

Synthesis of a Neamine Dimer Targeting the Dimerization Initiation Site of HIV-1 RNA

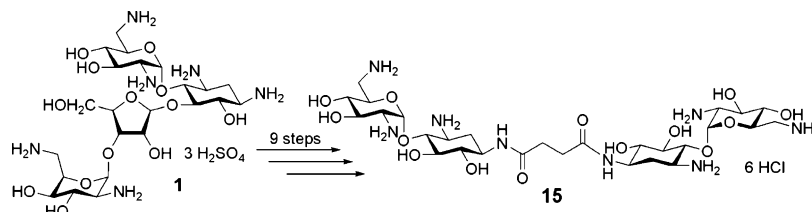
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ABSTRACT



A neamine dimer designed to bind to a specific sequence of HIV-1 RNA has been synthesized. Starting from neomycin B (1), a five-step synthesis efficiently provided a key protected neamine monomer 6 (28%). From the latter, coupling reactions with activated diacids gave dimers. After deprotection, a neamine dimer was obtained as the hexachlorohydrate salt 15 with 13% overall yield over nine steps.

Infectious viral particles of human immunodeficiency virus (HIV) contain two positive RNA strands linked together near their 5' end.¹ This organization is initiated by interactions between a conserved six nucleotide self-complementary sequence located in the dimerization initiation site (DIS), a stem-loop promoting formation of a loop–loop “kissing complex” (Figure 1a).² Since the DIS is essential for efficient viral replication at the RNA packaging and reverse transcrip-

tion stages,³ the dimerization step represents a new target for the development of future anti-HIV drugs.

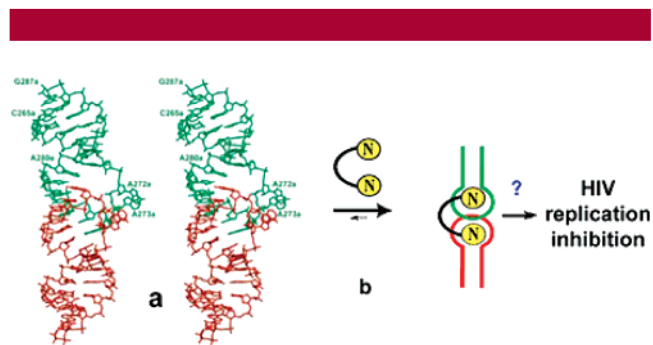


Figure 1. (a) Stereoview of the X-ray structure of the “kissing complex” of HIV-1, made of two HIV RNA dimerization initiation sites (DIS). (b) Concept of interfering with the RNA dimerization step (N: neamine; the two DIS are in green and red).

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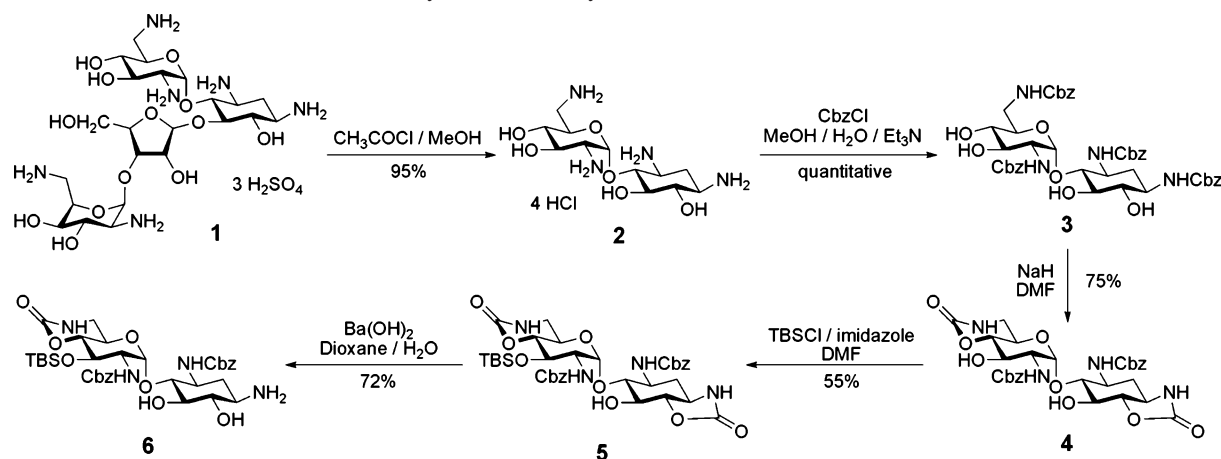
[‡] Architecture et réactivité de l'ARN, Université Louis Pasteur.

(1) Paillart, J.-C.; Shehu-Xhilaga, M.; Marquet, R.; Mak, J. *Nat. Rev. Microbiol.* **2004**, *2*, 461–472.

(2) (a) Skripkin, E.; Paillart, J.-C.; Marquet, R.; Ehresmann, B.; Ehresmann, C. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 4945–4949. (b) Laughrea, M.; Jette, L. *Biochemistry* **1994**, *33*, 13464–13474.

(3) (a) Paillart, J.-C.; Berthou, L.; Ottmann, M.; Darlix, J.-L.; Marquet, R.; Ehresmann, B.; Ehresmann, C. *J. Virol.* **1996**, *70*, 8348–8354. (b) Berkhout, B.; Van Wamel, J. L. B. *J. Virol.* **1996**, *70*, 6723–6732. (c) Clever, J. L.; Parslow, T. G. *J. Virol.* **1997**, *71*, 3407–3414. (d) Laughrea, M. *J. Virol.* **1997**, *71*, 3397–3406.

Scheme 1. Synthesis of a Key Monomer Derived from Neamine **6**



The DIS “kissing complex” crystal structure was solved, revealing a coaxial interaction of two stem-loops, two purines bulging out and stacking on one another, leaving a cavity in each stem-loop (Figure 1a).⁴ This feature led us to imagine blocking the DIS dimer with dimeric molecules able to simultaneously bind to each RNA strands. Over-stabilization of the DIS “kissing complex” could interfere with packaging and reverse transcription of the genomic RNA, thus interfering with viral replication (Figure 1b). Drug design as well as biological and crystallographic studies^{5,6} revealed that neamine tethered at N1 by a 4–6 Å chain would fit into the DIS “kissing complex” and thus interfere with it (Figure 2).

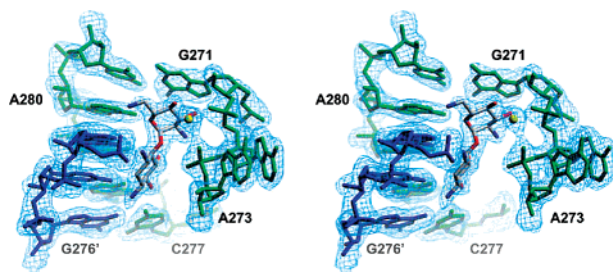


Figure 2. Stereoview of the X-ray structure of the neamine–DIS complex. The two DIS RNA strands are represented in green and blue, and the yellow sphere is a potassium cation. As expected, neamine occupies the RNA stem-loop cavity of the “kissing complex”, and interactions are mostly localized around the glucosamine moiety, with amine N1 pointing away.

On this basis, we designed, synthesized, and studied dimeric neamine derivatives, and herein, we report the first synthesis

of an N1 amide-linked neamine dimer which is capable of interacting with HIV-1 RNA. To maintain and even extend the interactions due to the N1 amino group, amide linkages 4–6 Å long were chosen to connect two neamine units at amine N1.

Surprisingly, only a few dimeric aminoglycosides have been described, but none of them are linked via N1.⁷ Moreover, approaches aiming at distinguishing the N1 amine from the other neamine functions are scarce.⁸ As they proved unsatisfactory in our hands, we relied on selective formation⁹ of cyclic carbamates and on their selective opening⁹ expecting that a strained carbamate opens up more readily than an unstrained one (Scheme 1).

The synthesis of the key protected neamine monomer **6** started with methanolysis of commercially available neomycin B trisulfate **1** to give neamine **2** as the tetrahydrochloride salt.¹⁰ The four amine groups of neamine were easily protected as benzyloxycarbamates using slightly modified conditions,¹¹ triethylamine being used here as base for purification reasons. A basic treatment of **3** with NaH under carefully controlled conditions allowed us to convert two of the four benzyloxycarbamates groups into five- and six-membered cyclic carbamates, producing compound **4** in good yield. The position of these carbamates were clearly established by HMBC correlations. It is worth noting that this

(7) (a) Michael, K.; Wang, H.; Tor, Y. *Bioorg. Med. Chem.* **1999**, *7*, 1361–1371. (b) Agnelli, F.; Sucheck, S. J.; Marby, K. A.; Rabuka, D.; Yao, S.-L.; Sears, P. S.; Liang, F.-S.; Wong, C.-H. *Angew. Chem., Int. Ed.* **2004**, *43*, 1562–1566. (c) Liu, X.; Thomas, J. R.; Hergenrother, P. J. *J. Am. Chem. Soc.* **2004**, *126*, 9196–9197.

(8) (a) Umezawa, S.; Ikeda, D.; Tsuchiya, T. *J. Antibiot.* **1973**, *26*, 304–306. (b) Haddad, J.; Kotra, L. P.; Liano-Sotelo, B.; Kim, C.; Azucena, E. F.; Meizheng, J. L.; Vakulenko, S. B.; Chow, C. S.; Mobashery, S. *J. Am. Chem. Soc.* **2002**, *124*, 3229–3237. (c) Li, J.; Chen, H.-N.; Chang, H.; Wang, J.; Chang, C. W. T. *Org. Lett.* **2005**, *7*, 905–910.

(9) For a review on the chemistry of carbamates, see: Agami, C.; Couty, F. *Tetrahedron* **2002**, *58*, 2701–2724.

(10) (a) Byron, E.; Leach, E.; Teeters, C. M. *J. Am. Chem. Soc.* **1951**, *73*, 2794–2797. (b) Dutcher, J.; Donin, M. *J. Am. Chem. Soc.* **1952**, *74*, 3420–3422. (c) Grapsas, I.; Cho, Y. J.; Mobashery, S. *J. Am. Chem. Soc.* **1994**, *59*, 1918–1922.

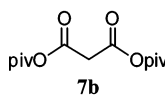
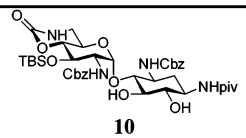
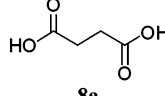
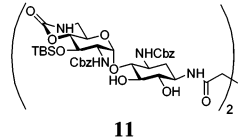
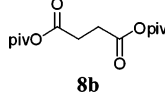
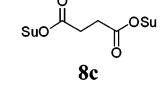
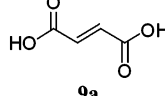
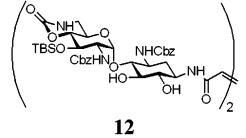
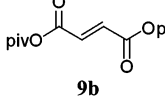
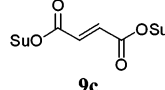
(11) Park, W. K. C.; Auer, M.; Jaksche, H.; Wong, C.-H. *J. Am. Chem. Soc.* **1996**, *118*, 10150–10155.

(4) (a) Ennifar, E.; Dumas, P. *J. Mol. Biol.* **2006**, *356*, 771–782. (b) Ennifar, E.; Walter, P.; Ehresmann, B.; Ehresmann, C.; Dumas, P. *Nat. Struct. Biol.* **2001**, *8*, 1064–1068.

(5) Ennifar, E.; Paillart, J.-C.; Marquet, R.; Ehresmann, B.; Ehresmann, C.; Dumas, P.; Walter, P. *J. Biol. Chem.* **2003**, *278*, 2723–2730.

(6) Ennifar, E.; Paillart, J.-C.; Bodlenner, A.; Walter, P.; Weibel, J.-M.; Aubertin, A.-M.; Pale, P.; Dumas, P.; Marquet, R. *Nucleic Acids Res.* **2006**, *34*, 2328–2339.

Table 1. Dimerization of the Key Monomer

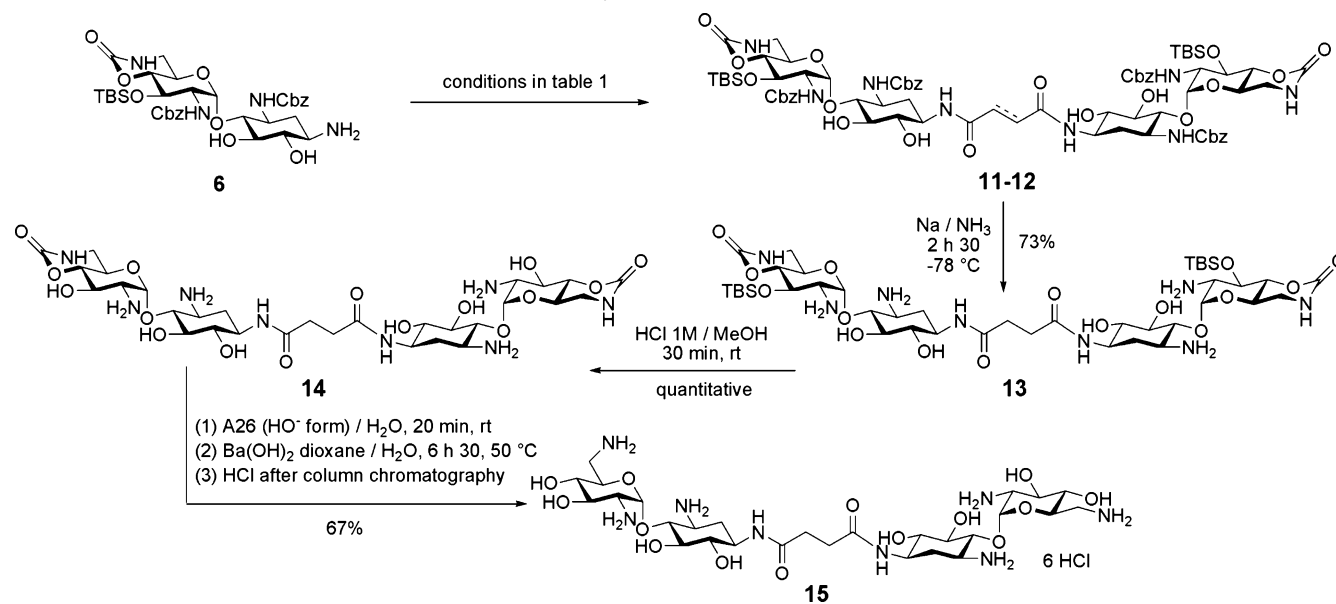
	reagent	cond ⁿ	product	yield (%) ^a
1	 7b	DIPEA CH ₂ Cl ₂ rt	 10	56
2	 8a	DIPC HOBT CH ₂ Cl ₂ rt	 11	71
3	 8b	DIPEA CH ₂ Cl ₂ rt	10	92
4	 8c	CH ₃ CN	11	70
5	 9a	DIPC HOBT CH ₂ Cl ₂ rt	 12	45
6	 9b	DIPEA CH ₂ Cl ₂ rt	12	68
7	 9c	DMF	12	92

^a Yield of chromatographically pure products; condⁿ, conditions; piv, pivaloyl; Su, *N*-succinimidyl; DIPEA, *N,N*-diisopropylethylamine; HOBT, 1-hydroxybenzotriazole; DIPC, diisopropylcarbodiimide.

sequence provided **4** in excellent overall yield and without chromatography, improving other routes to this compound.¹²

Compound **4**, having two amines (N1, N6') distinguished from the two others (N3, N2') as cyclic carbamates, was protected with a TBS group for solubility reasons. As expected from the known reactivity of the 5-hydroxyl group,¹¹ only the silylation at the 3' position was observed in the conditions used (see the Supporting Information). The trans-fused five-membered carbamate of the resulting **5** was selectively opened by treatment with barium hydroxide,¹² leading to **6** protected except at amine N1 and alcohols O5 and O6. The position of the silylation as well as the regioselectivity of the carbamate opening were again established by HMBC correlations. The remaining free hydroxyl groups could also be selectively protected, but the higher nucleophilicity of the amino group led us to directly attempt to introduce the required linker through amide bond formation.

Diacids, three to four carbons long, were used for the dimerization step, either directly in peptide coupling or as *N*-hydroxysuccinimide esters¹³ or pivaloyl anhydrides,¹⁴ known to be selective for amines in the presence of alcohols (Table 1). Dimerization with malonic acid **7a** or its bis-(pivaloyl) anhydride **7b** or bis(succinimidyl)diester **7c** did not yield the expected product. Decomposition often occurred, except with **7b**, which gave the *N*-pivaloyl product **10** (entry 1). In contrast, reactions between **6** and succinic acid **8a** or its activated diester **8c** selectively provide the expected dimer **11** in good yields (entries 2 and 4). The anhydride **8b** also gave the *N*-pivaloyl product **10** (entry 3). Fumaric acid **9a** was also investigated, since its *E* double bond induces a shorter chain length and should avoid intramolecular side reactions. Peptide coupling and the use of the anhydride **9b** gave almost the same good results (entries 5 and 6). Rewardingly, the diester **9c** provided the dimer **12** with an excellent yield (entry 7).

Scheme 2. Synthesis of Neamine Dimer **15**

Deprotection of the carbobenzyloxy groups (Cbz) in **11** or **12** could not be achieved under conventional hydrolysis conditions. Extensive investigations revealed that treatment with sodium in liquid ammonia led to deprotection of the four Cbz groups and also to hydrogenation of the double bond in **12** (Scheme 2). Desilylation also proved cumbersome and only acidic treatment with hydrogen chloride in methanol provided an efficient and clean reaction, cleaving both silyl groups in quantitative yield. The two remaining 6-membered cyclic carbamates were removed by treatment with barium hydroxide in carefully controlled conditions, leading to the hexamine, which was then treated with 1 M aqueous hydrochloric acid in order to recover good water solubility for biological tests and reliable NMR analysis. The corresponding hexahydrochloride salt **15** was obtained with an overall yield of 13% over nine steps from neomycin B.

Biochemical studies relying on a selective Pb^{2+} induced cleavage¹⁵ of the DIS loop showed that **15** binds to DIS, as anticipated. It indeed protected DIS from Pb^{2+} cleavage (Figure 3a). Dimer **15** also stabilizes the kissing complex at 37 °C in the context of 615 nucleotide RNA fragments (Figure 3b). Competition experiments between long and short RNA DIS fragments (615 and 311 nucleotides, respectively), performed in the presence or absence of either neamine or **15**, revealed that **15** is more efficient in preventing heterodimer formation (RNA615/RNA311) than neamine, indicating homodimer overstabilization, as expected.

In conclusion, we developed an efficient route toward a new class of dimeric compounds, designed to interfere with the HIV DIS function, and showed that a representative dimer interacts with DIS. Work is in progress to evaluate the potential antiviral activity of this new family of compounds.

(12) (a) Horton, D.; Weckerle, W. *Carbohydr. Res.* **1975**, *44*, 227–240. (b) Grewe, R.; Herberg, H. W. *Chem. Ber.* **1959**, *92*, 1195–1205.

(13) (a) Kawaguchi, H.; Naito, T.; Nakagawa, S.; Fujisawa, K.-I. *J. Antibiot.* **1972**, *25*, 695–708. (b) Katsarava, R. D.; Kharadze, D. P.; Avalishvili, L. M. *Makromol. Chem.* **1986**, *187*, 2053–2062. (c) Tanaka, H.; Nishida, Y.; Furuta, Y.; Kobayashi, K. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1723–1726.

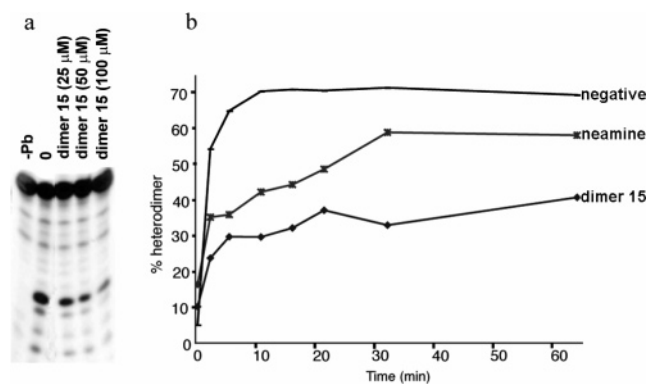


Figure 3. (a) Denaturing gel showing inhibition of Pb^{2+} -induced DIS cleavage by dimer **15**. Control experiments without Pb^{2+} or without dimer are indicated as -Pb and 0 respectively. (b) Stabilization of the kissing complex by **15** and neamine. Radiolabeled RNA 1–615 was incubated in dimerization buffer, and a 5-fold excess of unlabeled RNA1–311 was added to the solution in absence (“negative” curve) or in presence of dimer **15** or neamine.

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Supporting Information Available: General procedure for the preparation of unknown compounds, ^1H and ^{13}C NMR spectra of all compounds, and procedure for competition experiments are provided. This material is available free of charge via the Internet at <http://pubs.acs.org>.

OL701760K

(14) (a) Kocian, O.; Stransky, K.; Zavada, J. *Collect. Czech. Chem. Commun.* **1982**, *47*, 1356–1366. (b) Myers, A. G.; Gleason, J. L.; Yoon, T.; Kung, D. W. *J. Am. Chem. Soc.* **1997**, *119*, 656–673.

(15) Paillart, J.-C.; Westhof, E.; Ehresmann, C.; Ehresmann, B.; Marquet, R. *J. Mol. Biol.* **1997**, *270*, 36–49.