a**–**d**: Each

Table 1



R ⇨												
B	Pdt	R_t	Pdt	R_t	Pdt	R_t	Pdt	R_t	Pdt	R_t	Pdt	R_t
A	**1a**	30.5	**1e**	34.3	**1i**	47.8	**1m**	45.0	**1q**	36.0	**1u**	43.3
C	**1b**	28.6	**1f**	33.3	**1j**	47.5	**1n**	44.1	**1r**	35.1	**1v**	42.7
G	**1c**	28.1	**1g**	31.7	**1k**	46.5	**1o**	42.9	**1s**	33.6	**1w**	41.0
T	**1d**	29.7	**1h**	37.8	**1l**	48.2	**1p**	47.4	**1t**	38.2	**1x**	46.5

Representative library members (**1a**–**x**), and their HPLC retention time (R_t, min). Pdt=product, **B**= nucleobase, **A**=adenine, **C**=cytosine, **G**=guanine, **T**=thymine

CPG-bound nucleoside **3a**–**d** (0.228 mmol), along with CNP (0.36 ml, 1.14 mmol), bisdiisopropylammonium tetrazolide (855 mg, 5 mmol) in CH_2Cl_2 (50 mL) was placed in a round-bottomed flask and shaken at rt overnight in an orbital shaker. The CPG-bound phosphoramidite **2a**–**d** was collected by filtration and washed sequentially with CH_2Cl_2 (200 mL), CH_3CN (100 mL), and anhydrous ether (50 mL), and then dried in vacuo. The quality of **2a**–**d** was independently evaluated during solid-phase synthesis of representative dinucleotides by trityl assay and reversed-phase HPLC of the dinucleotides following deprotection.

(b) Synthesis of a 24-member library **1a**–**x**: (see also Fig. 2, Table 1) The CPG-bound phosphoramidites **2a**–**d** (5 mmol) were placed in spin columns under a blanket of argon. Then a solution of tetrazole (0.5 mL, 0.45 M in CH_3CN), followed by the appropriate alcohols **4a**–**f** (25 mmol) was added. The mixture was shaken for 5 min and the spin column mounted on receptacle vials and centrifuged. The CPG was washed with CH_3CN (2×0.5 mL) and then soaked in a solution of 3*H*-1,2-benzodithiole-3-one-1,1-dioxide in CH_3CN (0.1 M, 0.5 mL). The mixture was shaken at rt for 5 min. The solvent was removed and the CPG washed with acetonitrile (2×0.5 mL) and dried under argon. The CPG was transferred to a conical screwcap tube (1.5 mL, VWR) and ammonium hydroxide (28%, 1.5 mL) added. The mixture was heated at 55°C for 4 h. The suspension was cooled and centrifuged. The supernatant containing the desired products was collected and evaporated in a Speed Vac to give the crude diesters **1a**–**x**. The materials were dissolved in water and passed through a Sep-Pak Cartridge to give products with a purity of 90 to 95% as ascertained by reversed-phase HPLC (HPLC was performed on a Waters 600 system equipped with a photodiode-array UV detector 996, autosampler 717, and Millennium® 2000 software, using a Radial-Pak® liquid chromatography cartridge [8 mm I.D., 8NVC18]. Mobile phase: Buffer A: 0.1 M NH_4OAc; Buffer B: 20% A/80% CH_3CN, v/v: Gradient: 100% A, 0–3 min; 40% A, 40 min; 100% B, 49 min; 100% B).

Typical Spectral Data. Compound **1c**: ^{1}H NMR (D_2O): δ 8.04 (d, 1H, *J*=5.4 Hz), 6.24–6.28 (m, 1H), 4.19 (m, 1H), 3.96–4.05 (m, 2H), 3.63–3.68 (m, 2H), 2.78–2.85 (m, 1H), 2.47–2.52 (m, 1H), 1.34–1.41 (m, 2H), 1.07–1.13 (m, 2H), 0.71 (t, 3H, *J*=7.5 Hz) ppm; ^{31}P NMR (D_2O): δ 58.91, 58.61 ppm; MS (negative mode): calcd. for $C_{14}H_{21}N_5O_6PS$: 418 (M); found: *m/z*, 418. Compound **1g**: ^{1}H NMR (D_2O):

δ 8.10 (d, 1H, *J*=7.3 Hz), 6.24–6.27 (m, 1H), 4.19 (s, 1H), 3.97–4.04 (m, 2H), 3.38–3.52 (m, 2H), 2.79–2.86 (m, 1H), 2.48–2.53 (m, 1H), 1.89–1.94 (m, 1H), 1.45–1.50 (m, 2H), 1.34 (m, 4H), 0.87–1.00 (m, 2H) ppm; ^{31}P NMR (D_2O): δ 58.64, 58.23 ppm.

Acknowledgements

We thank Jennifer Abbott for expert assistance.

References

1. For selected publications on the combinatorial approach to drug discovery, see: (a) Geysen, H.; Meloen, R. H.; Barteling, S. J. *Proc. Natl. Acad. Sci. USA*, **1984**, *81*, 3998–4002; (b) Jung, G.; Beck-Sickinger, A. G. *Angew. Chem., Intl. Ed. Engl.* **1992**, *31*, 367–383; (c) Pavia, M. R.; Sawyer, T. K.; Moos, W. H. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 387–396; (d) Gordon, E. M.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gallop, M. A. *J. Med. Chem.* **1994**, *37*, 1385–1401; (e) Thompson, L. A.; Ellman, J. A. *Chem. Rev.* **1996**, *96*, 555–600.
2. For a recent review of nucleic acid-based compounds and their applications, see: Verma, S.; Eckstein, F. *Annu. Rev. Biochem.* **1998**, *67*, 99–134.
3. Beaucage, S. L.; Caruthers, M. H. *Tetrahedron Lett.* **1981**, *22*, 1859–1862.
4. For a review of phosphoramidite chemistry, see: Iyer, R. P., Beaucage, S. L. In *Comprehensive Natural Products Chemistry Vol. 7: DNA and Aspects of Molecular Biology*; Kool, E. T., Ed. Oligonucleotide synthesis. Elsevier Science: London, 1999; pp. 105–152.
5. Barone, A. D.; Tang, J.-Y.; Caruthers, M. H. *Nucl. Acids Res.* **1984**, *12*, 4051–4061.
6. Iyer, R. P.; Egan, W.; Regan, J. B.; Beaucage, S. L. *J. Am. Chem. Soc.* **1990**, *112*, 1253–1254.