nature chemistry

ARTICLES

PUBLISHED ONLINE: 15 JULY 2012 | DOI: 10.1038/NCHEM.1395

Designed, synthetically accessible bryostatin analogues potently induce activation of latent HIV reservoirs *in vitro*

Brian A. DeChristopher[1†], Brian A. Loy[1†], Matthew D. Marsden[2†], Adam J. Schrier[1†], Jerome A. Zack[2*] and Paul A. Wender[1*]

Bryostatin is a unique lead in the development of potentially transformative therapies for cancer, Alzheimer's disease and the eradication of HIV/AIDS. However, the clinical use of bryostatin has been hampered by its limited supply, difficulties in accessing clinically relevant derivatives, and side effects. Here, we address these problems through the step-economical syntheses of seven members of a new family of designed bryostatin analogues using a highly convergent Prins-macrocyclization strategy. We also demonstrate for the first time that such analogues effectively induce latent HIV activation *in vitro* with potencies similar to or better than bryostatin. Significantly, these analogues are up to 1,000-fold more potent in inducing latent HIV expression than prostratin, the current clinical candidate for latent virus induction. This study provides the first demonstration that designed, synthetically accessible bryostatin analogues could serve as superior candidates for the eradication of HIV/AIDS through induction of latent viral reservoirs in conjunction with current antiretroviral therapy.

HIV/AIDS is a global pandemic[1]. The Joint United Nations Programme on HIV/AIDS has estimated that the number of people living with HIV, the virus that causes AIDS, totalled 33.3 million in 2009 (see http://unaids.org/globalreport/Global_report.htm). In the same year, there were 1.8 million AIDS-related deaths.

The leading treatment for HIV is highly active antiretroviral therapy (HAART), a combination of drugs that halts viral proliferation by inhibiting several stages of the viral life cycle. For many patients, this treatment strategy has transformed HIV into a manageable, chronic disease by reducing their plasma viral loads, often to undetectable levels. However, HAART is not curative, as it does not address genomically integrated latent viral reservoirs that slowly resupply replication-competent active virus. As such, discontinuation of HAART results in viral rebound and disease progression[2]. In addition, HAART is costly, has associated side effects[3], and requires strict adherence to treatment regimens[4] to avoid the emergence of viral resistance[5].

The most formidable obstacle to the eradication of HIV is the persistence of various latent proviral reservoirs[6–8]. These are believed to be primarily established following integration of the HIV genome into that of activated $CD4^+$ T cells and other cell types. In rare cases, these infected cells transition to quiescent memory cells in a process that reversibly inhibits expression of the integrated HIV provirus, rendering it unsusceptible to HAART[9]. As a consequence, eradication of HIV in HAART-suppressed patients would require elimination or inactivation of these proviral reservoirs.

As HAART targets only actively replicating virus, it has little influence on latent viral reservoirs. It is estimated that decades of HAART treatment would be required for depletion of the reservoir source[10]. Therefore, agents that can controllably facilitate purging of the latent virus from these reservoirs could reduce the time for depletion and provide a strategy to eradicate HIV when used in combination with HAART. In this approach, which is now being actively pursued[11], the deliberate induction of viral replication from its latent state is proposed to eliminate HIV-harbouring cells either by direct viral cytopathic effects or by rendering those cells susceptible to immune system regulation. Concomitant HAART would prevent infection of healthy cells from the released virions. This combined approach would thus eliminate both the latent and active viral pools.

One experimental strategy for latent viral reactivation is direct immunological modulation of resting memory T cells. However, administration of cytokines and/or antibodies has thus far lacked clinical efficacy[12,13]. Another approach makes use of the pharmacological modulation of the signalling pathways associated with viral reactivation. To this end, protein kinase C (PKC) has emerged as an important target. Prostratin (Fig. 1), a non-tumour-promoting 12-deoxy phorbol ester that binds to and activates PKC, has demonstrated promising *in vitro* and *ex vivo* activities in reactivating latent HIV[14,15] and is currently being advanced as a clinical candidate for latent viral reservoir clearance[16]. A source of synthetic prostratin has recently been reported that also provides access to even more effective analogues[17].

Like prostratin, bryostatin 1 also targets PKC, but it is significantly more potent ($K_i = 1.35$ nM versus 50 nM) and thus represents a unique lead for HIV reservoir clearance. Bryostatin 1, the lead member of the bryostatin family, was reported in 1982 by Pettit and Clardy[18], and has subsequently been shown to demonstrate promising *in vitro*, *in vivo* and human clinical[19] activities (see http://clinicaltrials.gov) for a diverse array of indications[20] including cancer and Alzheimer's disease[21]. Early reports also suggested that bryostatin might induce HIV reservoir clearance[22,23], and this has been further supported in more recent studies[24]. An

[1]Departments of Chemistry and of Chemical and Systems Biology, Stanford University, Stanford, California, 94305, USA, [2]Department of Medicine, Division of Hematology and Oncology, University of California Los Angeles, Los Angeles, California, 90095, USA; †These authors contributed equally to this work. *e-mail: wenderp@stanford.edu; jzack@ucla.edu

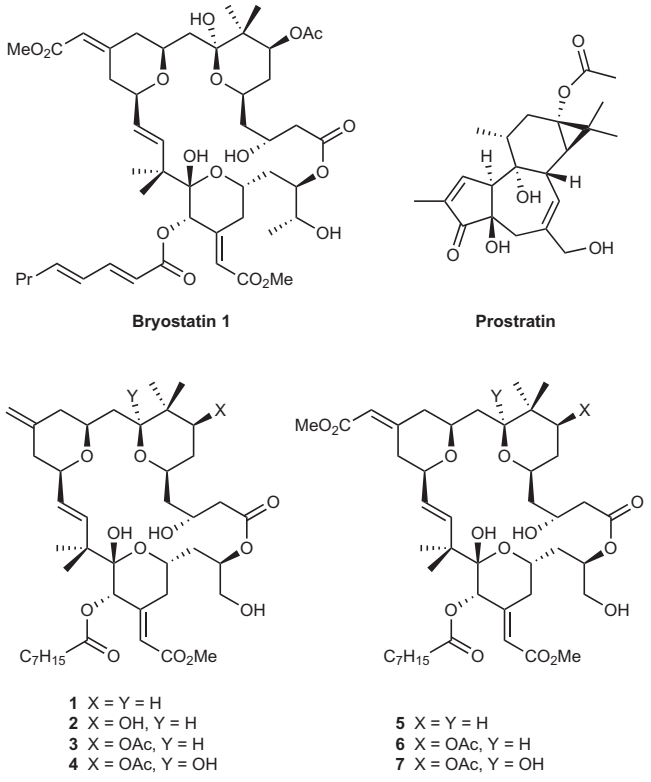

Figure 1 | Bryostatin, prostratin and synthetic analogues.

attractive aspect of bryostatin is that it has already been tested in human trials, albeit for other cancer-related indications. However, further underscoring the need for a reliable supply of a more effective agent, patient accrual in a recent bryostatin clinical trial was terminated by the National Cancer Institute and the clinical investigators 'given the more potent bryostatin analogs in development', a focus of this current study[25].

Unlike prostratin and its analogues, for which a practical five-step synthesis has been reported[17], bryostatin has not been reliably available in the quantities needed for research and sustained clinical studies. Isolated yields from natural sources are low (10^{-3} to 10^{-8}%) and variable; the good manufacturing practices (GMP) production required 14 tons of the marine bryozoan *Bugula neritina* to provide just 18 g of bryostatin 1 (ref. 26). Although this supply was sufficient to initiate preclinical and clinical research on bryostatin, economic and environmental factors have severely limited further development of this source and related aquaculture production. Engineered biosynthesis, while promising, is still in development and has yet to have an impact on clinical supply[27]. Impressive progress has also recently been made in the realm of bryostatin total synthesis[28–34], but approaches to the potent bryostatins require, at best, a total of ~40 steps. Importantly, clinical studies have also revealed off-target toxicities associated with bryostatin. In principle, issues of both supply and undesirable effects could be overcome by the design of simplified, clinically superior, functional analogues that can be produced in a more step-economical fashion.

To address these supply and performance issues, our group proposed in 1988 that the activities of bryostatin could arise from only a subset of its functionality[35]. As such, bryostatin-like activity could be achieved with simplified and thus more synthetically accessible designed analogues dubbed 'bryologs', which incorporate the key activity-determining functionalities[36,37]. Using this function-oriented synthesis[38] approach, we reported in 1998 the first simplified bryostatin analogues[39] and, more recently, similar bryologs that are even more potent than the natural product[40–42]. Significantly, these analogues, several of which were prepared with less than 30 steps, are currently the most synthetically accessible agents with bryostatin-like activity, PKC translocation selectivity[43] and potency. Moreover, a related bryostatin analogue has been shown to be well tolerated and efficacious in an *in vivo* mouse cancer model[44]. To date, there has been no reported study of simplified bryostatin analogues for the activation of latent HIV reservoirs as required for disease eradication. Here, we report the convergent syntheses of seven members (**1**–**7**) of a new family of bryostatin analogues incorporating a tetrahydropyranyl B-ring formed through a versatile Prins macrocyclization. We also disclose for the first time that such designed, synthetically accessible analogues can induce activation of latent HIV expression in a cellular latency model with potencies similar to or better than bryostatin 1. We also demonstrate that these simplified bryostatin analogues share the functional activity of the clinical candidate prostratin in this assay, but are up to four orders of magnitude more potent than prostratin. These findings establish that bryologs, in addition to their therapeutic potential for cancer and Alzheimer's disease[45], could serve as synthetically accessible and potentially superior clinical candidates as adjuvants with HAART for the eradication of HIV infection.

Results

The assembly of the new bryostatin analogues **1**–**7** was proposed to entail a highly convergent strategy in which a spacer domain (Fig. 2: **8**, **9** or **10**) would be conjoined with a recognition domain (**11**) through a two-step esterification/Prins-driven macrocyclization[46] sequence. This process would form a readily diversifiable B-ring pyran and set C15 stereochemistry to produce the key intermediates **20**–**22**. Support for this strategy comes from our earlier work on the syntheses of simplified bryostatin analogues[41] as well as bryostatin 9 (ref. 33). As the name implies, the recognition domain is proposed to directly contact the PKC, thereby influencing analogue–PKC affinity, while the spacer domain is proposed to control bryolog conformation and to influence translocation of the bryolog/PKC complex and its insertion into the cellular membrane. The preparation of analogues **1**–**7** was proposed to start from a common A-ring intermediate **12**, which was prepared and elaborated as described previously[42] into hydroxyesters **13** and **14**. Hydroxyester **13** would be used to synthesize the C7-deoxy analogues **1** and **5**, while hydroxyester **14** would be used to prepare the C7-oxy analogues **2**, **3** and **6**. Analogues **4** and **7**, which have bryostatin 1-like A-ring functionalization, would be prepared from spacer domain **10** (ref. 33).

Our studies started with previously prepared hydroxyesters **13** and **14** (ref. 42), which were converted into allylsilanes **15** and **16**, respectively, in three steps involving C11 silylation, $CeCl_3$·2LiCl-mediated[47] double nucleophilic addition of $TMSCH_2MgCl$, and Peterson olefination of the carbinol product. Knochel's $CeCl_3$·2LiCl salt provided optimal yields for the nucleophilic addition step[33]. Intermediates **15** and **16** were then separately debenzylated with lithium naphthalenide (84–91% yield), and the resultant C1 alcohols oxidized with TPAP/NMO followed by $NaClO_2$ to provide spacer domains **8** and **9** (91–97% yield over two steps).

Spacer domains **8**, **9** and **10** were then each esterified with recognition domain **11** using Yamaguchi's esterification to provide the Prins macrocyclization precursors **17**, **18** and **19**, respectively. Significantly, we found that C11 deprotection and Prins-driven macrocyclization of **17**, **18** and **19** can be promoted in one operation and in excellent yields (up to 90%) using mild conditions (catalytic PPTS in alcohol solvent), providing macrocycles **20**, **21** and **22**, respectively. Importantly, only a single diastereomer is obtained in each case. The Prins-driven macrocyclizations of **17** and **18** proceeded rapidly with PPTS/EtOH without C26 desilylation. Macrocyclization of **19** with PPTS/MeOH occurred less rapidly giving macrocycle **22** and some C26-desilylated product; this crude mixture of macrocycles was either further deprotected to

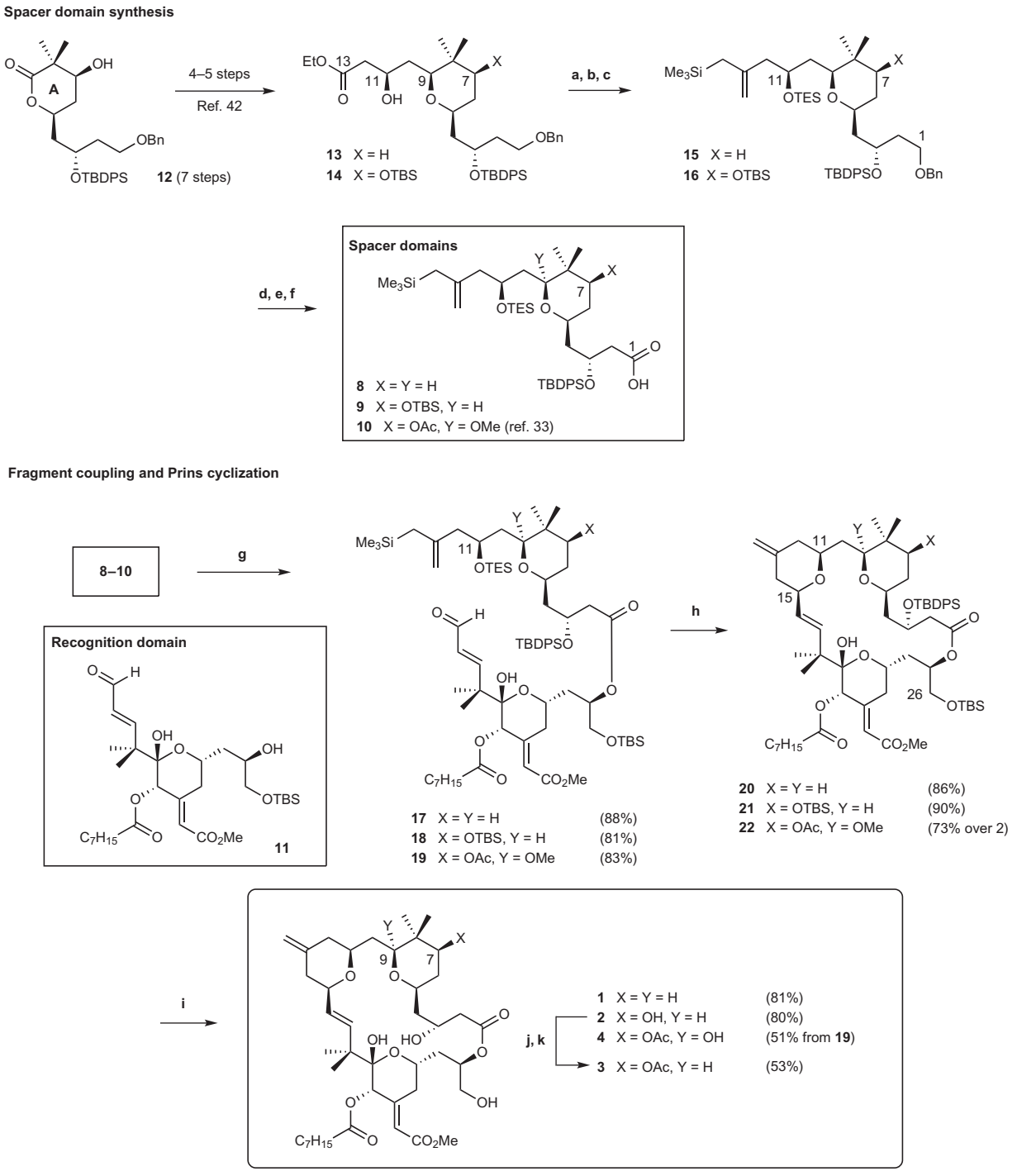

Figure 2 | Synthesis of analogues 1–4 via Prins-driven macrocyclization. Reagents and conditions. **a**–**f**, Spacer domain synthesis. When X = H, TESCl, imidazole, CH_2Cl_2, 95% (**a**); $CeCl_3$·2LiCl, $TMSCH_2MgCl$, THF (**b**); silica gel, CH_2Cl_2, 85% over 2 steps (**c**); lithium naphthalenide, THF, 84% (**d**); TPAP (10 mol%), NMO, 4 Å MS, CH_2Cl_2 (**e**); $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, 2:1 *t*-BuOH:H_2O, 91% over 2 steps (**f**). When X = OTBS, conditions as before: >99% (**a**); 82% over 2 steps (**b**,**c**); 91% (**d**); 97% over 2 steps (**e**,**f**). **g**, Fragment coupling: 2,4,6-trichlorobenzoyl chloride, Et_3N, then DMAP, recognition domain **11**, $PhCH_3$, rt. **h**, Prins macrocyclization: from **17** and **18**, PPTS, EtOH, rt; from **19**, i. PPTS, MeOH, rt, ii. TBSCl, imidazole, CH_2Cl_2. **i**–**k**, Analogue synthesis: from **20** and **21**, HF·pyridine, THF, rt; from **19**, i. PPTS, MeOH, rt, ii. HF·pyridine, THF, rt, iii. PPTS, 4:1 THF:H_2O (**i**); i. TESCl, imidazole, CH_2Cl_2, ii. Ac_2O, DMAP, pyridine, CH_2Cl_2 (**j**); HF·pyridine, THF (**k**).

directly provide analogue **4** (51% over three steps) or reprotected to provide intermediate **22** (73% overall yield) en route to analogue **7**. Analogues **1** and **2** were prepared by deprotection of macrocycles **20** and **21**, respectively. The C7-OH analogue **2** was converted into the C7-OAc analogue **3** by C26 silylation followed by C7 acylation and desilylation.

Stoichiometric ozonolysis of the C13-methylidene subunits of bryopyrans **20**, **21** and **22** followed by Horner–Wadsworth–Emmons olefination afforded intermediates **26**, **27** and **28**, respectively (Fig. 3). Although we have shown that reagent-controlled olefination can afford greater selectivity for the *Z*-enoate[33], preparation of both *E*- and *Z*-enoate isomers was required for ongoing

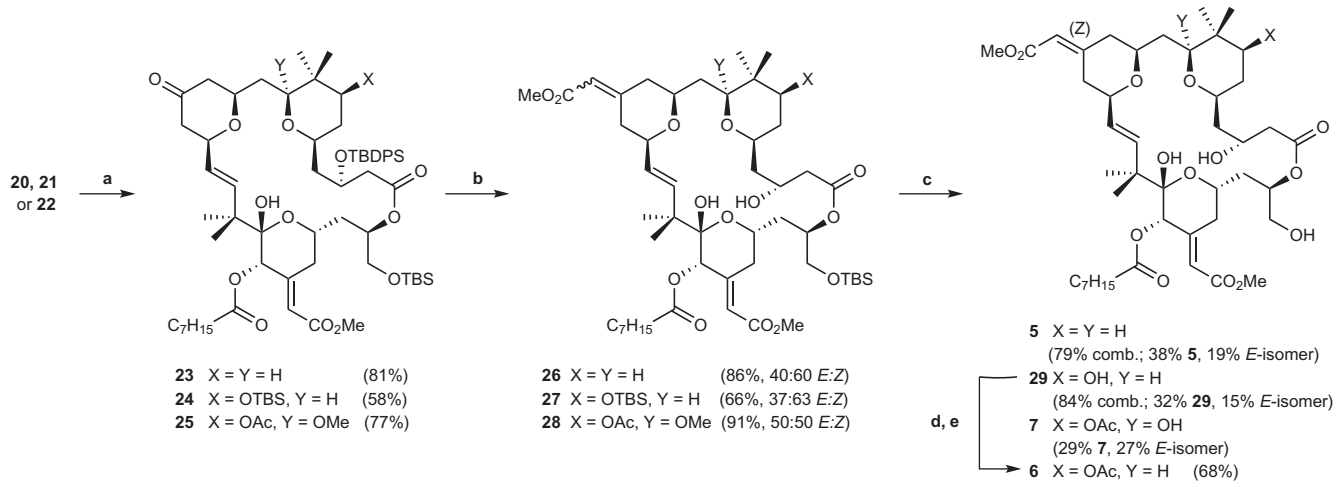

Figure 3 | Synthesis of analogues 5–7 via ozonolysis followed by Horner–Wadsworth–Emmons olefination. Reagents and conditions. **a**, O_3, CH_2Cl_2, -78 °C, then thiourea, MeOH:CH_2Cl_2. **b**, Trimethyl phosphonoacetate, NaHMDS, THF, 0 → 4 °C. **c**, From **26** or **27**: HF·pyridine, THF, rt; from **28**: i. HF·pyridine, THF, rt, then ii. PPTS, 4:1 THF:H_2O, rt. **d**, i. TESCl, imidazole, CH_2Cl_2, ii. Ac_2O, DMAP, pyridine, CH_2Cl_2. **e**, HF·pyridine, THF.

comparative activity studies. In this study, the activities of only the *Z*-enoates are reported (vide infra). Deprotection of intermediates **26**–**28** followed by reverse-phase HPLC purification afforded enoate analogues **5** and **7** and intermediate **29** en route to analogue **6**. The C7-OH intermediate **29** could be converted to the C7-OAc analogue **6** using the protocol described above involving C26 silylation followed by C7 acylation and desilylation.

Analogues **1**–**7** were found to exhibit excellent affinities for a rat brain mixture of PKC isoforms with single-digit nanomolar or sub-nanomolar K_i values (Table 1). This PKC mixture was selected because it allows for calibration of the potency of the new bryostatin analogues **1**–**7** with data obtained previously for other bryostatin analogues[39–42]. Importantly, this represents the first quantitative comparison of the relative affinities of bryostatin 1, prostratin and synthetic bryostatin analogues using a common PKC mixture. Significantly, all of the new bryostatin analogues (**1**–**7**) were more potent in this assay than the clinical candidate prostratin. Similar to previous observations on simplified substrates[41], *Z*-enoate analogues **5**, **6** and **7** were found to be slightly more potent than their *des*-enoate counterparts **1**, **3** and **4**. As we had observed for the B-ring dioxane scaffold[42], and similar to the potency difference between bryostatin 1 (C7-OAc, lit. $K_i = 1.4$ nM, ref. 35) and bryostatin 2 (C7-OH, lit. $K_i = 5.9$ nM, ref. 35), the C7-OH analogue **2,** while highly potent ($K_i = 3.4$ nM), was less so than the C7-OAc analogue **3** ($K_i = 0.42$ nM) or the C7-deoxy analogue **1** ($K_i = 0.58$ nM).

After establishing that the novel bryostatin analogues bind effectively to PKC, we tested their HIV latency induction activity. Several cellular models have been developed to assess the ability of particular agents to activate HIV from post-integration latency[48]. These models typically employ stable cell lines harbouring an integrated, yet transcriptionally silent, partial HIV genome. On treatment with an appropriate agent, viral reactivation is assessed by observation of either the production of natural viral proteins or other reporter proteins whose expression is coupled to that of the virus. J-Lat cell lines harbour near full-length latent HIV proviruses that express green fluorescent protein (GFP) upon stimulation of virus expression[49]. Treatment of J-Lat cells with certain PKC activators such as prostratin[50] or bryostatin 1 (ref. 24) results in the induction of HIV expression[51]. Alongside these benchmark agents, we examined the ability of our novel bryostatin analogues to induce HIV expression in J-Lat cells (clone 10.6) at varying concentrations. In this assay, bryostatin induced expression of HIV at concentrations as low as 1 nM (Table 1). Significantly, several of the bryostatin analogues displayed comparable or better potency, when compared to bryostatin, in both half maximum effective concentration (EC_{50}) and the percentage of cells induced to express latent HIV. For example, analogues **4** and **7** both had EC_{50} values below 1 nM and induced HIV expression in a higher percentage of cells than bryostatin at all concentrations tested between 0.1 nM and 1 μM (Supplementary Fig. S1). Similar to the results obtained in the PKC binding affinity assay, *Z*-enoate analogues **5**, **6** and **7** were found to be more potent than their *des*-enoate counterparts **1**, **3** and **4** in their ability to activate latent HIV expression in the J-Lat cell line. Moreover, all of the bryostatin analogues evaluated in this assay were at least 25-fold more potent than prostratin in

Table 1 | Analogue PKC affinity and activity in the J-Lat cell line model of HIV latency.

Analogue	X (C7)	Y (C9)	B-ring	PKC K_i (nM)*	J-Lat EC_{50} (nM)†
Bryostatin 1	OAc	OH	*Z*-Enoate	0.28 (0.18–0.44)	1.61 (0.92–2.84)
Prostratin	–	–	–	6.6 (4.1–10.6)	>1,000
1	H	H	Pyran	0.58 (0.41–0.81)	37.4 (21.6–64.9)
2	OH	H	Pyran	3.4 (1.7–6.6)	15.2 (7.5–30.8)
3	OAc	H	Pyran	0.42 (0.22–0.77)	32.0 (16.3–62.8)
4	OAc	OH	Pyran	0.95 (0.67–1.4)	0.46 (0.31–0.69)
5	H	H	Z-Enoate	0.46 (0.28–1.1)	1.9 (0.91–3.95)
6	OAc	H	Z-Enoate	0.32 (0.17–0.60)	1.15 (0.42–3.18)
7	OAc	OH	Z-Enoate	0.79 (0.58–1.1)	0.38 (0.21–0.69)

*Determined in a rat brain isoform mixture. Results from single experiments are presented. Error ranges presented in parentheses indicate 95% confidence intervals from nonlinear regression analysis.
†EC_{50} for induction of GFP transcription in the J-Lat cell line. GFP expression indicates transcription of the HIV-Long Terminal Repeat and correlates with viral reactivation from latency. Error ranges presented in parentheses indicate 95% confidence intervals.

inducing HIV from latency, with analogues **4** and **7** being over 1,000-fold more potent.

Discussion

Bryostatin 1 shows great promise as a lead candidate in the search for transformative therapies aimed at cancer and neurodegenerative disorders such as Alzheimer's disease. More recently, Bryostatin 1 was shown to induce the expression of HIV from latent viral reservoirs, an activity of potential importance for the eradication of HIV infection. However, a sustainable, cost-effective supply of bryostatin 1 has not been established. More importantly, an agent that does not exhibit the side effects of bryostatin is desirable. To address these issues of both supply and therapeutic performance, we have designed a series of bryostatin analogues (**1**–**7**) that can be synthesized on scale and can be tuned for optimal clinical performance. Analogues **1**–**7** were found to exhibit excellent affinities for PKC with single-digit nanomolar or subnanomolar K_i values, and all were more potent in this assay than the preclinical candidate prostratin. Significantly, these simplified bryologs were shown to induce the expression of latent HIV *in vitro*, with potencies similar to or better than the natural product, bryostatin 1, and at doses up to 1,000-fold lower than that of prostratin.

The study presented here demonstrates that bryostatin analogues potently activate latent HIV reservoirs *in vitro*. Notably, none of the compounds evaluated in this assay showed overt toxicity at concentrations up to 1 μM based on flow cytometry forward and side scatter profiles. While *in vivo* studies of these bryostatin analogues **1**–**7** in an animal viral induction model are in progress, a related analogue has been shown to be well tolerated and efficacious in a mouse cancer model, with no toxicity observed at doses in excess of that reported here (up to 1 mg kg^{-1})[44]. Moreover, the convergent nature of this strategy allows for additional tuning if needed to improve therapeutic performance. Further evaluation of these compounds in additional *in vitro*, *ex vivo* and *in vivo* latency induction assays is in progress.

This study is the first demonstration that designed, simplified analogues of bryostatin can serve as therapeutic leads for the eradication of HIV/AIDS. Given the problems associated with the supply of bryostatin 1 and with its side effects, this study provides a reliable synthetic source of agents that are comparable or superior to bryostatin in activity and can be tuned to accommodate clinical needs. Viral reactivation with these agents, performed in combination with HAART, would purge latent viral reservoirs while simultaneously depleting active virus. Coupling this approach with a targeted therapeutic such as an immunotoxin to more rapidly kill any resulting virus-expressing cell represents an additional therapeutic opportunity[52]. This type of approach, now enabled by the availability of highly potent and tunable analogues, could be used to clear all replication-competent HIV from infected individuals, thereby providing a strategy for disease eradication.

Methods

Experimental details for the synthesis of all new compounds, including experimental procedures, characterization and spectral data are provided in the Supplementary Information. Assay protocols for the PKC competitive binding assay and HIV latency activation assay are also provided in the Supplementary Information.

Received 27 February 2012; accepted 28 May 2012; published online 15 July 2012

References

1. Fauci, A. S. *et al.* HIV vaccine research: the way forward. *Science* **321,** 530–532 (2008).
2. Davey, R. T. Jr *et al.* HIV-1 and T cell dynamics after interruption of highly active antiretroviral therapy (HAART) in patients with a history of sustained viral suppression. *Proc. Natl Acad. Sci. USA* **96,** 15109–15114 (1999).
3. Carr, A. Toxicity of antiretroviral therapy and implications for drug development. *Nature Rev. Drug Discov.* **2,** 624–634 (2003).
4. Mills, E. J. *et al.* Adherence to HAART: a systematic review of developed and developing nation patient-reported barriers and facilitators. *PLoS Med.* **3,** 2039–2064 (2006).
5. Bangsberg, D. R. *et al.* High levels of adherence do not prevent accumulation of HIV drug resistance mutations. *AIDS* **17,** 1925–1932 (2003).
6. Ruff, C. T. *et al.* Persistence of wild-type virus and lack of temporal structure in the latent reservoir for human immunodeficiency virus type 1 in pediatric patients with extensive antiretroviral exposure. *J. Virol.* **76,** 9481–9492 (2002).
7. Bailey, J. R. *et al.* Residual human immunodeficiency virus type 1 viremia in some patients on antiretroviral therapy is dominated by a small number of invariant clones rarely found in circulating $CD4^+$ T cells. *J. Virol.* **80,** 6441–6457 (2006).
8. Dinoso, J. B. *et al.* Treatment intensification does not reduce residual HIV-1 viremia in patients on highly active antiretroviral therapy. *Proc. Natl Acad. Sci. USA* **160,** 9403–9408 (2009).
9. Coiras, M., Lopez-Huertas, M. R., Perez-Olmeda, M. & Alcami, J. Understanding HIV-1 latency provides clues for the eradication of long-term reservoirs. *Nature Rev.* **7,** 798–812 (2009).
10. Finzi, D. *et al.* Latent infection of $CD4^+$ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. *Nature Med.* **5,** 512–517 (1999).
11. Richman, D. D. *et al.* The challenge of finding a cure for HIV infection. *Science* **323,** 1304–1307 (2009).
12. Stellbrink, H-J. *et al.* Effects of interleukin-2 plus highly active antiretroviral therapy on HIV-1 replication and proviral DNA (COSMIC trial). *AIDS* **16,** 1479–1487 (2002).
13. Van Praag, R. M. E. *et al.* OKT3 and IL-2 treatment for purging of the latent HIV-1 reservoir *in vivo* results in selective long-lasting CD41 T cell depletion. *J. Clin. Immunol.* **21,** 218–226 (2001).
14. Kulkosky, J. *et al.* Prostratin: activation of latent HIV-1 expression suggests a potential inductive adjuvant therapy for HAART. *Blood* **98,** 3006–3015 (2001).
15. Korin, Y. D., Brooks, D. G., Brown, S., Korotzer, A. & Zack, J. A. Effects of prostratin on T-cell activation and human immunodeficiency virus latency. *J. Virol.* **76,** 8118–8123 (2002).
16. Brow, S. J. & Hezarah, M. Methods of administering prostratin and structural analogs thereof. US patent no. 2009126949 (2009).
17. Wender, P. A., Kee, J-M. & Warrington, J. M. Practical synthesis of prostratin, DPP, and their analogs, adjuvant leads against latent HIV. *Science* **320,** 649–652 (2008).
18. Pettit, G. R. *et al.* Isolation and structure of bryostatin 1. *J. Am. Chem. Soc.* **104,** 6846–6848 (1982).
19. Kortmansky, J. & Schwartz, G. K. Bryostatin-1: a novel PKC inhibitor in clinical development. *Cancer Invest.* **21,** 924–936 (2003).
20. Hale, K. J., Hummersone, M. G., Manaviazar, S. & Frigerio, M. The chemistry and biology of the bryostatin antitumour macrolides. *Nat. Prod. Rep.* **19,** 413–453 (2002).
21. Etcheberrigaray, R. *et al.* Therapeutic effects of PKC activators in Alzheimer's disease transgenic mice. *Proc. Natl Acad. Sci. USA* **101,** 11141–11146 (2004).
22. Kinter, A. L., Poli, G., Maury, W., Folks, T. M. & Fauci, A. S. Direct and cytokine-mediated activation of protein kinase C induces human immunodeficiency virus expression in chronically infected promonocytic cells. *J. Virol.* **64,** 4306–4312 (1990).
23. Quatsha, K. A., Rudolph, C., Marme, D., Schachtele, C. & May, W. S. Go 6976, a selective inhibitor of protein kinase C, is a potent antagonist of human immunodeficiency virus 1 induction from latent/low-level-producing reservoir cells *in vitro*. *Proc. Natl Acad. Sci. USA* **90,** 4674–4676 (1993).
24. Mehla, R. *et al.* Bryostatin modulates latent HIV-1 infection via PKC and AMPK signaling but inhibits acute infection in a receptor independent manner. *PLoS One* **5,** e11160 (2010).
25. Barr, P. M. *et al.* Phase II study of bryostatin 1 and vincristine for aggressive non-Hodgkin lymphoma relapsing after an autologous stem cell transplant. *Am. J. Hematol.* **84,** 484–487 (2009).
26. Schaufelberger, D. E. *et al.* The large-scale isolation of bryostatin 1 from *Bugula neritina* following current good manufacturing practices. *J. Nat. Prod.* **54,** 1265–1270 (1991).
27. Trindade-Silva, A. E., Lim-Fong, G. E., Sharp, K. H. & Haygood, M. G. Bryostatins: biological context and biotechnological prospects. *Curr. Opin. Biotechnol.* **21,** 834–842 (2010).
28. Kageyama, M. *et al.* Synthesis of bryostatin 7. *J. Am. Chem. Soc.* **112,** 7407–7408 (1990).
29. Evans, D. A. *et al.* Total synthesis of bryostatin 2. *J. Am. Chem. Soc.* **121,** 7540–7552 (1999).
30. Ohmori, K. *et al.* Total synthesis of bryostatin 3. *Angew. Chem. Int. Ed.* **39,** 2290–2294 (2000).
31. Trost, B. M. & Dong, G. Total synthesis of bryostatin 16 using atom-economical and chemoselective approaches. *Nature* **456,** 485–488 (2008).
32. Keck, G. E., Poudel, Y. B., Cummins, T. J., Rudra, A. & Covel, J. A. Total synthesis of bryostatin 1. *J. Am. Chem. Soc.* **133,** 744–747 (2011).

33. Wender, P. A. & Schrier, A. J. Total synthesis of bryostatin 9. *J. Am. Chem. Soc.* **133,** 9228–9231 (2011).
34. Lu, Y., Woo, S. & Krische, M. J. Total synthesis of bryostatin 7 via C–C bond-forming hydrogenation. *J. Am. Chem. Soc.* **133,** 13876–13879 (2011).
35. Wender, P. A. *et al.* Modeling of the bryostatins to the phorbol ester pharmacophore on protein kinase C. *Proc. Natl Acad. Sci. USA* **85,** 7197–7201 (1988).
36. Wender, P. A. in *Drug Discovery Research: New Frontiers in the Post-Genomic Era* (ed. Huang, Z.) Ch. 6 (Wiley-VCH, 2007).
37. Wender, P. A., Loy, B. A. & Schrier, A. J. Translating nature's library: the bryostatins and function-oriented synthesis. *Isr. J. Chem.* **51,** 453–472 (2011).
38. Wender, P. A., Verma, V. A., Paxton, T. J. & Pillow, T. H. Function-oriented synthesis, step economy, and drug design. *Acc. Chem. Res.* **41,** 40–49 (2008).
39. Wender, P. A. *et al.* Synthesis of the first members of a new class of biologically active bryostatin analogues. *J. Am. Chem. Soc.* **120,** 4534–4535 (1998).
40. Wender, P. A. *et al.* The practical synthesis of a novel and highly potent analogue of bryostatin. *J. Am. Chem. Soc.* **124,** 13648–13649 (2002).
41. Wender, P. A., DeChristopher, B. A. & Schrier, A. J. Efficient synthetic access to a new family of highly potent bryostatin analogues via a Prins-driven macrocyclization strategy. *J. Am. Chem. Soc.* **130,** 6658–6659 (2008).
42. Wender, P. A. *et al.* Design, synthesis, and evaluation of potent bryostatin analogs that modulate PKC translocation selectivity. *Proc. Natl Acad. Sci. USA* **108,** 6721–6726 (2011).
43. Mackay, H. J. & Twelves, C. J. Targeting the protein kinase C family: are we there yet? *Nature Rev. Cancer* **7,** 554–562 (2007).
44. DeChristopher, B. A., Fan, A. C., Felsher, D. W. & Wender, P. A. 'Picolog', a synthetically-available bryostatin analog, inhibits growth of MYC-induced lymphoma *in vivo*. *Oncotarget* **3,** 58–66 (2012).
45. Khan, T. K., Nelson, T. J., Verma, V. A., Wender, P. A. & Alkon, D. L. A cellular model of Alzheimer's disease therapeutic efficacy: PKC activation reverses Aβ-induced biomarker abnormality on cultured fibroblasts. *Neurobiol. Dis.* **34,** 332–339 (2009).
46. Crane, E. A. & Scheidt, K. A. Prins-type macrocyclizations as an efficient ring-closing strategy in natural product synthesis. *Angew. Chem. Int. Ed.* **49,** 8316–8326 (2010).
47. Krasovskiy, A., Kopp, F. & Knochel, P. Soluble lanthanide salts ($LnCl_3$·2LiCl) for the improved addition of organomagnesium reagents to carbonyl compounds. *Angew. Chem. Int. Ed.* **45,** 497–500 (2006).
48. Marsden, M. D. & Zack, J. A. Establishment and maintenance of HIV latency: model systems and opportunities for intervention. *Future Virol.* **5,** 97–109 (2010).
49. Jordan, A., Bisgrove, D. & Verdin, E. HIV reproducibly establishes a latent infection after acute infection in T cells *in vitro*. *EMBO J.* **22,** 1868–1877 (2003).
50. Williams, S. A. *et al.* Prostratin antagonizes HIV latency by activating NF-κB. *J. Biol. Chem.* **279,** 42008–42017 (2004).
51. Kovochich, M., Marsden, M. D. & Zack, J. A. Activation of latent HIV using drug-loaded nanoparticles. *PLoS One* **6,** e18270 (2011).
52. Brooks, D. G. *et al.* Molecular characterization, reactivation, and depletion of latent HIV. *Immunity* **19,** 413–423 (2003).

Acknowledgements

This research was supported by the National Institutes of Health (CA31845 to P.A.W., AI070010 to J.A.Z.) and the UCLA Center for AIDS Research (AI028697). Additional funding was provided by the ACS Division of Organic Chemistry Graduate Fellowship sponsored by Bristol-Myers Squibb, the Eli Lilly Organic Chemistry Graduate Fellowship, and the Stanford University Center for Molecular Analysis and Design.

Author contributions

B.A.D., B.A.L., M.D.M., A.J.S., J.A.Z. and P.A.W. conceived and designed the experiments. B.A.D., B.A.L., M.D.M. and A.J.S. performed the experiments and analysed the data. B.A.L., M.D.M., A.J.S. and P.A.W. co-wrote the paper. All authors commented on the manuscript.

Additional information

The authors declare no competing financial interests. Supplementary information and chemical compound information accompany this paper at www.nature.com/naturechemistry. Reprints and permission information is available online at http://www.nature.com/reprints. Correspondence and requests for materials should be addressed to J.A.Z. and P.A.W.