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Graphical Abstract

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Stephen C. Pelly

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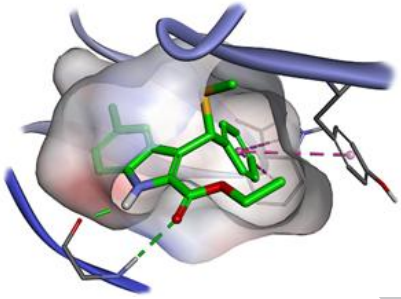

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Novel Indole Sulfides as Potent HIV-1 NNRTIs

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ABSTRACT

In a previous communication we described a series of indole based NNRTIs which were potent inhibitors of HIV replication, both for the wild type and K103N strains of the virus. However, the methyl ether functionality on these compounds, which was crucial for potency, was susceptible to acid promoted indole assisted S_N1 substitution. This particular problem did not bode well for an orally bioavailable drug. Here we describe bioisosteric replacement of this problematic functional group, leading to a series of compounds which are potent inhibitors of HIV replication, and are acid stable.

Human immunodeficiency virus type-1 (HIV-1) remains the major cause of the life threatening condition known as acquired immunodeficiency syndrome (AIDS). With no cure predicted for the near future, this disease remains a significant pandemic with a heavy socio-economic toll, especially for developing nations where it is most prevalent.[1] Fortunately, due to an extensive amount of research in the area and the subsequent development of numerous therapeutic agents, it is possible to control the viral levels in afflicted individuals with chronic medication.[2-4] Front line anti-retroviral treatment usually consists of a cocktail of three drug entities, all of which target the essential enzyme HIV reverse transcriptase (RT).[1] This enzyme is in fact unique in HIV therapy in that it offers two sites for therapeutic intervention, which have been well exploited. The first of these is at the actual catalytic site and here several licensed inhibitors in the form of DNA base mimics, or nucleoside reverse transcriptase inhibitors (NRTIs), are highly effective inhibitors of HIV replication. Secondly, located just 10 Å from this catalytic site is a small cleft in the enzyme which is amenable to the binding of small hydrophobic inhibitors. These non-nucleoside reverse transcriptase inhibitors (NNRTIs) cause an allosteric twist in the enzyme, inactivating its DNA polymerase function, and these compounds are also potent inhibitors of HIV replication.[5] The first-line highly active anti-retroviral therapy (HAART) regimen usually consists of two NRTIs as well as an NNRTI, but unfortunately all the drugs in these classes are susceptible to resistance inactivation, and many of them display undesirable side effects with long term use.[6, 7] For this reason, until a cure for the disease is found, there is a constant need to fill the drug development pipeline with new inhibitors capable of tackling problematic resistant strains of the virus. This is of particular need in sub-Saharan Africa which has the highest incidence of HIV, and where patient compliance, especially in rural areas, is problematic due to a variety of logistical and socio-economic reasons.[8]

In a previous communication, we discussed the design and synthesis of several indole methyl ether derivatives (for example **1**, Figure 1) which exhibited low nano-molar potency against wild type and the problematic K103N strains of HIV-1.[9, 10] Although the indole nucleus has been well validated as a scaffold for the design of novel NNRTIs,[11-18] we discovered a serious problem with our particular compounds during acidic workup. In this process, acid activation of the methyl ether would lead to the elimination of methanol facilitated by the indole, and subsequent attack by water would produce the corresponding alcohol **2** (Figure 1), which was poorly effective against HIV RT. In fact, having discovered this instability we exploited it in our synthesis of these analogues. Although this was convenient from a synthetic chemistry perspective, it did not bode well for an orally bioavailable drug. In this article we describe the preparation of a bioisosteric replacement of the problematic labile methyl ether to afford potent and stable HIV NNRTIs.

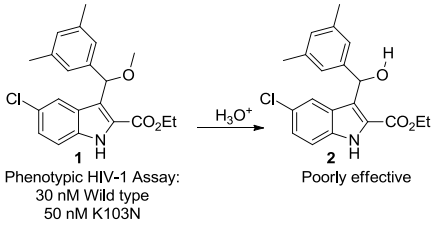


Figure 1. Our previously synthesized NNRTI **1** undergoes S_N1 substitution in the presence of aqueous acid leading to the alcohol **2** which is a poorly effective NNRTI.

In our first attempt at synthesizing a more stable analogue, we made use of a bioisosteric replacement of the labile methyl ether **3**, opting instead for the ethyl amine analogue **4** (Figure 2). With the nitrogen attached directly to the indole's 3-position, acid promoted elimination of this functional group would not be possible. Molecular modelling of this compound indicated that it would indeed be well accommodated in the non-nucleoside inhibitor binding pocket (NNIBP),[19] with the desired double hydrogen bond interactions to the backbone of Lys101 maintained, as well as π-π interactions to Tyr181 and the conserved residue, Trp229. Furthermore, the ethyl chain extending off the nitrogen neatly occupied the small hydrophobic pocket in the region of Val179. However, a concerning revelation regarding the binding mode was that in order to adopt the perfect binding conformation, the newly introduced nitrogen's p-orbital would be required to be almost transverse to both the phenyl and indole aromatic ring systems. We anticipated that this may result in poor p-orbital overlap and impede the desired sp^2 trigonal planar character of the nitrogen, lending itself more toward an sp^3 trigonal pyramidal nitrogen. Although the change in geometry of the newly introduced nitrogen system could also be well accommodated in the NNIBP, the concern lay in the fact that a nitrogen with more sp^3 character would be basic, and susceptible to protonation, resulting in a mismatch in terms of an ionized nitrogen now being required to fill the hydrophobic Val179 pocket. Nevertheless, we felt that the elegant simplicity in this design in terms of overcoming the acid lability problem warranted an investigation. Furthermore, if this strategy proved successful it would eliminate the complication of the stereogenic carbon found in the methyl ether analogues.

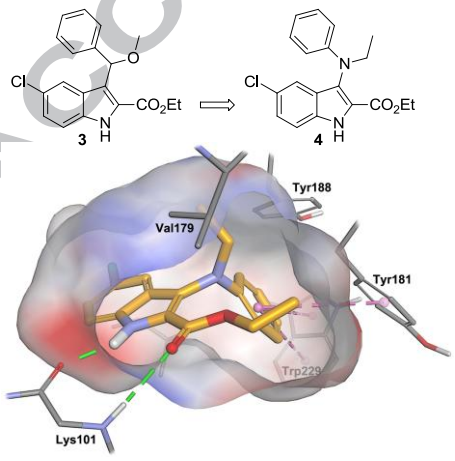
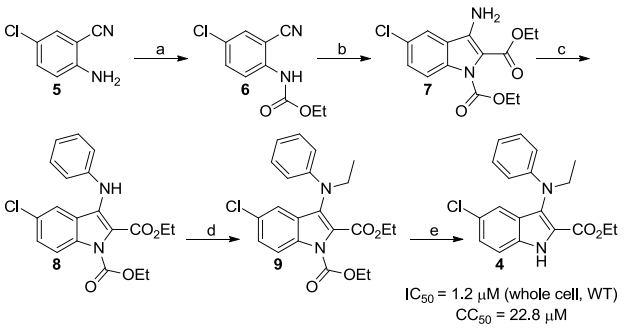


Figure 2. Bioisosteric replacement of the acid labile methyl ether **3** provided compound **4** which modelled equally well.

Synthesis of the amine derivative **4** however, turned out to be significantly more challenging than originally anticipated. The initially planned route followed a simple strategy outlined by Hiremath *et al.*,[20] involving a copper mediated coupling of aniline to the appropriate 3-bromoindole. However, in our hands this coupling could not be repeated and after a significant effort, we reverted to the more complicated procedure shown in Scheme 1.[21] To this end, commercially available 5-chloro-anthranilonitrile **5** was converted into the carbamate **6** using ethyl chloroformate. In the presence of sodium hydride, ethyl chloroacetate was substituted onto the carbamate nitrogen with concomitant intramolecular nucleophilic addition to the nitrile, leading to the desired indole scaffold **7** in good yield. Functionalization of the resulting amine however proved to be problematic. In the end, the phenyl substituent was introduced by way of a Buchwald coupling reaction, providing **8**, albeit in poor yield. Introduction of the ethyl substituent proved even more troublesome and here we had to settle for a base promoted substitution in very poor yield to afford **9**. Part of the reason for the poor yield was due to hydrolysis of the carbamate at the indole nitrogen under these strongly basic conditions, which was followed by the undesirable alkylation of the indole. Nevertheless, following a potassium phosphate mediated deprotection of the indole which proceeded smoothly, the desired 3-amino indole derivative **4** was in hand and could be evaluated in our HIV-1 assay to establish proof of concept. To this end, we utilized an *in vitro* single-cycle, non-replicative phenotypic assay to determine IC_{50} values,[22, 23] and toxicity values were determined using HEK293T cells and a tetrazolium-based colorimetric assay.[24] Disappointingly, this compound performed poorly with an IC_{50} = 1.2 μM. Possible explanations for this severe decrease in potency could firstly be associated with the aforementioned problem regarding the hybridization state of the nitrogen and secondly, the presence of this electron rich nitrogen at the 3-position of the indole could in fact weaken the hydrogen bond donor character of the indole N–H, a crucial interaction on our compound design strategy. Therefore, we abandoned this scaffold as a viable option to overcome the acid lability problem.



Scheme 1. Reagents and conditions: a) ethyl chloroformate, 93°C, 18 h, 85%; b) ethyl chloroacetate, NaH, DMF, 0°C – r.t, 18 h, 82%; c) bromobenzene, $Pd(dba)_2$, Xphos, K_3PO_4, toluene, 130°C, 48 h, 32%; d) NaO*t*Bu, EtI, DMF, 0°C – r.t, 18 h, 11%; e) K_3PO_3, EtOH, 70°C, 4 h, quant.

We now turned our attention to a new strategy, that being to install a poorer Lewis base in the form of a methyl sulfide **10** rather than the methyl ether **3** (Figure 3). Molecular modelling of the methyl sulfide derivatives indicated that there was indeed enough room to accommodate the larger sulfur atom, with its methyl substituent extending into the small hydrophobic Val179 pocket. In addition, the desired double hydrogen bonding interactions to the backbone of Lys101 were maintained, as well as π interactions to the side chains of Tyr181 and the important conserved residue, Trp229. Molecular modelling using Discovery Studio's CDocker suggested that although the sulfide analogues could be accommodated in the pocket, they would be slightly

poorer inhibitors, as attested to by slightly poorer energy scores (Table 1). There are two possible reasons as to why this may be the case, the first of which is simply that the larger methyl sulfide may not be as well accommodated in the small hydrophobic Val179 pocket. Secondly, the more electronegative ether located at the three position of the indole draws more electron density from the indole scaffold, rendering the indole N-H a better hydrogen bond donor than is the case when the methyl sulfide is located at this same position. It should also be mentioned that in general, the *R*-enantiomers scored just slightly better according to CDocker, however at this stage our assay tests were performed on the racemates for all compounds.

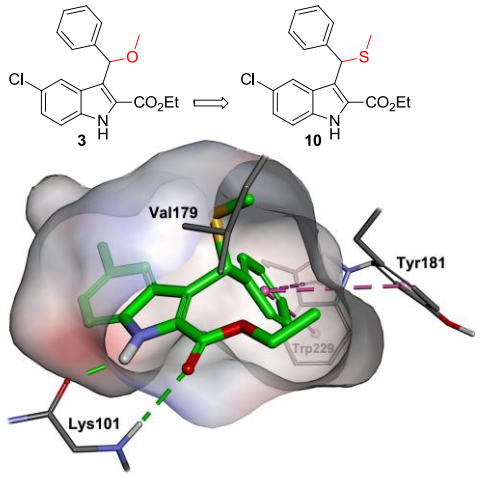
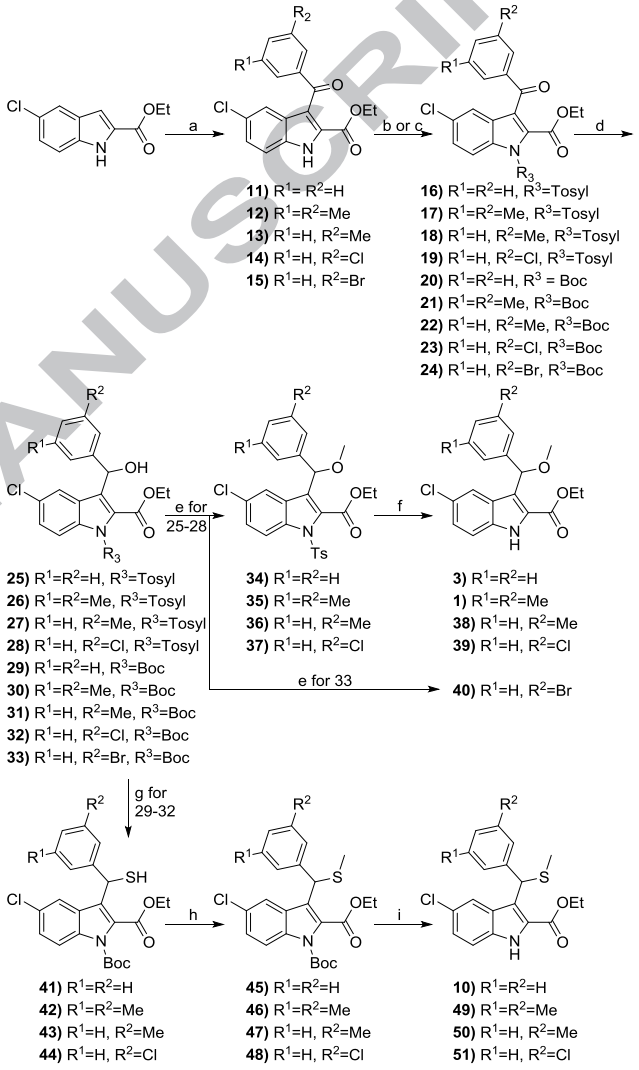


Figure 3. Replacement of the methyl ether **3** with methyl sulfide **10** was envisaged to lead to a more stable compound given the poorer Lewis basicity of the sulfur. Modelling indicated that the larger sulfide group could still be well accommodated in the small Val179 pocket.

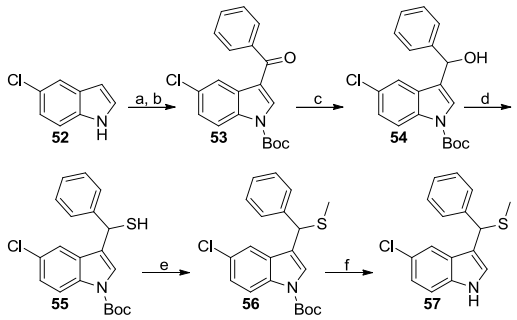
To evaluate the effectiveness of this strategy, a small library of methyl sulfide compounds was synthesized, as well as the corresponding methyl ether compounds for comparative purposes (Scheme 2). To this end, commercially available ethyl 5-chloro-2-indole-carboxylate was acylated in the presence of aluminium trichloride thereby affording the 3-acyl derivatives **11-15**. Subsequent protection of the indole nitrogen using either toluene sulfonyl chloride or Boc anhydride afforded compounds **16-24**. Simple reduction of the ketone using sodium borohydride provided the alcohols **25-33**. For the synthesis of the methyl ether analogues, the alcohols **25-28** were subjected to an acid catalyzed S_N1 substitution, facilitated by the indole, leading to compounds **34-37**. This reaction proved to be particularly difficult for compounds bearing an electron withdrawing group on the phenyl ring (e.g. **37**) no doubt due to the higher energy transition state leading to the required S_N1 intermediate. This interesting revelation provided some insight into a possible means of overcoming the acid lability of these methyl ether derivatives. Finally, deprotection of the indole using potassium hydroxide furnished the methyl ether indoles **1**, **3**, **38** and **39**. For the synthesis of the sulfide analogues, the Boc-protected alcohols **29-32** were treated with Lawesson's reagent thereby converting the alcohol into the corresponding thiol. Unfortunately, this reaction proved to be somewhat low yielding throughout the series. Nevertheless, methylation of the thiol provided the sulfides **45-48** and finally potassium phosphate mediated Boc deprotection furnished the desired sulfides **10** and **49-51**. In addition, having stumbled upon a possible method of stabilizing the methyl ether derivatives by incorporating an electron withdrawing substituent on the phenyl ring system (compound **37**), we decided to expand upon this and synthesize the bromo derivative **40**. To this end we followed a very similar synthetic route although we utilized a Boc protecting group and not the tosyl as had been employed for the other methyl ether derivatives. In summary, the Boc protected indole **33** was subjected to the acid promoted S_N1 substitution reaction in the presence of methanol. Similarly to the chloro-derivative **28**, this reaction was particularly reluctant to proceed and the large excess of toluene sulfonic acid employed in the procedure resulted in simultaneous removal of the Boc protecting group, leading directly to the desired methyl ether indole **40**.



Scheme 2. Reagents and conditions: a) aryl acid chloride, $AlCl_3$, DCE, 2 h, 0°C - 85°C, 47% - 65%; b) *p*-TsCl, NaH, DMF, 18 h, 0°C - r.t., 42% - 79%; c) Boc_2O, DMAP, THF, 3 h, r.t., 63% - 94%; d) $NaBH_4$, EtOH, THF, 3 h, 0°C - r.t., 75% - 99%; e) *p*-TsOH, MeOH, 18 h, r.t., 11% - 63%; f) KOH, EtOH, THF, 4 h, r.t., 72% - 100%; g) Lawesson's Reagent, toluene, 3 h, reflux, 27% - 53%; h) MeI, Et_3N, DCM, 18 h, r.t., 76% - 85%; i) K_3PO_4, EtOH, 1 h, 70°C, 67% - quant.

To evaluate the importance of the ester functionality at the indole's 2-position on the sulfide compounds, an analogue was synthesized lacking this group (Scheme 3). As would be expected, molecular modelling indicated that this compound would be a significantly less effective inhibitor of HIV RT. To this end, commercially available 5-chloroindole **52** was acylated using benzoyl chloride and then the indole was protected as the Boc carbamate, affording **53**. Reduction of the ketone proceeded smoothly giving alcohol **54** which was converted into the thiol **55**

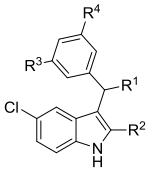
using Lawesson's reagent. Finally, methylation of the thiol afforded the sulfide **56** and following a Boc-deprotection facilitated by potassium phosphate, the desired sulfide **57** was in hand ready for testing.



Scheme 3. Reagents and conditions: a) aryl acid chloride, $AlCl_3$, DCE, 2 h, 0°C - 85°C, 47%; b) Boc_2O, DMAP, THF, 3 h, r.t., 83%; c) $NaBH_4$, EtOH, THF, 3 h, 0°C - r.t., 90%; d) Lawesson's Reagent, toluene, 3 h, reflux, 46%; e) MeI, Et_3N, DCM, 18 h, r.t., 71%; f) K_3PO_4, EtOH, 1 h, 70°C, 72%.

Having synthesized a small library of methyl ether and methyl sulfide analogues, we were now in a position to compare their anti-viral efficacy in our phenotypic assay (Table 1).[22-24] Consistent with our previous results,[9] the methyl ether derivatives were all very effective low nano-molar inhibitors of HIV-1 replication. Interestingly, the modelling results correlated well with the observed HIV assay data. In general, the methyl sulfide compounds obtained slightly poorer CDocker Energy values in comparison to the methyl ether derivatives (Table 1), and similarly, their IC_{50} values were all just slightly less potent than their corresponding methyl ether analogues. Nevertheless, the methyl sulfide compounds remained potent inhibitors of HIV-1 replication with the exception of compound **57**, which lacks the ester group at the 2-position of the indole. Since this compound cannot form a second hydrogen bond to Lys101 it scores significantly less well in the CDocker docking protocol, and as expected, also performs significantly more poorly in the HIV assay.

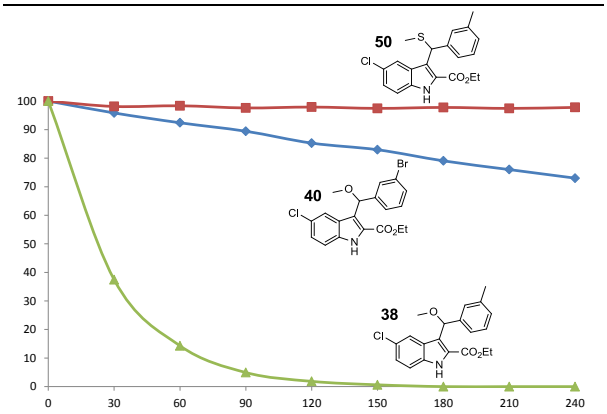
Table 1. Modelling data and HIV-1 phenotypic assay data and toxicity data

Compound		R1	R2	R3	R4	CDocker Energy	IC_{50} /μM	CC_{50} /μM
	**3**	-OMe	$-CO_2Et$	-H	-H	-49.8	0.016	26.7
	1	-OMe	$-CO_2Et$	$-CH_3$	$-CH_3$	-51.8	0.034	35.9
	38	-OMe	$-CO_2Et$	$-CH_3$	-H	-53.1	0.020	27.4
	39	-OMe	$-CO_2Et$	-Cl	-H	-51.1	0.033	26.8
	40	-OMe	$-CO_2Et$	-Br	-H	-50.6	0.020	26.4
	10	-SMe	$-CO_2Et$	-H	-H	-43.7	0.039	26.1
	49	-SMe	$-CO_2Et$	$-CH_3$	$-CH_3$	-41.5	0.060	32.0
	50	-SMe	$-CO_2Et$	$-CH_3$	-H	-44.5	0.038	29.6
	51	-SMe	$-CO_2Et$	-Cl	-H	-41.9	0.042	28.6
	57	-SMe	-H	-H	-H	-30.0	9.95	52.7

Note: CDocker Energy score values ($KCal.mol^{-1}$) are calculated using the CDocker docking protocol,[25] launched from within Biovia Discovery studio 2016. Lower values imply a better docking. All CDocker Energy values are for the *R*-enantiomers, which generally score just slightly better than the *S*-enantiomers. The IC_{50} and CC_{50} describe the concentration of each compound that inhibits viral infection by 50% or decreases cell viability by 50%, respectively. These assays were performed on the racemates in each case. Compounds **1** and **3** have been reported in a previous communication,[9] but are included here for comparative purposes to the sulfide analogues.

Having established that the methyl sulfide compounds were potent inhibitors of HIV-1 replication, we now set about investigating whether these compounds would be less susceptible to acid promoted degradation by way of the indole assisted S_N1 mechanism discussed earlier. To this end, the methyl ether **38**, and its sulfide analogue, **50** were suspended in equivalent amounts of ethanol and the solutions were spiked with equivalent amounts of concentrated sulfuric acid. Under these identical conditions, the reactions were stirred and sampled at regular intervals in order to monitor the progress of the S_N1 substitution by HPLC. Interestingly, within two hours, the methyl ether **38** had been fully consumed, yet the methyl sulfide remained essentially unreacted showing no conversion to the ethyl ether over this time period (Chart 1). In fact, after 72 hours there was less than 5% conversion of the sulfide **50** to the ethyl ether**.** As per our hypothesis, the sulfide was indeed a far poorer Lewis base and therefore resulted in significantly more stable compounds than the ether derivatives. Also of interest was compound **40**, a methyl ether derivative with a bromine substituent on the phenyl ring system. We noticed during the synthetic campaign that it was particularly difficult to convert from the alcohol **33** into the methyl ether **40** (Scheme 2). Therefore, we were similarly interested to see how this compound would fair in our stability test. Although this compound is also a methyl ether derivative it was significantly more stable than its analogue **38**, with only 17% conversion to the corresponding ethyl ether over a four-hour period. This enhanced stability for a methyl ether derivative can be explained by virtue of the fact that the bromine on the phenyl ring decreases the electron density in this ring system resulting in a much higher energy transition state leading towards the S_N1 intermediate. It was however, not nearly as stable as the sulfide derivative **50**.

Chart 1: Acid lability studies



Suspension of the methyl ether **38** and methyl sulfide **50** in acidic ethanol shows rapid degradation of the methyl ether, yet the methyl sulfide remains stable under these conditions. Interestingly, the methyl ether **40**, with the electron withdrawing bromine on the phenyl ring system is also more stable than **38**, but not as stable as the sulfide **50**.

In conclusion, we have prepared a series of methyl sulfide indole based NNRTIs compounds, which, like their methyl ether analogues, are potent inhibitors of HIV replication. However, unlike the methyl ether analogues, these sulfides are stable under acidic conditions.

Acknowledgments

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Supplementary Material

Detailed experimental conditions as well as compound characterization data can be found in the supplementary information (PDF file).

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