Published in final edited form as:
Bioorg Med Chem. 2012 September 1; 20(17): 5181–5187. doi:10.1016/j.bmc.2012.07.006.

Transition state analogue inhibitors of human methylthioadenosine phosphorylase and bacterial methylthioadenosine/*S*-adenosylhomocysteine nucleosidase incorporating acyclic ribooxacarbenium ion mimics

Keith Clinch[a,*], **Gary B. Evans**[a], **Richard F. G. Fröhlich**[a], **Shivali A. Gulab**[a], **Jemy A. Gutierrez**[b], **Jennifer M. Mason**[a], **Vern L. Schramm**[b], **Peter C. Tyler**[a,*], and **Anthony D. Woolhouse**[a]

[a]Carbohydrate Chemistry, Industrial Research Limited, P O Box 31310, Lower Hutt 5040, New Zealand [b]Department of Biochemistry, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, New York 10461, USA

Abstract

Several acyclic hydroxy-methylthio-amines with 3 to 5 carbon atoms were prepared and coupled *via* a methylene link to 9-deazaadenine. The products were tested for inhibition against human MTAP and *E. coli* and *N. meningitidis* MTANs and gave K_i values as low as 0.23 nM. These results were compared to those obtained with 1st and 2nd generation inhibitors (1*S*)-1-(9-deazaadenin-9-yl)-1,4-dideoxy-1,4-imino-5-methylthio-D-ribitol (MT-Immucillin-A, **3**) and (3*R*, 4*S*)-1-[9-deazaadenin-9-yl)methyl]3-hydroxy-4-methylthiomethylpyrrolidine (MT-DADMe-Immucillin-A, **4**). The best inhibitors were found to exhibit binding affinities of approximately 2- to 4-fold those of **3** but were significantly weaker than **4**. Cleavage of the 2,3 carbon–carbon bond in MT-Immucillin-A (**3**) gave an acyclic product (**79**) with a 21,500 fold loss of activity against *E. coli* MTAN. In another case, *N*-methylation of a side chain secondary amine resulted in a 250-fold loss of activity against the same enzyme [(±)-**65** vs (±)-**68**]. The inhibition results were also contrasted with those acyclic derivatives previously prepared as inhibitors for a related enzyme, purine nucleoside phosphorylase (PNP), where some inhibitors in the latter case were found to be more potent than their cyclic counterparts.

Keywords

Human MTAP; Bacterial MTANs; Ribooxacarbenium ion mimics; Inhibitors; Acyclic hydroxy-methylthio-amines

*Corresponding authors. Fax.: +64-4-931-3055; k.clinch@irl.cri.nz; Fax.: +64-4-931-3055 p.tyler@irl.cri.nz.

Supporting information

The full experimental details and the ^{1}H and ^{13}C NMR spectra of **17 I** (**17 II**, **17 III**), (±)-**25**, **33**, (*ent*-**33**), **40**, (*ent*-**40**), **47**, **54**, (±)-**65**, (±)-**68**, (±)-**72**, (±)-**78** and **79** and of the Mosher amide derivatives of **14 I** and **14 II** can be found, in the online version, at

1. Introduction

The human methylthioadenosine phosphorylase (MTAP)[1,2] and bacterial methylthioadenosine/*S*-adenosylhomocysteine nucleosidase (MTAN)[3,4] enzymes are targets for drug design. MTAP is a target for cancer therapy through its importance in polyamine biosynthesis and *S*-adenosylmethionine (SAM) metabolism.[5–10] Efficient inhibitors of MTAP are expected to lead to accumulation of methylthioadenosine (MTA) and consequent inhibition of spermidine and spermine synthases while MTA itself has antiproliferative properties.[11–17] In addition, the metabolism of SAM is likely to be prevented along with the disruption of SAM-dependent methylation. We have developed potent inhibitors of MTAP that show strong anti-cancer effects[18,19] thereby providing validation for MTAP as a cancer target.

MTAN is involved in bacterial polyamine biosynthesis and quorum sensing.[20–22] Certain gram-negative organisms utilize species-specific acyl-homoserine lactones as quorum sensing molecules. These are synthesized from *N*-acylated *S*-adenosylmethionine generating MTA as a by-product of the reaction.[23] Efficient inhibitors of MTAN will lead to elevated concentrations of MTA which may induce feedback inhibition of acyl-homoserine lactone synthesis. *S*-Ribosylhomocysteine, the product of MTAN-catalysed hydrolysis of *S*-adenosylhomocysteine, is catabolized by *S*-ribosylhomocysteinase (LuxS) into L-homocysteine and (4*S*)-4,5-dihydroxy-2,3-pentanedione.[24] The latter forms the basis of the bacterial quorum sensing signal AI-2.[21,25,26] The LuxS enzyme has been identified in more than 55 gram-negative and gram-positive bacteria and the evidence suggests that the autoinducing signal AI-2 is common to many bacterial species. Thus, without MTAN activity there can be no synthesis of AI-2. In consequence, inhibition of MTAN would be expected to inhibit polyamine biosynthesis, block salvage pathways for methionine and adenine as well as blocking the synthesis of two types of quorum sensing autoinducer molecules.

We have been engaged for a number of years in the design and synthesis of transition state analogue inhibitors of a number of *N*-ribosyltransferase enzymes, including those mentioned above. The use of measured kinetic isotope effects has enabled the identification of the nature of the transition states of many of these enzymes.[27–29] With this knowledge, inhibitor design and synthesis has provided exceedingly potent inhibitors of human and *Plasmodium falciparum* purine nucleoside phosphorylases (PNPs),[30–33] human MTAP,[34–37] protozoan nucleoside hydrolases[38,39] and bacterial MTANs.[36,40–47] Immucillin-H (**1**, Forodesine) and DADMe-Immucillin-H (**2**, BCX4208) are first and second generation inhibitors of the PNPs, and are in clinical development for the treatment of T-cell proliferative disorders and gout (Fig. 1). MT-Immucillin-A (**3**) and MT-DADMe-Immucillin-A (**4**) are corresponding first and second generation inhibitors of MTAP and bacterial MTANs and the second generation inhibitor **4** is in preclinical development for oncology indications.[18,19] Recently, we reported on third generation inhibitors of PNP with acyclic aza-sugar mimics, some of which showed surprising activity. For example, DATMe-Immucillin-H **5** and SerMe-Immucillin-H **6** had exceptional activity with the achiral serinol derivative **7** being the most potent PNP inhibitor yet discovered (K_i^* = 2.1 pM).[48]

Therefore we wished to investigate whether replacement of the hydroxymethyl and 9-deazahypoxanthine or 9-deazaguanine units, found in acyclic PNP inhibitors, with methylthio and 9-deazaadenine ones, respectively, would lead to potent inhibitors of the related MTAP and MTAN enzymes. In our previous work on the preparation of acyclic PNP inhibitors, including compounds **5** to **7**, we found reductive amination/alkylation and Mannich reactions useful in their construction.[48] In both cases several primary and secondary amino alcohols were prepared and coupled through their amino functions, *via* a

methylene link, to the 9-position of either deazahypoxanthine or deazaguanine. In this paper we describe the synthesis of several hydroxymethylthio-substituted primary and secondary amines and their couplings to aldehyde **10** or 9-deazaadenine,[49] substrates for the reductive amination/alkylation (Schemes 1 to 8) and Mannich reactions (Schemes 9 to 11), respectively. In addition, the direct convertion of MT-Immucillin-A (**3**) into an acyclic derivative is also described (Scheme 12).

2. Chemistry

2.1. Synthesis using the reductive amination/alkylation reaction

The key aldehyde **10** was synthesized by brominating chlorodeazapurine **8**[50] followed by trapping of the lithium-bromine-exchanged derivative with *N*,*N*-dimethylformamide (DMF) (Scheme 1). The structure of bromide **9** was characterized spectroscopically and further confirmed by X-ray crystallography.[51]

Serinol-linked 9-deazahypoxanthine or 9-deazaguanine derivatives **6** and **7** (Fig. 1) have been shown to be low pM inhibitors of human PNP[48] and so we prepared the analogous methylthio-serinol-based 9-deazaadenine derivative **17 III** to see if this would also be the case with human MTAP and bacterial MTANs. Treatment of racemic methylthio-serinol derivative **13 III**[52] with hydrochloric acid gave amine salt **14 III**. This was reductively alkylated with aldehyde **10** using sodium cyanoborohydride[53], after adjusting the pH of the mixture to between 6 and 7 with sodium hydrogen carbonate, to give the benzyloxymethyl (BOM) protected chloride **15 III** (Scheme 2). Displacement of the chloride in **15 III** with ammonia was effected in a sealed tube at 135 °C to give **16 III**. Finally, the BOM protecting group was efficiently removed hydrogenolytically using hydrazine hydrate and palladium black in methanolic ammonia solution to produce **17 III**.

Encouraged by the enzyme inhibition results (Table 1) obtained for **17 III**, we next prepared the individual enantiomers **17 I** and **17 II**. Readily obtainable (*S*)-alcohol **11 I**, prepared as for its enantiomer,[54] was converted through its mesylate into methylthio compound **12 I** and then into (*R*)-amine salt **14 I** on treatment with hydrochloric acid (Scheme 2). Similarly, (*R*)-alcohol **11 II**[54] was transformed into (*S*)-amino hydrochloride **14 II**.

Conversions of **14 I** and **14 II** into enantiomeric products **17 I** and **17 II**, respectively, were achieved as for the racemate above except 2-picoline-borane complex[55] was used as a less toxic alternative to sodium cyanoborohydride, and triethylamine was used as a more convenient base for adjusting the pH. Treatment of amines **14 I** and **14 II** with (*S*)-α-methoxy-α-trifluoromethylphenylacetyl chloride [(*S*)-MTPACl][56] gave Mosher amides with d.e.'s of ~94%.

The (*S*)-enantiomer **17 II** structurally resembles **3** with a CHOH group removed from the latter's pyrrolidine ring. Similarly, the (*S*)-enantiomer present in (±)-**25** resembles the structure resulting from the formal removal of a methylene at the 2-position of the pyrrolidine ring in **4**. We therefore synthesized (±)-**25** as shown (Scheme 3). Acetonide alcohol **18**[57] was methylthiolated *via* its mesylate, then the isopropylidene protecting group was removed by acid-catalysed transacetalization, and the resulting diol selectively mono-silylated[58] to give alcohol (±)-**20**. Displacement of the mesylate derivative of (±)-**20** with azide followed by hydrogenation furnished amine (±)-**22** which was de-silylated then reductively alkylated with aldehyde **10** to afford (±)-**23**. Transformations (±)-**23** → (±)-**24** → (±)-**25** were carried out as described for the conversions of **15 I** to **III** → **17 I** to **III** above.

DATMe-Immucillin-H (**5**) has been identified, amongst its enantiomer and diastereomers, as a powerful PNP inhibitor (Fig. 1)[48]. Human MTAP and PNP share similar active sites and overall structural homology[59] and this, together with a crystal structure of **5** in the active site of human PNP[60], suggested that the methylthio 9-deazadenine analogue **33** was preferred as a target for MTAP/ MTANs inhibition, rather than the structure in which the alternative hydroxymethyl was substituted by methylthio (Scheme 4). The free amine present in salt **26**, prepared as for its enantiomer[61] and liberated from the benzoic acid with basic ion exchange resin, was converted to the oxazolidinone **27** with triphosgene, then deacetalized under acid-catalysed conditions to give diol **28**. Tosylation of the primary hydroxyl then displacement with sodium thiomethoxide in DMF led unexpectedly to the rearranged oxazolidinone **29**. X-ray crystallography[62] of **29** with molybdenum *K*α radiation confirmed both the relative and absolute stereochemistry. Base-catalysed hydrolysis of the oxazolidinone ring in **29** led to amine **30** which was reductively alkylated with aldehyde **10** to give **31**. The standard conversion of **31** → **32** → **33** proceeded uneventfully as shown in the Scheme. The enantiomer (*ent*-**33**) of **33** was also prepared from *ent*-**26**[61] in the same way as described for **33** (Scheme 4).

Two further diastereomers (**40** and its enantiomer *ent*-**40**) of **33** were also prepared (Scheme 5). Azido triol **34**[63] was converted into methylthio derivative **36** through regioselective formation of an intermediate 5-membered ring stannylene acetal. The structure of **36** was confirmed by ^{1}H-^{1}H DQF-COSY NMR. After reduction of the azido group in **36**, the resulting amine **37** was reductively alkylated with aldehyde **10** to give **38** then further transformed into product by the standard end sequence **38** → **39** → **40**. The enantiomer (*ent*-**40**) of **40** was made in the same way starting from *ent*-**34**[63]

In order to explore steric congestion effects on the side chain adjacent to the amino group, the site that when protonated mimics the ribosyl cation in the transition state, we prepared compound **47** - formally equivalent to adding a hydroxymethyl group to compounds **17 I** to **III**. In similar fashion to the methods presented above, the tris(hydroxymethyl)aminomethane acetonide **41**[64] was converted into the *N*-donor **44** and coupled to aldehyde **10** to provide the chloro-deazapurine derivative **45** (Scheme 6). The usual transformation of the deazapurine Cl → NH_2 (**45** → **46**) then BOM removal furnished **47**.

On the other hand, removal of a hydroxymethyl group from **33** would lead to compound **54** containing just one hydroxyl on a 3-carbon acyclic framework. The synthesis of **54** was accomplished through conversion of *N*-benzyl amine **48**[48] to the known *tert*-butyl carbamate (Boc) protected amino alcohol **49**[65] followed by successive treatment with tosyl chloride then sodium thiomethoxide to give oxazolidinone **51** in addition to the expected product **50** (Scheme 7). After removal of the *N*-Boc group in **50** with hydrochloric acid, the resulting amine salt was reductively alkylated with aldehyde **10** and the deazapurine ring modified as shown in the Scheme to give **54**.

The compounds described so far were constructed with either 3- or 4-carbon atoms in their acyclic side chains. We expanded the scope of the study by preparing products with 5-carbon side chains (Scheme 8). The 1,3-dioxepine acetal **55**[66] underwent a 1,3-dipolar cycloaddition with *N*-benzyl hydroxylamine and formaldehyde to afford (±)-**56** which after reductive cleavage of the 1,2-isoxazolidine ring, then amino protection as a benzyl carbamate (Cbz), gave (±)-**57**. Acid-catalysed rearrangement of the acetonide moiety in (±)-**57** was accomplished in acetone and gave a separable mixture of the 1,3-dioxane (±)-**58** and 1,3-dioxolane (±)-**59**. Standard transformation of the hydroxyl in (±)-**59** into a methylthio substituent followed by base-catalysed hydrolysis of the carbamate moiety gave amine (±)-**61**. Reductive amination of aldehyde **10** with (±)-**61**, using sodium

triacetoxyborohydride[67] as a less toxic alternative to sodium cyanoborohydride, gave the expected chloro-deazapurine (±)-**62**. At this stage we tried a different end sequence that avoided the use of sealed pressure vessels. Treatment of chloride (±)-**62** with sodium azide gave (±)-**63** which underwent a Staudinger reaction to produce protected amine (±)-**64**. The acetonide and BOM protecting groups were then removed by acid-catalysed hydrolysis and hydrogenolysis, respectively, to furnish (±)-**65**.

2.2. Synthesis using the Mannich reaction

N-Methylation of the secondary amine products above would provide derivatives with an altered affinity towards protonation whilst at the same time removing an *N*-H bond which may be important for enzyme inhibitor interaction. We therefore prepared a small subset of such compounds. Carbamate (±)-**60** was *N*-methylated under anhydrous, basic conditions then the Cbz group was removed by basic solvolysis to give *N*-methyl amine (±)-**66** (Scheme 9). This latter compound underwent a Mannich reaction with formaldehyde and 9-deazaadenine[49] to afford (±)-**67**, then the acetal function was hydrolysed with hydrochloric acid to give, after neutralization, (±)-**68**, the *N*-methyl analogue of (±)-**65** as the free base form.

An isomer [(±)-**72**] of (±)-**68** was also prepared by Mannich reaction except in this case the amine precursor (±)-**70** was obtained by direct reduction of the *N*-Cbz group in (±)-**69** with lithium aluminium hydride (Scheme 10).

By way of further example we also prepared (±)-**78**, equivalent to the formal removal of a methylene from the hydroxymethyl side chain found in (±)-**72** (Scheme 11). It was conveniently prepared from racemic diethyl tartrate-derived amino alcohol (±)-**73**[48] using similar chemistry to that used in the previous two schemes. In this case *N*-methylation was accomplished by reduction of the *N*-Boc function in (±)-**75** with lithium aluminium hydride.

2.3. Synthesis from a cyclic precursor

MT-Immucillin-A (**3**), a first generation MTAP/MTAN inhibitor, is ideally set up for conversion to an acyclic system through oxidative cleavage of the vicinal diol. In a one-pot reaction **3** was selectively oxidized with periodate and the resulting dialdehyde reduced with sodium borohydride to afford **79** (Scheme 12).

3. Biological evaluation

The inhibition of the phosphorolysis or hydrolysis of MTA catalysed by human MTAP or bacterial MTANs, respectively, was evaluated with 3rd generation acyclic inhibitors and the results compared with 1st and 2nd generation inhibitors, **3** and **4** (Table 1). Compounds **17 I – III**, (±)-**25** and **33** were the most potent inhibitors with the (*R*)-enantiomer (**17 I**) having K_i's of 4.4 (K_m/K_i = 1200) and 0.23 (K_m/K_i = 1870) nM against human MTAP and *E. coli* MTAN, respectively. Although **17 I** was not tested against *N. meningitidis* MTAN it is likely that this compound would also be a strong inhibitor of this enzyme given that the racemate (**17 III**) was the strongest inhibitor tested with a K_i of 0.80 nM (K_m/K_i = 1750). Racemic compound (±)-**25** was found to be just 1.5 times weaker an inhibitor than **17 I** against *E. coli* MTAN suggesting one of the enantiomers present in (±)-**25** could be of similar potency to **17 I**.

As shown in the schemes, compounds **17 I** and (±)-**25** can be drawn such that they resemble cyclic compounds **3** and **4**, respectively, with one carbon atom removed. Compound **17 I** had binding affinities in the order of 3- and 4-fold that of **3** aginst *E. coli* MTAN and human MTAP, respectively. The racemate **17 III** was about half as potent against *N. meningitidis*

MTAN indicating **17 I** could have a similar potency to **3** against the latter enzyme. Thus it appeared that compounds **17**, despite the larger number of degrees of freedom, were able to adopt conformations similar to that taken by **3** in the enzymes' active sites, resulting in comparable enzyme inhibition. Compared with **4** however, **17 I** and (±)-**25** showed approximately 110- and 180-fold weaker binding affinities, respectively, against *E. coli* MTAN and approximately 50- and 110-fold weaker binding affinities, respectively, against human MTAP. In contrast **17 III** and (±)-**25**, had smaller 6- and 9-fold differences, respectively, with *N. meningitidis* MTAN. The large differences seen with the first two enzymes were attributed to compound **4** being a better match for the late dissociative transition state found with human MTAP[37] and *E. coli* MTAN[44] than for the early transition state of *N. meningitidis* MTAN[40].

Compound **54** contains a side chain one carbon atom shorter than one of the enantiomers found in (±)-**25** and had significantly reduced inhibitory activity, particularly against *E. coli* MTAN, experiencing a drop in binding affinity in the region of 110-fold. Alternatively, compound **54** can be viewed as the product produced by the removal of a hydroxymethyl group from **33**. In contrast, compound **47** which had an extra hydroxymethyl group compared to compounds **17 I** to **III** still retained moderate activity.

Compounds **33**, **40** and their respective enantiomers *ent*-**33** and *ent*-**40** are the structures that would be formed from the formal cleavage of the 1,2-bond in the pyrrolidine ring of **3** with subsequent transposition of the terminal CH_2SMe and CH_2OH groups. Compound **33** was the strongest inhibitor of this group having approximately 7- to 17-fold greater affinity over the other compounds for human MTAP and approximately 3- to 13-fold increased affinity for the bacterial MTANs. Again compound **33** showed comparable efficacy to compound **3** being within about 2 to 10 fold of its activity against all three enzymes. Compound **33** was also of a similar potency as **17 I** was against human MTAP and as **17 III** was against *N. meningitides* MTAN, respectively, but was about 4-fold less active than **17 I** was against *E. coli* MTAN. By comparison, oxidative cleavage of the vicinal diol bond in **3** without transposition of the CH_2SMe and CH_2OH units gave **79**, and resulted in a severe loss of inhibitory activity against *E. coli* MTAN together with more moderate losses suffered against the other two enzymes.

A dramatic 250-fold loss of binding affinity occured in *E. coli* MTAN when (±)-**65**, a moderate inhibitor against all three enzymes, was *N*-methylated to (±)-**68**, making it the weakest inhibitor with a K_i of 2300 nM (K_m/K_i = 19). In contrast, inhibition of *N. meningitidis* MTAN suffered only an approximate 12-fold loss in activity by this change but inhibition improved about 3-fold against human MTAP. This loss of activity against *E. coli* MTAN may be due to removal of a critical *N*-H bond. A similar case was also observed in the related enzyme PNP where *N*-methylation of DATMe-Immucillin-H (**5**) and SerMe-Immucillin-H (**6**) also resulted in a significant loss of activity[48].

The isomeric *N*-methyl compounds (±)-**68** and (±)-**72** differ only by having their CH_2SMe and CH_2OH groups transposed. The latter compound showed a gain in binding affinity in the order of 6-fold with *E. coli* MTAN but a reduction of approximately 2- and 9-fold for *N. meningitidis* MTAN and human MTAP, respectively. On the other hand compound (±)-**78** which differs from (±)-**72** by one carbon atom, resulting in the transformation of a primary alcohol to a secondary one, produced around a 2-fold increases in potency for the bacterial MTANs but an approximate 4-fold loss in activity with human MTAP.

4. Conclusions

Cyclic compounds **3** and **4** are known powerful transition state analogue inhibitors of human MTAP and bacterial MTANs and therefore we have prepared a series of acyclic compounds to see if improved activity and simpler structures could be found as was the case with the related enzyme PNP[48]. Compounds **17 I** to **III**, (±)-**25** and **33** were identified as the best inhibitors with **17 I** being around 3- and 4-fold as active as compound **3** against *E. Coli* MTAN human MTAP, respectively, but significantly weaker than **4** against the same enzymes. (*S*)-enantiomer **17 II**, the product of the formal removal of a CHOH group from the pyrrolidine ring of **3**, was found to be a weaker inhibitor than its (*R*)-enantiomer **17 I**. Racemate (±)-**25** containing the enantiomer present in the product of formal removal of the 2-methylene from the pyrrolidine in **4** was the second most potent inhibitor found against *E. coli* MTAN but it was not determined which enantiomer had the best activity. It was also found that simple bond cleavage of a cyclic inhibitor (**3** → **79**) or *N*-methylation of a secondary amine to a tertiary one [(±)-**65** → (±)-**68**] led to dramatic losses in activity. In our previous work on the related PNP enzyme, acyclic compounds were found that were comparable or better inhibitors than the best cyclic forms and it was further shown that unfavourable binding entropies present in these acyclic derivatives were offset by favourable enthalpies of binding.[68] In the present study such thermodynamic properties remain to be determined but it is clear that with MTAP/MTANs the acyclic inhibitors described here have not achieved the same level of potency as seen with our previous PNP study, particularly when compared to **4**. The best compounds were nevertheless novel and potent inhibitors of human MTAP and *E. coli* and *N. meningitidis* MTANs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by research grant C08X0701 from the New Zealand Foundation for Research, Science & Technology and by research grants GM41916 and CA135405 from the National Institutes for Health. We are grateful to Drs Herbert Wong and Yinrong Lu for NMR and MS measurements, respectively.

References

1. Della Ragione F, Carteni-Farina M, Gragnaniello V, Schettino MI, Zappia V. J. Biol. Chem. 1986; 261:12324. [PubMed: 3091600]
2. Toorchen D, Miller RL. Biochem. Pharmacol. 1991; 41:2023. [PubMed: 1903946]
3. Miller CH, Duerre JA. J. Biol. Chem. 1968; 243:92. [PubMed: 4867478]
4. Ragione D, Porcelli FM, Carteni-Farina M, Zappia V, Pegg AE. Biochem. J. 1985; 232:335. [PubMed: 3911944]
5. Marton LJ, Pegg AE. Ann. Rev. Pharmacol. Toxicol. 1995; 35:55. [PubMed: 7598507]
6. Seiler N, Atanassov CL, Raul F. Int. J. Oncol. 1998; 13:993. [PubMed: 9772292]
7. Gupta S, Ahmad N, Marengo SR, MacLennan GT, Greenberg NM, Mukhtar H. Cancer Res. 2000; 60:5125. [PubMed: 11016639]
8. Belting M, Borsig L, Fuster MM, Brown JR, Persson L, Fransson LA, Esko JD. Proc. Natl. Acad. Sci. U SA. 2002; 99:371.
9. Wallace HM, Fraser AV. Biochem. Soc. Trans. 2003; 31:393. [PubMed: 12653646]
10. Meyskens FL Jr, Gerner EW. Clin. Cancer Res. 1999; 5:945. [PubMed: 10353725]
11. Wolford RW, MacDonald MR, Zehfus B, Rogers TJ, Ferro A. J. Cancer Res. 1981; 41:3035.
12. Williams-Ashman HG, Seidenfeld J, Galletti P. Biochem. Pharmacol. 1982; 31:277. [PubMed: 6803807]

13. Pegg AE, Borchardt RT, Coward JK. Biochem. J. 1981; 194:79. [PubMed: 7305994]
14. Pajula RL, Raina A. FEBS Letters. 1979; 99:343. [PubMed: 428559]
15. Carteni-Farina M, Cacciapuoti G, Porcelli M, Ragione FD, Lancieri M, Geraci G, Zappia V. Biochim. Biophys. Acta (BBA) – Mol. Cell Res. 1984; 805:158.
16. Avila MA, Garcia-Trevijano ER, Lu SC, Corrales FJ, Mato JM. Int. J. Biochem. Cell Biol. 2004; 36:2125. [PubMed: 15313459]
17. Andreu-Pérez P, Hernandez-Losa J, Moliné T, Gil R, Grueso J, Pujol A, Cortés J, Avila MA, Recio JA. BMC Cancer. 2010; 10:265. [PubMed: 20529342]
18. Basu I, Cordovano G, Das I, Belbin TJ, Guha C, Schramm VL. J. Biol. Chem. 2007; 282:21477. [PubMed: 17548352]
19. Basu I, Locker J, Cassera MB, Belbin TJ, Merino EF, Dong X, Hemeon I, Evans GB, Guha C, Schramm VL. J. Biol. Chem. 2011; 286:4902. [PubMed: 21135097]
20. Xavier KB, Bassler BL. Curr. Opin. Microbiol. Rev. 2003; 6:191.
21. Chen X, Schauder S, Potier N, Dorsselaer VA, Pelczer I, Bassler BL, Hughson FM. Nature. 2002; 415:545. [PubMed: 11823863]
22. Miller MB, Bassler BL. Ann. Rev. Microbiol. 2001; 55:165. [PubMed: 11544353]
23. Fuqua C, Parsek MR, Greenberg EP. Annu. Rev. Genet. 2001; 35:439. [PubMed: 11700290]
24. Zhu J, Dizin E, Hu X, Wavreille AS, Park J, Pei D. Biochemistry. 2003; 42:4717. [PubMed: 12705835]
25. Miller ST, Xavier KB, Campagna SR, Taga ME, Semmelhack MF, Bassler BL, Hughson FM. Mol. Cell. 2004; 15:677. [PubMed: 15350213]
26. Meijler MM, Horn LG, Kaufmann GF, McKenzie KM, Sun C, Moss JA, Matsushita M, Janda KD. Angew. Chem. Int. Ed. 2004; 43:2106.
27. Schramm VL. Acc. Chem. Res. 2003; 36:588. [PubMed: 12924955]
28. Schramm VL. Arch. Biochem. Biophys. 2005; 433:13. [PubMed: 15581562]
29. Schramm, VL.; Tyler, PC. In iminosugars from synthesis to therapeutic applications. Compain, P.; Martin, OR., editors. England: John Wiley & Sons Ltd; p. 177-208.
30. Kline PC, Schramm VL. Biochemistry. 1993; 32:13212. [PubMed: 8241176]
31. Lewandowicz A, Tyler PC, Evans GB, Furneaux RH, Schramm VL. J. Biol. Chem. 2003; 278:31465. [PubMed: 12842889]
32. Lewandowicz A, Schramm VL. Biochemistry. 2004; 43:1458. [PubMed: 14769022]
33. Miles RW, Tyler PC, Furneaux RH, Bagdassarian CK, Schramm VL. Biochemistry. 1998; 37:8615. [PubMed: 9628722]
34. Evans GB, Furneaux RH, Schramm VL, Singh V, Tyler PC. J. Med. Chem. 2004; 47:3275. [PubMed: 15163207]
35. Evans GB, Furneaux RH, Lenz DH, Painter GF, Schramm VL, Singh V, Tyler PC. J. Med. Chem. 2005; 48:4679. [PubMed: 16000004]
36. Singh V, Shi W, Evans GB, Tyler PC, Furneaux RH, Almo SC, Schramm VL. Biochemistry. 2004; 43:9. [PubMed: 14705926]
37. Singh V, Schramm VL. J. Am. Chem. Soc. 2006; 128:14691. [PubMed: 17090056]
38. Horenstein BA, Parkin DW, Estupinan B, Schramm VL. Biochemistry. 1991; 30:10788. [PubMed: 1931998]
39. Parkin DW, Schramm VL. Biochemistry. 1995; 34:13961. [PubMed: 7577992]
40. Gutierrez JA, Luo M, Singh V, Li L, Brown RL, Norris GE, Evans GB, Furneaux RH, Tyler PC, Painter GF, Lenz DH, Schramm VL. ACS Chemical Biology. 2007; 2:725. [PubMed: 18030989]
41. Lee JE, Singh V, Evans GB, Tyler PC, Furneaux RH, Cornell KA, Riscoe MK, Schramm VL, Howell PL. J. Biol. Chem. 2005; 280:18274. [PubMed: 15746096]
42. Luo M, Schramm VL. J. Am. Chem. Soc. 2008; 130:11617. [PubMed: 18693725]
43. Singh V, Lee JE, Nunez S, Howell PL, Schramm VL. Biochemistry. 2005; 44:11647. [PubMed: 16128565]
44. Singh V, Evans GB, Lenz DH, Mason JM, Clinch K, Mee S, Painter GF, Tyler PC, Furneaux RH, Lee JE, Howell PL, Schramm VL. J. Biol. Chem. 2005; 280:18265. [PubMed: 15749708]

45. Singh V, Shi W, Almo SC, Evans GB, Furneaux RH, Tyler PC, Painter GF, Lenz DH, Mee S, Zheng R, Schramm VL. Biochemistry. 2006; 45:12929. [PubMed: 17059210]
46. Singh V, Luo M, Brown RL, Norris GE, Schramm VL. J. Am. Chem. Soc. 2007; 129:13831. [PubMed: 17956098]
47. Singh V, Schramm VL. J. Am. Chem. Soc. 2007; 129:2783. [PubMed: 17298059]
48. Clinch K, Evans GB, Fröhlich RFG, Furneaux RH, Kelly PM, Legentil L, Murkin AS, Li L, Schramm VL, Tyler PC, Woolhouse AD. J. Med. Chem. 2009; 52:1126. [PubMed: 19170524]
49. Evans GB, Kelly PM, Luxenburger A, Guan R, Suarez J, Thomas K, Schramm VL, Tyler PC. Manuscript in preparation.
50. Evans GB, Furneaux RH, Hutchison TL, Kezar HS, Morris PE, Schramm VL, Tyler PC. J. Org. Chem. 2001; 66:5723. [PubMed: 11511245]
51. Gainsford GJ, Mason JM, Gulab SA. Acta Crystallogr. Sect. E. 2010; 66:o138.
52. Murkin AS, Clinch K, Mason JM, Tyler PC, Schramm VL. Bioorg. Med. Chem. Lett. 2008; 18:5900. [PubMed: 18778937]
53. Borch RF, Bernstein MD, Durst HD. J. Am Chem. Soc. 1971; 93:2897.
54. Dondoni A, Perrone D. Org. Synth. 2004; Coll. Vol. 10:320. *Org. Synth.***2000**, *77*, 64.
55. Sato S, Sakamoto T, Miyazawa E, Kikugawa Y. Tetrahedron. 2004; 60:78–99. 2-Picoline-borane complex is now the reagent of choice for conducting reductive amination/alkylation reactions in our laboratories.
56. Ward DE, Rhee CK. Tetrahedron Lett. 1991; 32:7165.
57. Harnden MR, Wyatt PG, Boyd MR, Sutton D. J. Med. Chem. 1990; 33:187. [PubMed: 2153202]
58. McDougal PG, Rico JG, Oh YI, Condon BD. J. Org. Chem. 1986; 51:3388.
59. Appleby TC, Erion MC, Ealick SE. Structure. 1999; 7:629. [PubMed: 10404592]
60. Ho M-C, Shi W, Rinaldo-Matthis A, Tyler PC, Evans GB, Clinch K, Almo SC, Schramm VL. Proc. Nat. Acad. Sci. 2010; 107:4805. [PubMed: 20212140]
61. Inaba T, Yamada Y, Abe H, Sagawa S, Cho H. J. Org. Chem. 2000; 65:1623. [PubMed: 10750488]
62. Gainsford GJ, Clinch K. Acta Crystallogr. Sect. E. 2012; 68:o2082.
63. Calderón F, Doyagüez EG, Fernández-Mayoralas A. J. Org. Chem. 2006; 71:6258. [PubMed: 16872215]
64. Ooi H, Ishibashi N, Iwabuchi Y, Ishihara J, Hatakeyama S. J. Org. Chem. 2004; 69:7765. [PubMed: 15498013]
65. Kokotos G, Verger R, Chiou A. Chem. Eur. J. 2000; 6:4211. [PubMed: 11128286]
66. Elliott WJ, Fried J. J. Org. Chem. 1976; 41:2469. [PubMed: 932865]
67. Abdel-Magid AF, Maryanoff CA, Carson KG. Tetrahedron Lett. 1990; 31:5595.
68. Edwards AA, Mason JM, Clinch K, Tyler PC, Evans GB, Schramm VL. Biochemistry. 2009; 48:5226. [PubMed: 19425594]

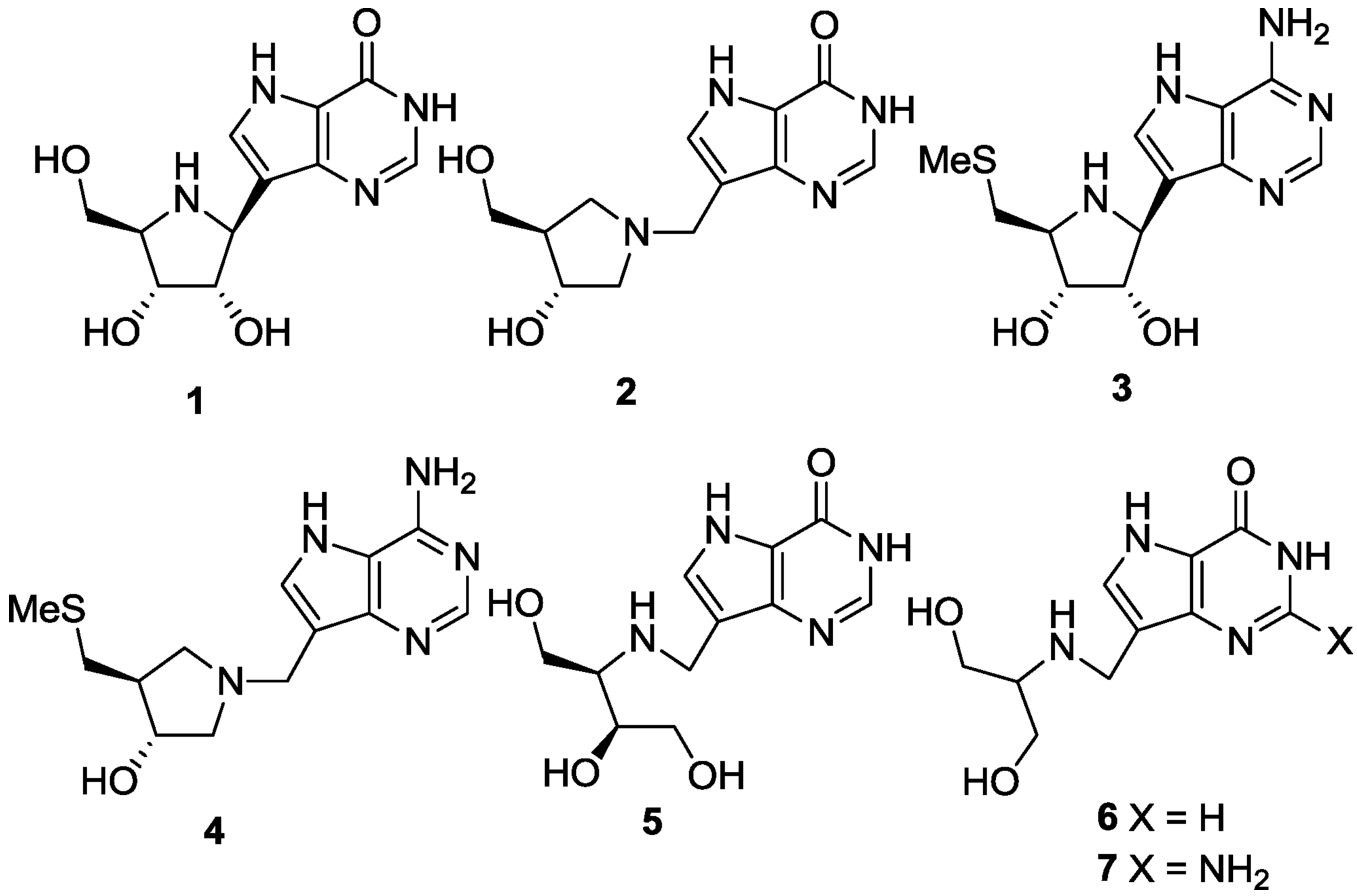
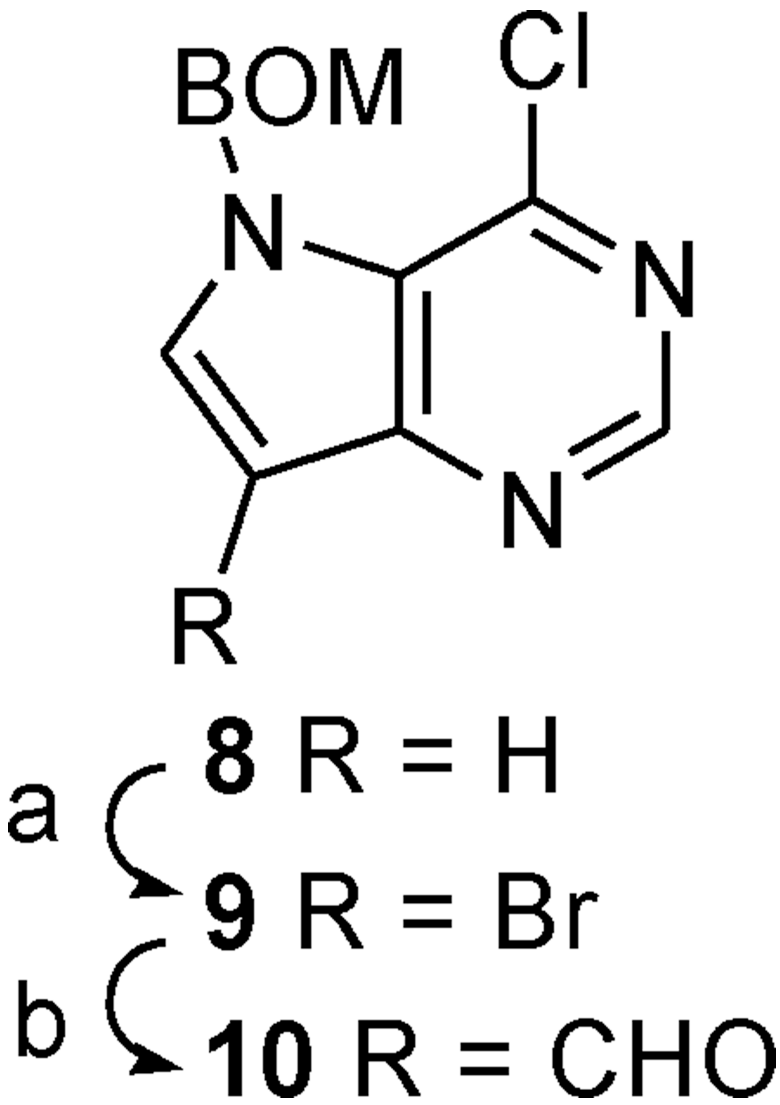
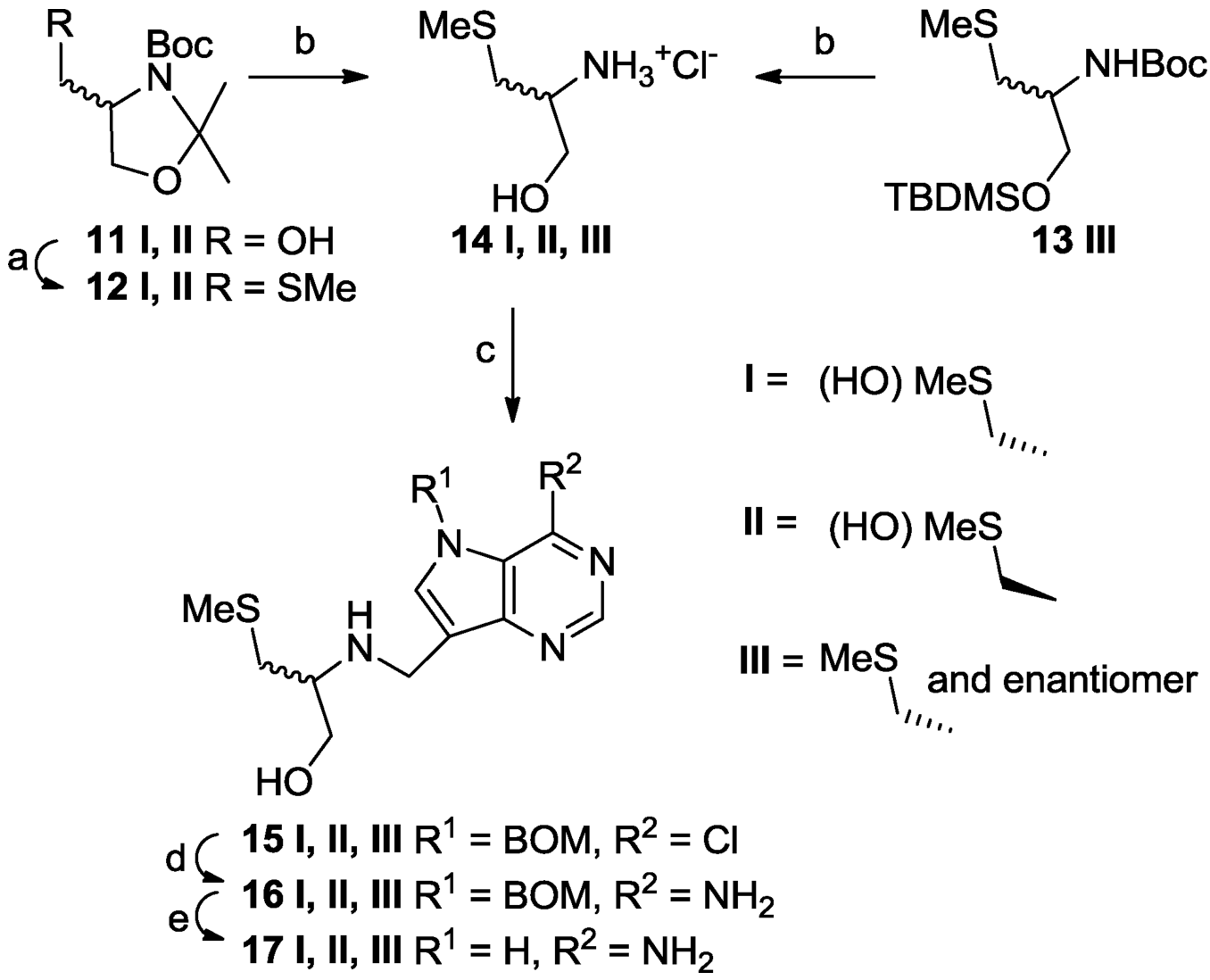


Figure 1.
Structures of known transition state inhibitors of various *N*-ribosyltransferases.

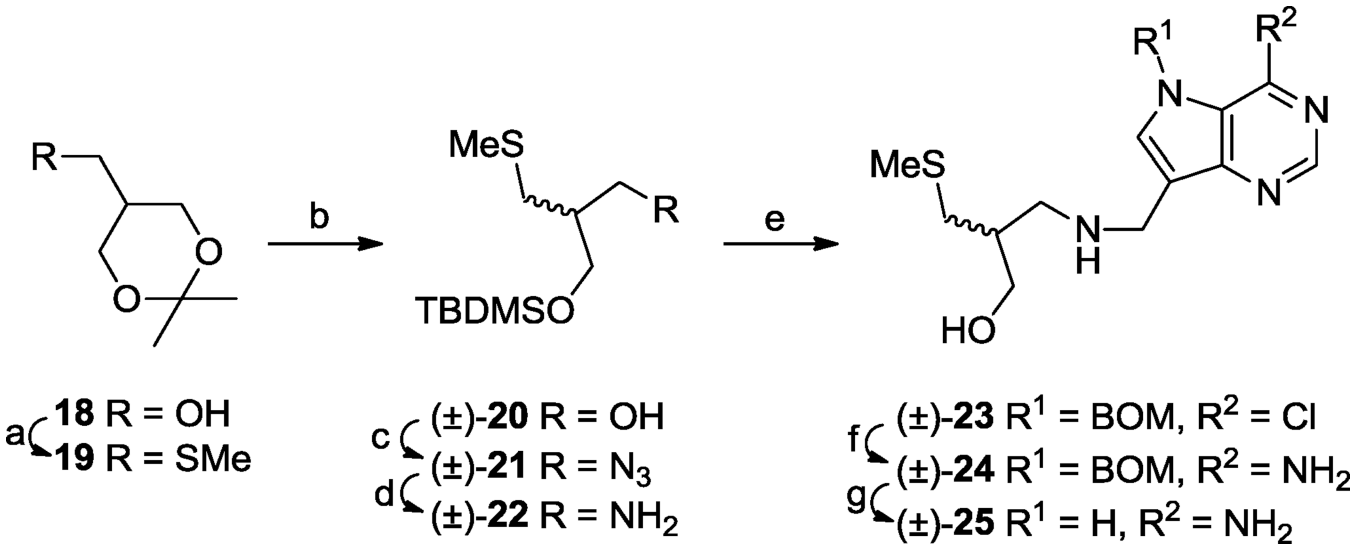


Scheme 1.
(a) NBS, 0 °C → rt, 1 h, 71%; (b) (i) *n*-BuLi, anisole-Et_2O (1:3), −78 °C, 3 min, (ii) DMF, −78 → −40 °C, (iii) H_2O, −40 °C → rt, 57%.



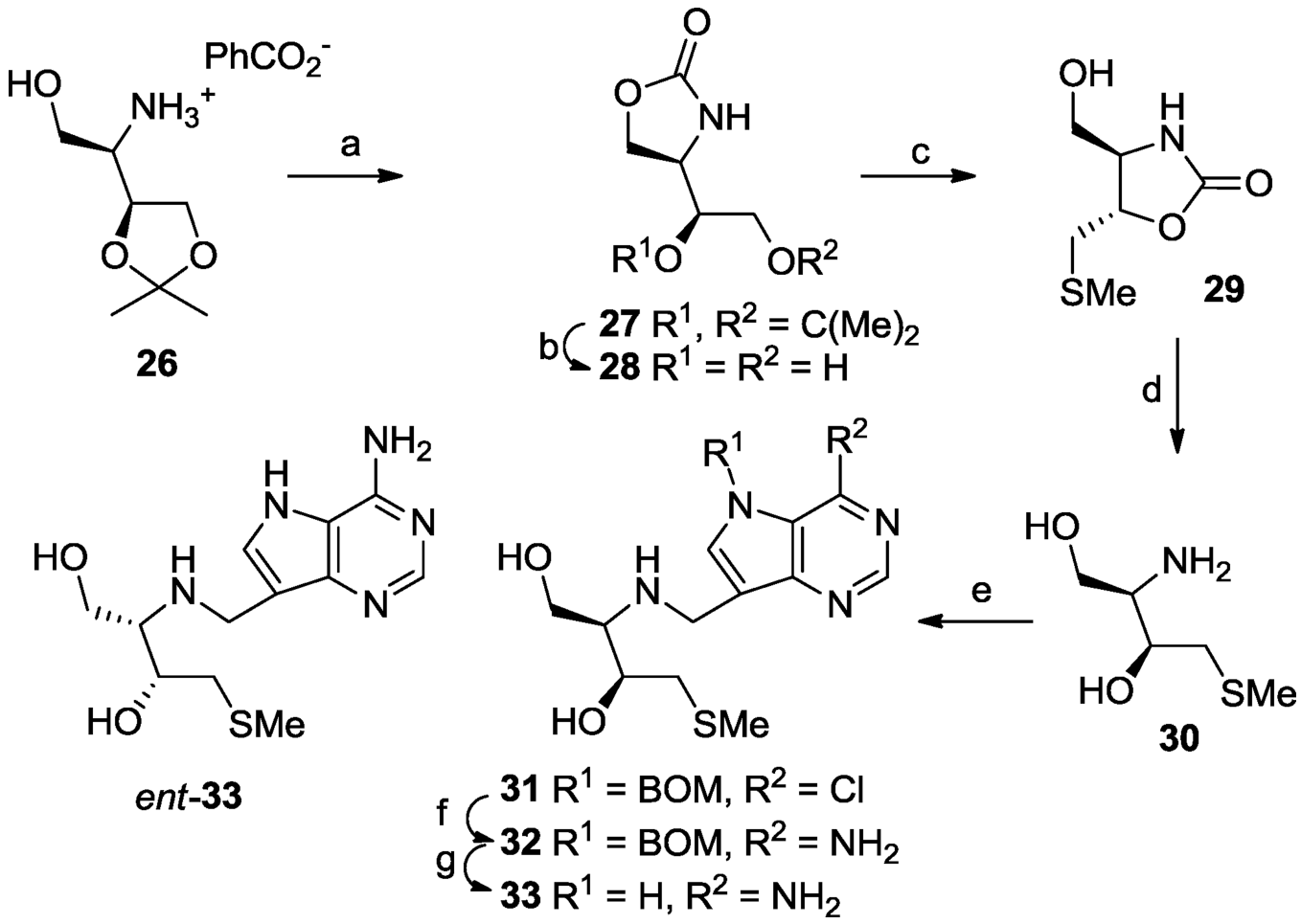
Scheme 2.

(a) (i) MsCl, Et_3N, CH_2Cl_2, 0 °C → rt, 30 min, (ii) NaSMe, DMF, rt, 1 h, 75% (**I**), 91% (**II**); (b) aq. HCl (37%), MeOH, 0 °C → rt, 1 h, 100% (**I**, **II** and **III**); (c) **10**, 2-picoline-borane complex (for **I** and **II**) or $NaCNBH_3$ (for **III**), MeOH, Et_3N (for **I** and **II**) or $NaHCO_3$ (for **III**), rt, 16 h, 67% (**I**), 78% (**II**), 71% (**III**); (d) 7M NH_3-MeOH, 135 °C, sealed tube, 24–30 h, 74% (**I**), 86% (**II**), 59% (**III**); (e) NH_2NH_2•H_2O, Pd black, 7M NH_3-MeOH, rt 1 h, 84% (**I**), 79% (**II**), 76% (**III**).



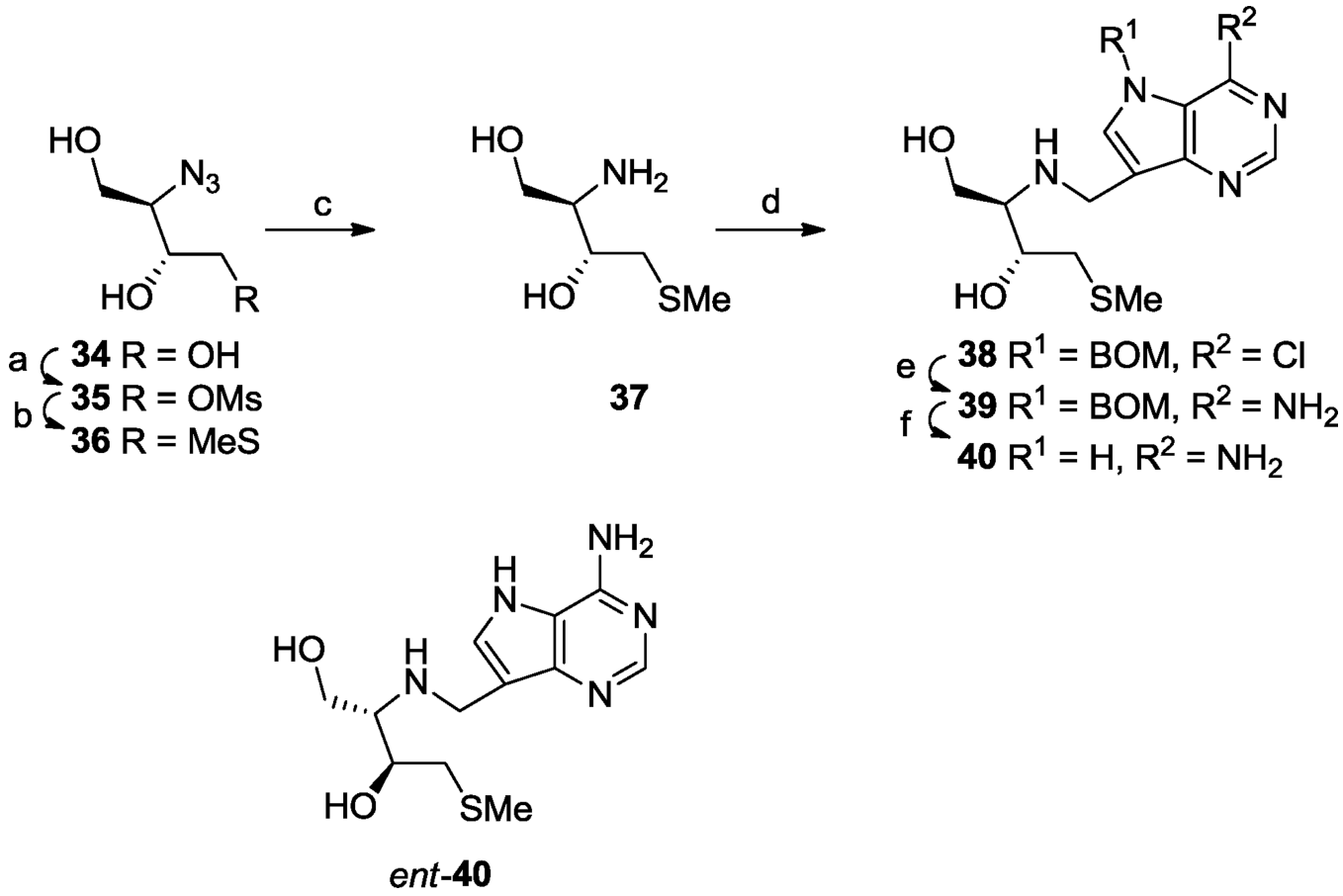
Scheme 3.

(a) (i) MsCl, Et_3N, 0 °C → rt, 30 min, (ii) NaSMe, DMF, rt, 16 h, 76%; (b) (i) AcCl, MeOH, rt, 1 h, (ii), NaH, TBDMSCl, rt, 2 h, 75%; (c) (i) MsCl, Et_3N, 0 °C → rt, 30 min, (ii) NaN_3, DMF, 80 °C, 3 h, 80%; (d) $NH_2NH_2•H_2O$, Pd black, MeOH, rt 1 h, 82%; (e) (i) aq. HCl (37%), MeOH, rt, 1 h, (ii) **10**, $NaCNBH_3$, $NaHCO_3$, MeOH, (iii) 7M NH_3-MeOH, 135 °C, sealed tube, 24 h, 20%; (f) $NH_2NH_2•H_2O$, Pd black, 7M NH_3-MeOH, rt 1 h, 54%.



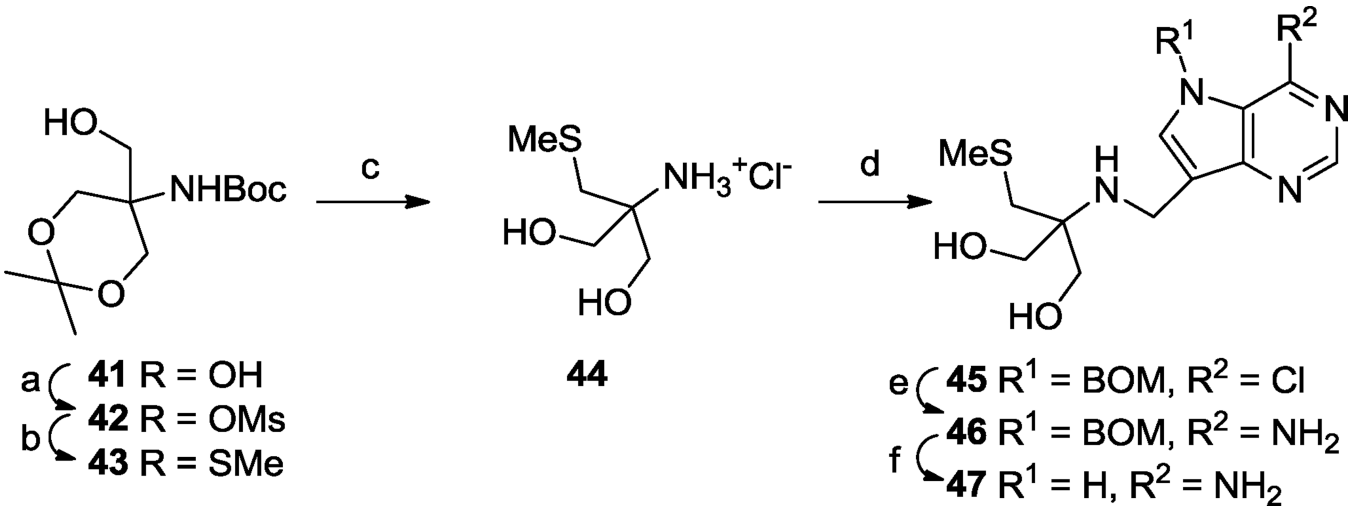
Scheme 4.

(a) (i) Amberlyst A26 (OH^-) resin, MeOH; (ii) triphosgene, CH_2Cl_2, Et_3N, rt, 90 min, 92%; (b) AcCl, MeOH, rt, 5 h, 93%; (c) (i) TsCl, pyridine, 0 °C → rt, 16 h; (ii) NaSMe, DMF, rt, 3 h, 44%; (d) KOH, *i*-PrOH, 80 °C, 4 h, 94%; (e) **10**, AcCl, MeOH, $NaCNBH_3$, rt, 3 h, 52%; (f) 7M NH_3-MeOH, 135 °C, sealed tube, 24 h, 60%; (g) NH_2NH_2•H_2O, Pd black, 7M NH_3-MeOH, rt 1 h, 74%.



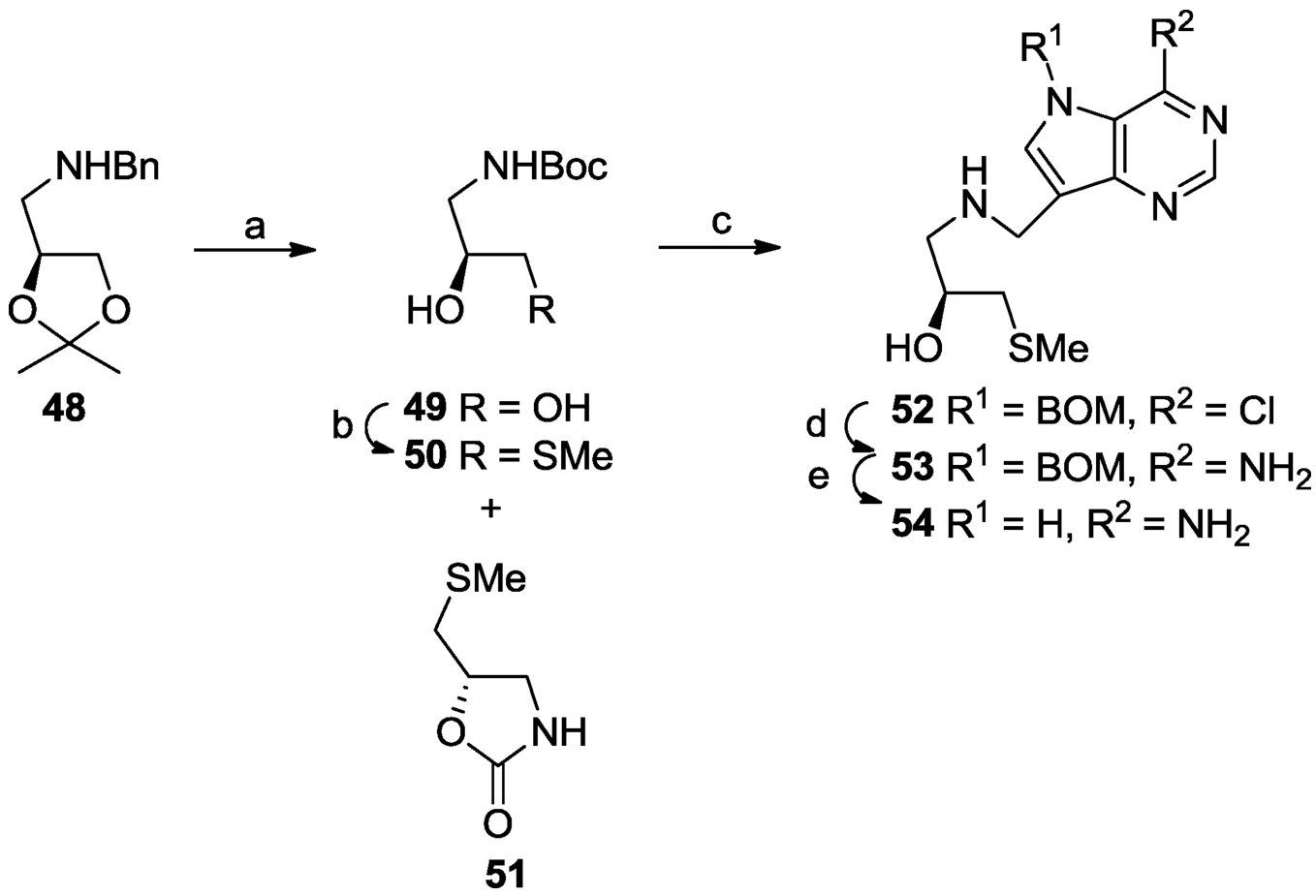
Scheme 5.

(a) (i) Bu_2SnO, toluene, reflux, 30 min, (ii) MsCl, rt, 16 h; (b) NaSMe, DMF, rt, 2 h, 37% over 2 steps; (c) LAH, THF, 0 °C → rt, 1 h, 65%; (d) **10**, $NaCNBH_3$, HCl, MeOH, 61%; (e) 6M NH_3-EtOH, 130 °C, sealed tube, 48 h, 73%; (g) $NH_2NH_2•H_2O$, Pd black, 7M NH_3-MeOH, rt 1 h, 61%.



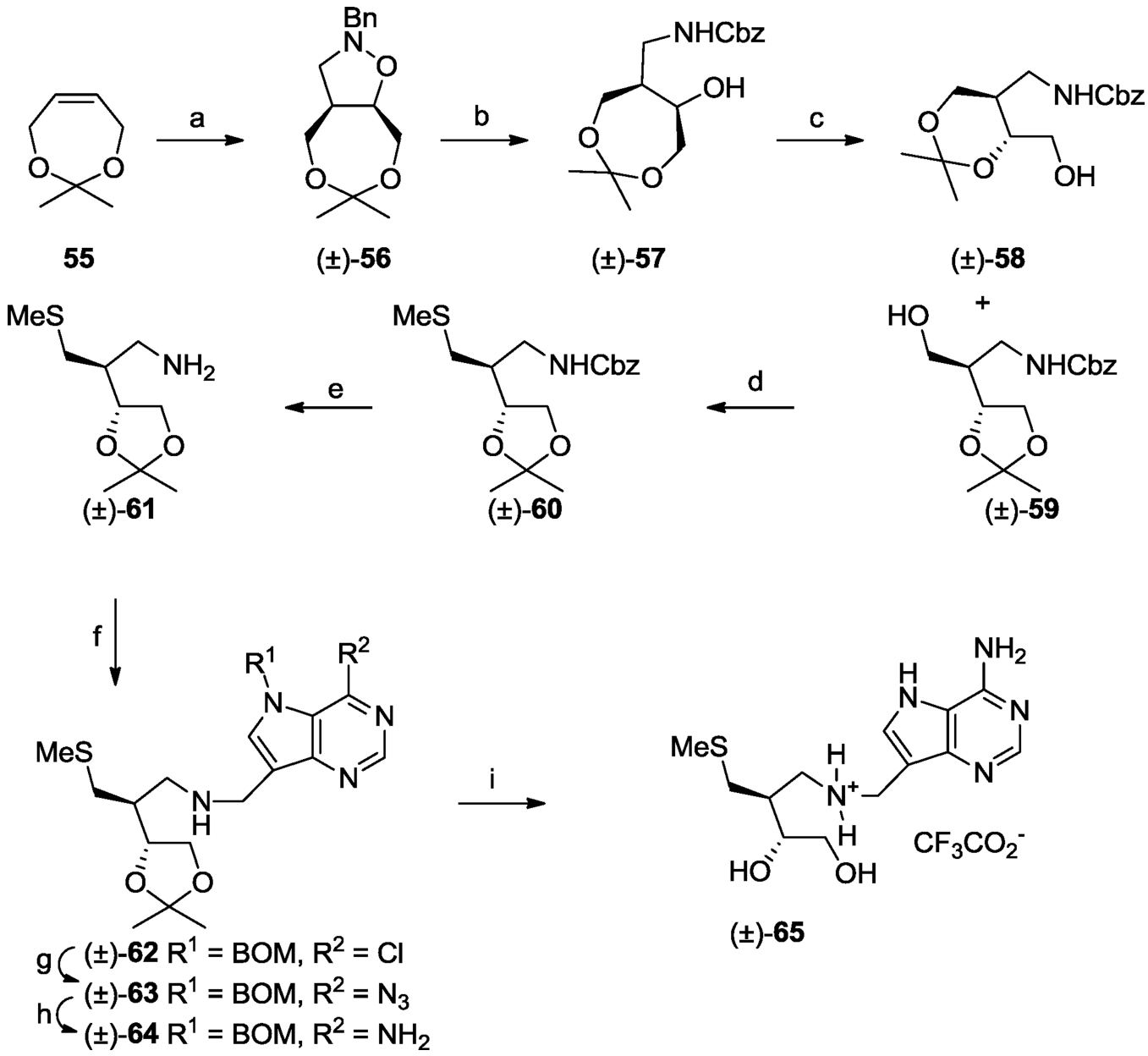
Scheme 6.

(a) MsCl, Et_3N, CH_2Cl_2, 0 °C → rt, 90 min; (b) NaSMe, DMF, rt, 15 h; (c) aq. HCl (37%), MeOH, rt, 65% over 3 steps; (d) **10**, $NaCNBH_3$, MeOH, rt, 15 h; (e) 7M NH_3-MeOH, 135 °C, sealed tube, 20 h, 78% over 2 steps; (f) $NH_2NH_2•H_2O$, Pd black, 7M NH_3-MeOH, rt 1 h, 56%.



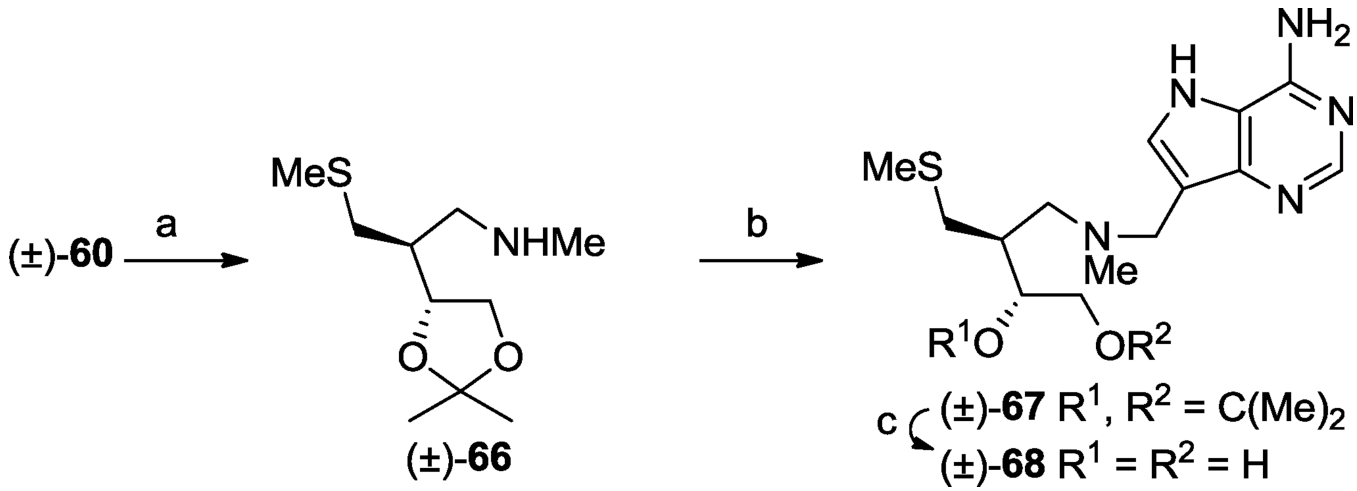
Scheme 7.

(a) (i) H_2, Pd/C, EtOH, rt, 16 h, (ii) 6M HCl, 100 °C, 30 min, (iii) $(Boc)_2O$, Et_3N, MeOH, rt, 2 h, 66%; (b) (i) TsCl, pyridine, 0 °C → rt, 42 h, (ii) NaSMe, DMF, rt, 2 h, 22% (**50**), 16% (**51**); (c) (i) aq. HCl (37%), MeOH, rt, 5 min, (ii) **10**, $NaCNBH_3$, MeOH, rt, 60 h, 44%; (d) 7M NH_3-MeOH, 135 °C, sealed tube, 24 h, 72%; (e) NH_2NH_2•H_2O, Pd black, 7M NH_3-MeOH, rt, 40 min, 76%.



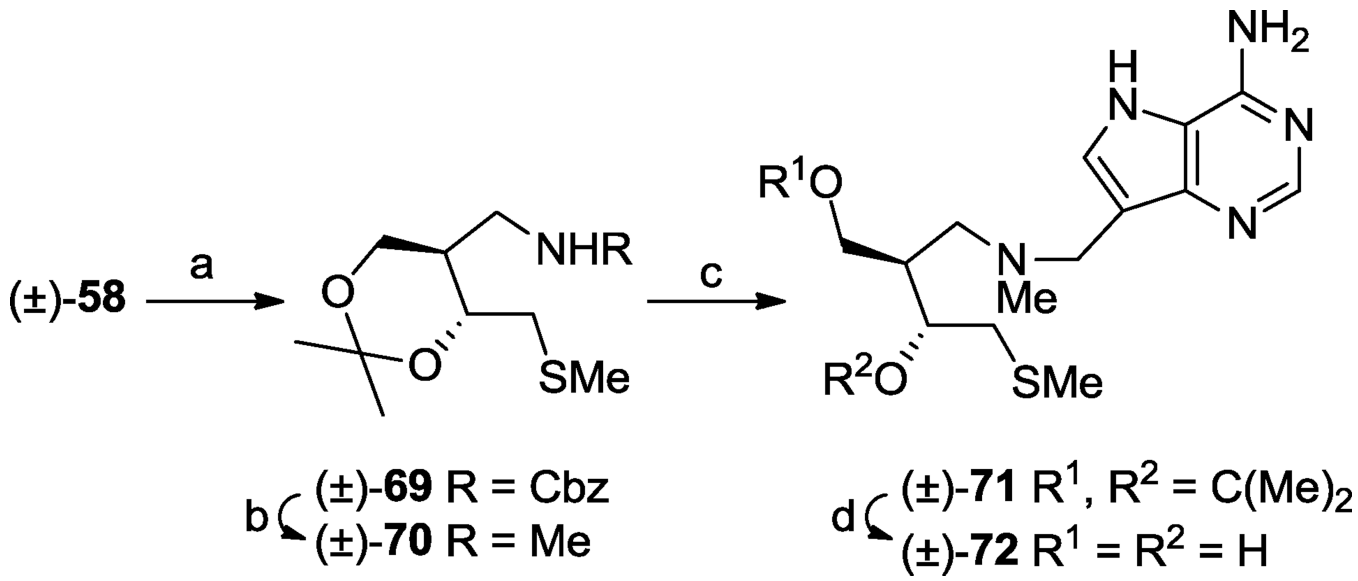
Scheme 8.

One enantiomer series present in the racemic mixtures is drawn to illustrate relative stereochemistry. (a) BnNHOH•HCl, aq. CH_2O, NaOAc, EtOH, reflux, 6 h, 43%; (b) (i) H_2, Pd/C. EtOH, rt, 2 days, (ii) BnOCOCl, Et_3N, 0 °C → rt, 30 min, 63%; (c) camphor sulfonic acid, Me_2CO, rt, 40 min, (±)-**58** (52%), (±)-**59** (44%); (d) (i) MsCl, *i*-Pr_2NEt, CH_2Cl_2, rt 15 min, (ii) NaSMe, DMF, rt, 1 h, 83%; (e) KOH, *i*-PrOH, reflux, 2 h, 83%; (f) **10**, $Na(OAc)_3BH$, 1,2-dichloroethane, rt, 40 min, 64%; (g) NaN_3, DMF, 90 °C, 1 h, 100%; (h) PMe_3, THF, aq. NH_3, rt, 1.5 h, 91%; (i) TFA-H_2O, rt, 10 min, then NH_2NH_2•H_2O, Pd black, 7M NH_3-MeOH, rt 1 h, 100%.



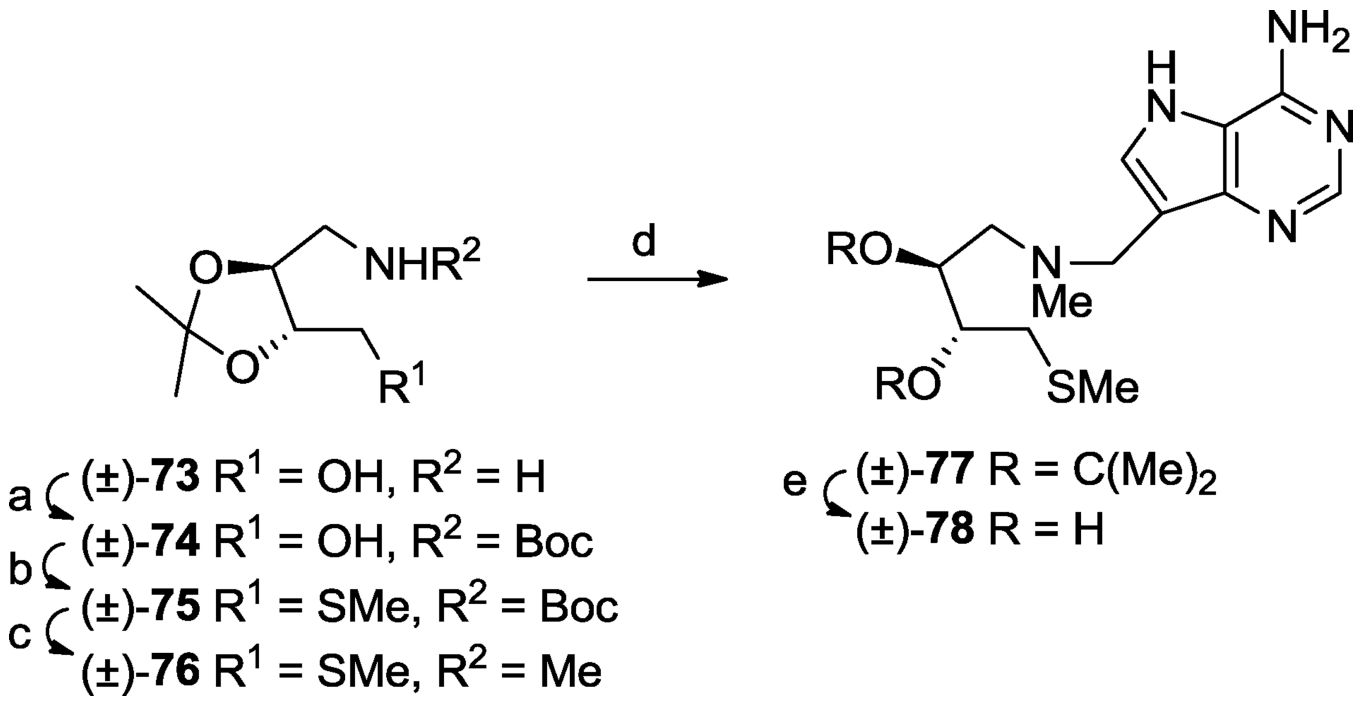
Scheme 9.

One enantiomer series present in the racemic mixtures is drawn to illustrate relative stereochemistry. (a) (i) NaH, THF, MeI, rt, 30 min, (ii) KOH, *i*-PrOH, reflux, 5 h, 77%; (b) 9-deazaadenine, CH_2O, 1,4-dioxane, H_2O, 90 °C, 1 h, (c) aq. HCl (37%)- MeOH, rt, 61% over 2 steps.



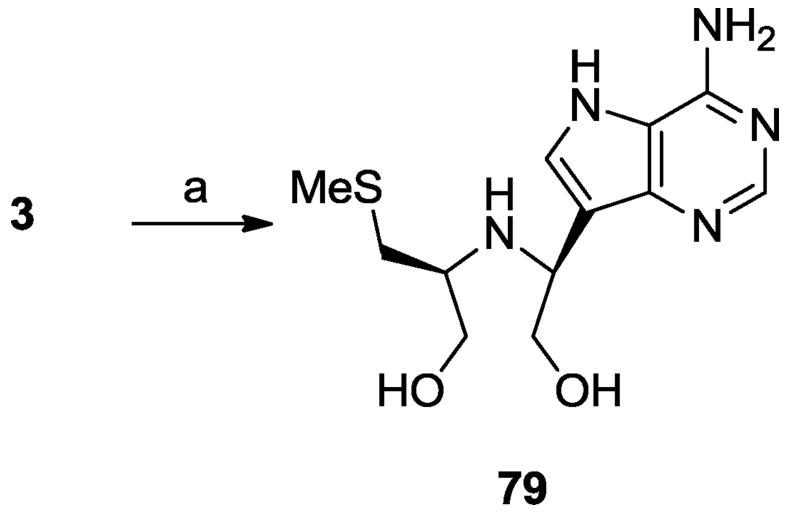
Scheme 10.

One enantiomer series present in the racemic mixtures is drawn to illustrate relative stereochemistry. (a) (i) MsCl, *i*-Pr_2NEt, CH_2Cl_2, rt 15 min, (ii) NaSMe, DMF, rt, 1 h, 86%; (b) LAH, THF, rt, 18 h, 57%; (c) 9-deazaadenine, CH_2O, 1,4-dioxane, H_2O, 85 °C, 15 min, 68%; (d) aq. HCl (37%), MeOH, rt, 79%.



Scheme 11.

One enantiomer series present in the racemic mixtures is drawn to illustrate relative stereochemistry. (a) $(Boc)_2O$, MeOH, rt, 1 h, 85%; (b) (i) MsCl, Et_3N, CH_2Cl_2, rt, 30 min, (ii) NaSMe, DMF, rt 2 h, 60%; (c) LAH, THF, reflux, 1 h, 74%; (d) 9-deazaadenine, CH_2O, 1,4-dioxane, H_2O, 90 °C, 1 h; (e) aq. HCl (37%), MeOH, rt, 63% over 2 steps.



79

Scheme 12.
(a) (i) $NaIO_4$, H_2O, rt, 1 h, (ii) $NaBH_4$, rt, 15 min, 47%.

Table 1

Inhibitory constants (nM) for acyclic compounds *vs* human MTAP and bacterial MTANs

Compound	K_i *human* MTAP[a] nM	K_i *E. coli* MTAN[a] nM	K_i *N. men.* MTAN[a] nM
3	1.0 ± 0.5[b,c]	0.08 ± 0.02[d]	0.36[e]
4	0.09 ± 0.01[c]	0.002 ± .0002[d]	0.14[e]
17 III	12 ± 1	1.7 ± 0.2	0.80 ± .06
17 I	4.4 ± 0.2	0.23 ± 0.2	ND[f]
17 II	34 ± 3	1.1 ± 0.4	ND[f]
(±)-**25**	10 ± 1	0.36 ± 0.04	1.2 ± 0.1
33	5.2 ± 0.4	0.8 ± 0.1	0.9 ± 0.1
ent-**33**	87 ± 11	3.9 ± 0.5	6 ± 1
40	87 ± 8	10 ± 2	10 ± 1
ent-**40**	34 ± 18	2.1 ± 0.5	1.8 ± 0.4
47	34 ± 24	5.8 ± 0.8	4 ± 2
54	602 ± 83	40 ± 5	36 ± 4
(±)-**65**	34 ± 6	9 ± 2	5 ± 1
(±)-**68**	12 ± 2	2300 ± 600	61 ± 5
(±)-**72**	105 ± 30	401 ± 37	132 ± 9
(±)-**78**	368 ± 153	202 ± 35	85 ± 6
79	130 ± 20	1720 ± 850	47 ± 4

[a] for compounds **3** and **4** these are K_i* values;

[b] Ref 34;

[c] Ref 35;

[d] Ref 44;

[e] Ref 40;

[f] ND – not determined