Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 2608–2613
Bioorganic & Medicinal Chemistry Letters

Tetrazole and ester substituted tetrahydoquinoxalines as potent cholesteryl ester transfer protein inhibitors

C. Todd Eary,* Zachary S. Jones, Robert D. Groneberg, Laurence E. Burgess, David A. Mareska, Mark D. Drew, James F. Blake, Ellen R. Laird, Devan Balachari, Michael O'Sullivan, Andrew Allen and Vivienne Marsh

Array BioPharma, 3200 Walnut Street Boulder, CO 80301, USA

Received 3 January 2007; revised 30 January 2007; accepted 31 January 2007
Available online 8 February 2007

Abstract—Cholesteryl ester transfer protein is a plasma glycoprotein that transfers cholesterol ester between lipoprotein particles. Inhibition of this protein, in vitro and in vivo, produces an increase in plasma high density lipoprotein cholesterol (HDL-C). This communication will describe the SAR and synthesis of a series of substituted tetrahydroquinoxaline CETP inhibitors from early μ lead to advanced enantiomerically pure analogs.

Atherosclerosis is a systemic disease that consists of the accumulation of lipid-rich plaques within the walls of large arteries; it contributes to coronary heart disease, stroke, and peripheral vascular disease. Atherosclerosis is the leading cause of deaths in industrialized nations and the standard for treatment is the administration of statins to decrease low density lipoprotein cholesterol levels (LDL-C). In addition to reducing the number of coronary events through the reduction of LDL-C, statins have been shown to modestly increase (5–10%) high density lipoprotein cholesterol (HDL-C).[1]

The anti-atherogenic role of HDL in reverse cholesterol transport is now well established and the elevation of HDL has become a therapeutic target. Studies have shown an inverse relationship between HDL and cardiovascular disease, even a modest 1 mg/dL elevation of HDL may reduce the risk of cardiovascular disease by 2–3 percent.[2] At present, niacin and Niaspan, extended release niacin, are the most effective therapeutic agents to elevate HDL-C.[3] These treatments can elevate HDL-C from 15% to 35% however; niacin can produce undesirable side effects such as flushing and hepatotoxicity.[4]

Cholesteryl ester transfer protein (CETP) is a plasma glycoprotein that transfers cholesteryl ester between lipoprotein particles with a balanced exchange of triglyceride (TG). The net effect of CETP is to remove cholesteryl ester from HDL and transfer it to LDL and VLDL. Populations deficient in CETP do show increased levels of HDL.[5] Several research programs are currently targeting the inhibition of CETP as a method to raise HDL-C levels and several potent inhibitors have been disclosed (Fig. 1).[6–8] The most advanced programs are Pfizer's Torcetrapib and Roche/Japan Tobacco's JTT-705, these programs entered into phase III with significant elevations of HDL-C observed in phase II clinical trials.[9]

We recently became interested in pursuing a research program on CETP inhibition and, following an evaluation of published work, we chose a substituted tetrahydroquinoxaline as a potentially novel lead structure (Fig. 2). This type of core would provide multiple points for further synthetic diversification as shown.

The substituted tetrahydroquinoxaline cores were prepared in a three-step sequence beginning with S_NAr reactions on commercially available *ortho* nitro aryl fluorides (Scheme 1). The appended alcohols **1** and **2** were converted to mesylates under standard conditions. The tetrahydroquinoxalines **5** and **6** were formed through reduction of the nitro group followed by intramolecular S_N2 displacement of the mesylate group. Compound **7**

Keywords: Cholesteryl ester transfer protein; CETP; HDL; Tetrahydoquinoxalines; Cholesterol.
* Corresponding author. Tel.: +1 303 386 1101; e-mail: teary@arraybiopharma.com

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.01.112

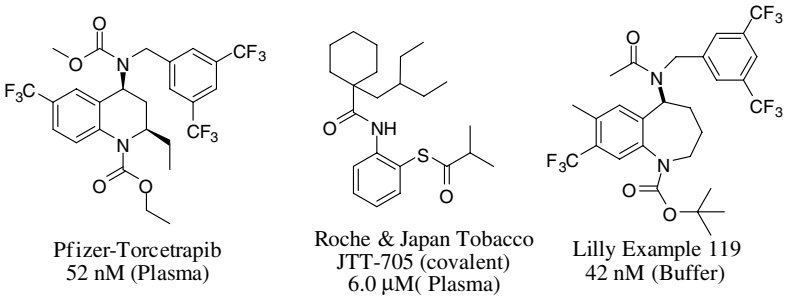


Figure 1. IC_{50} values for cholesteryl ester transfer protein inhibitors.

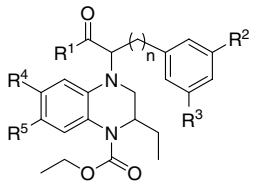


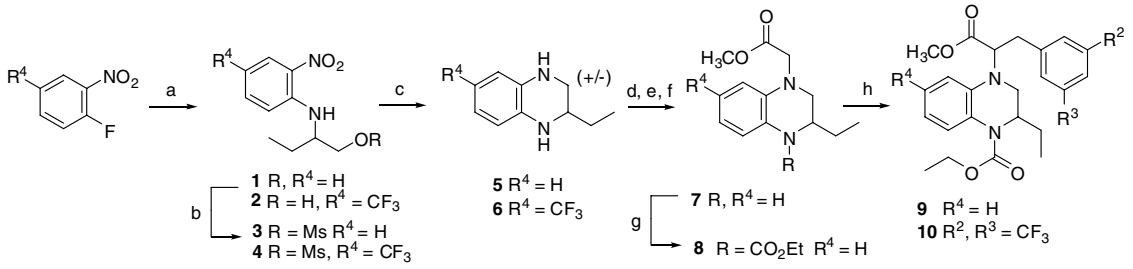
Figure 2. Substituted tetrahydroquinoxalines as CETP inhibitors.

was prepared from selective reductive amination of the less hindered nitrogen with ethyl oxoacetate and conversion to the methyl ester. The remaining aniline was capped with an ethyl carbamate group followed by subsequent enolate alkylation to give **9** and **10**.[10]

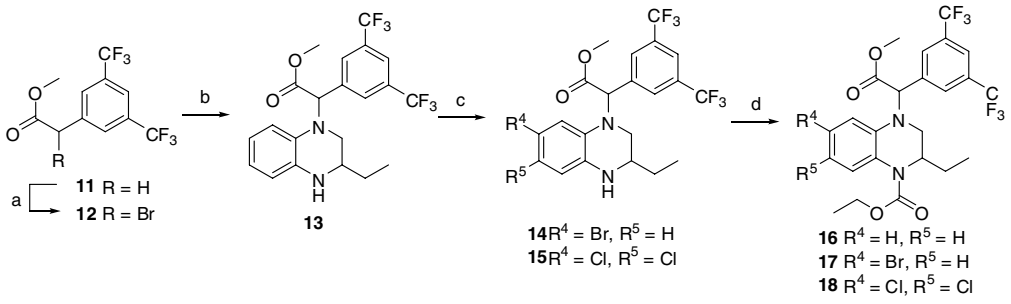
The chain-shortened analogs ($n = 0$) were prepared from benzylic bromination of methyl 3,5-bis(trifluoromethyl)phenyl acetate (**11**) (Scheme 2).[11] The resulting bromides were treated with tetrahydroquinoxaline core **5** under S_N2 conditions to provide **13**.[12] Compound **13** underwent electrophilic aromatic halogenation to give **14**[13] and **15**. The remaining unsubstituted anilines were capped with the ethyl carbamate functionality to give analogs **16**–**18**.

Since the S_N2 approach utilized to prepare **13** was unsuccessful for the 6-trifluoromethyl series an alternate route was undertaken (Scheme 3). Selective reductive amination with 3,5-bis(trifluoromethyl)benzaldehyde followed by acylation with ethyl chloroformate gave compound **21**. Selective benzylic lithiation and subsequent trapping with methyl chloroformate produced the desired carbon–carbon bond in **22**.

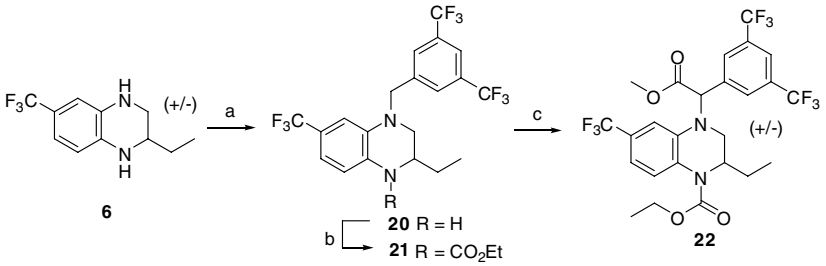
The preliminary SAR of these series (Table 1) from a human plasma CETP assay[14] indicated that direct attachment of the aryl ring to the N2 methine was optimal to inhibit CETP. Additionally it was found that deviation away from an ester to methyl, dimethyl, and



Scheme 1. Reagents and conditions: (a) 2-aminobutan-1-ol, K_2CO_3, DMF rt to 80 °C (75–90%); (b) MsCl, py, DCM (50–70%); (c) H_2, Pd/C, NMP, then TBAI, 80 °C (50–60%); (d) ethyl 2-oxoacetate, $NaCNBH_3$, MeOH, Δ (50–80%); (e) LiOH, MeOH, 70–85%; (f) $TMSCHN_2$, THF/MeOH (90%); (g) $ClCO_2Et$, py, DCM, (76%); (h) LDA, HMPA, THF, 3,5-bis CF_3 benzyl bromide or benzyl bromide, −78 °C (50–60%).

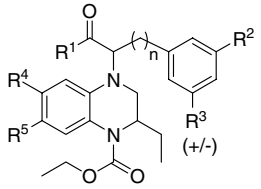


Scheme 2. Reagents and condition: (a) NBS, AIBN, CCl4, Δ (50%); (b) **5**, TBAI, K_2CO_3, DMF (40–60%); (c) **15**, NBS, DMF or NCS, DMF (5–22%); (d) $ClCO_2Et$, py, DCM (44–65%).



Scheme 3. Reagents and conditions: (a) 3,5-bis CF_3 benzaldehyde, $NaCNBH_3$, MeOH rt to 80 °C (50%); (b) $ClCO_2Et$, Cs_2CO_3, DCM (50%); (c) sec-BuLi, HMPA, THF, $ClCO_2Me$, −78 °C (10–35%).

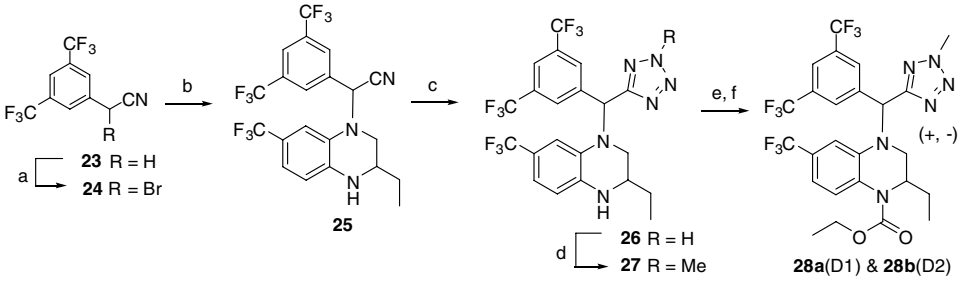
Table 1. Activity of preliminary analogs in the plasma CETP assay



Compound	R^1	R^2	R^3	R^4	R^5	n	IC_{50} (μM)	
							D1[a]	D2[a]
9a,**9b**	OCH_3	H	H	H	H	1	>100	>100
10a,**10b**	OCH_3	CF_3	CF_3	H	H	1	>100	>100
16a,**16b**	OCH_3	CF_3	CF_3	H	H	0	5.0	8.6
17	OCH_3	CF_3	CF_3	Br	H	0	1.9[b]	
18a,**18b**	OCH_3	CF_3	CF_3	Cl	Cl	0	92.5	93.4
22a,**22b**	OCH_3	CF_3	CF_3	CF_3	H	0	0.5	0.56

[a] The D1 and D2 nomenclature denotes the first and second eluting diastereomers undergoing chromatography. This convention is used throughout this work.

[b] Diastereomers were not separated (~1:1 mixture).



Scheme 4. Reagents and condition: (a) NBS, AIBN, CCl_4, reflux (25–38%); (b) racemic or *R* or *S* **6**, DIEA, DMP, argon (74%); (c) NaN_3, NH_4Cl, DMF (65%); (d) $TMSCHN_2$, THF/MeOH (80–95%); (e) SiO_2; (f) $EtCO_2Cl$, py, DCM (40–70%).

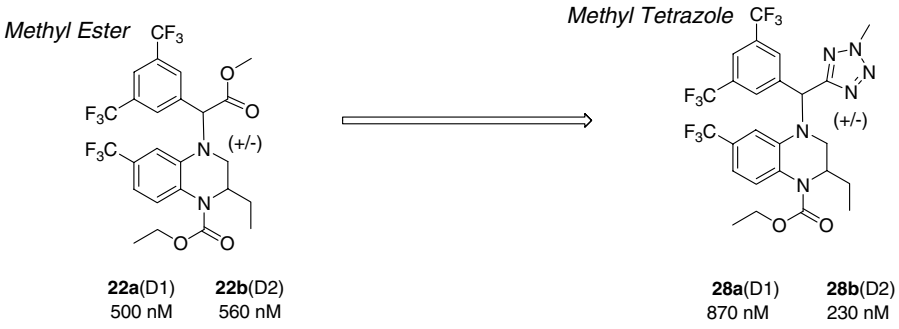
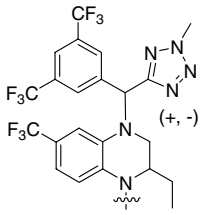


Figure 3. Separated methyl tetrazole diastereomers differ in potency.

hydroxyethyl amides resulted in a complete loss of activity (data not shown). The introduction of aryl substituents (R^4 and R^5) increased potency, except for chloro disubstitution. The R^4 trifluoromethyl group was optimal with both diastereomers providing an IC_{50} of about 500 nM, a 10-fold increase in potency over compounds **16a**, **16b**.

To suppress potential metabolic liabilities we became interested in substituting a methyl tetrazole group in place of the methyl ester.[15] The tetrazole group was prepared in a five-step sequence which began with the radical bromination of 3,5-bis-trifluoromethyl benzonitrile (**23**) (Scheme 4). Alkylation of the more reactive aniline with **24** produced the desired derivative for cycloaddition with sodium azide to the free tetrazole. Subsequent selective[16] N2 methylation with TMS-diazomethane produced diastereomers that could be separated via silica gel chromatography.[17] Finally, the free aniline was capped as the ethyl carbamate group. The methyl tetrazole substitution did produce a boost in potency over the ester series for one of the diastereomers, D2. The second eluting diastereomer **28b** (D2) was found to be

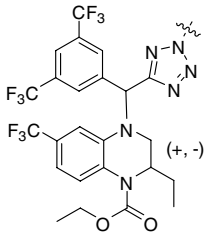
Table 2. Activity of Nl derivatives in the plasma CETP assay



Compound	Method[a]	IC_{50} (nM)	
		D1	D2
28a,**28b**	A	870	230
29	A	1110	
30	A	4400	
31	A	232	
32	A	62,590	
33a,**33b**	B	100,000	4570
34	B	46,380	
35	B	100,000	
36	B	100,000	
37	B	36,900	
38	C	854	
39a,**39b**	C	1300	5700
40	C	2915	

[a] Prepared from compound **26** utilizing method (A) RCO_2Cl, py, DCM; (B) Cl_2CO, DIEA, DCM then ROH or RNH (R or H); (C) RCHO, $NaCNBH_3$, MeOH, Δ.

Table 3. Activity of tetrazole analogs in the plasma CETP assay



Compound		IC_{50} (nM)	
		D1	D2
28a,**28b**		870	230
43[a]		32,500	
44[a]		4420	
45[a]		14,580	
46a,**46b**[b]		2265	2225
50a,**50b**[a]		5220	100,000
51[a]		7815	
52[a]		54,570	
53[a]		3420	
54[a]		4555	

[a] Analog prepared by converting the aniline of compound **27** to the ethyl carbamate ($EtCO_2Cl$, pyridine, DCM) followed by tetrazole formation (N_3, NH_4Cl, DMF, Δ). This material was then alkylated[16] with the corresponding alkyl halide utilizing DIEA or NaH as base.

[b] This analog was prepared through the reduction of compound **44** with $LiBH_4$.

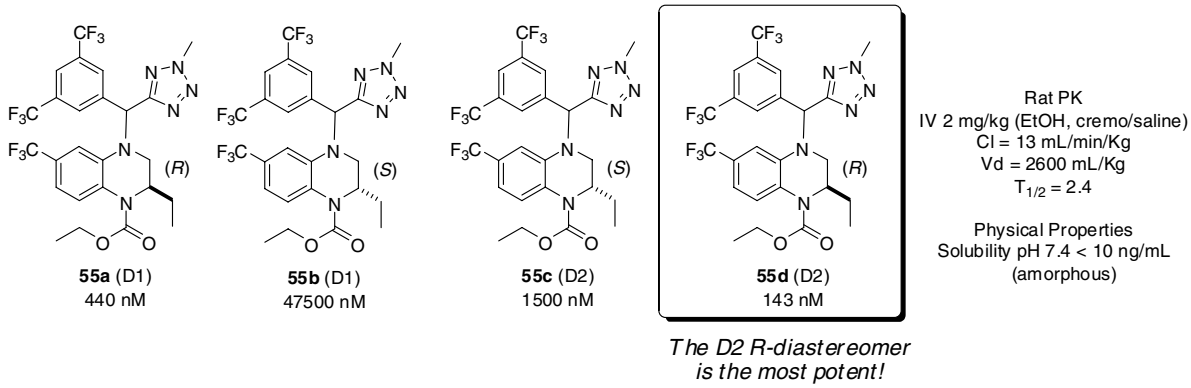


Figure 4. Preparation of all four stereoisomers of analog **28** reveals a preferred enantiomer **55d**.

~4-fold more potent as compared to the first eluting diasteromer **28a** (D1) (Fig. 3).

Since the diastereomerically pure tetrazole analog was more potent than the corresponding methyl ester and offered esterase stability we conducted a more thorough exploration of the SAR. Our strategy was to explore the N1 position of the tetrahydroquinoxaline core as well as substitution of the tetrazole ring. The synthetic routes were amenable to these substitutions since both sites are elaborated late in the synthesis.

In addition to the ethyl carbamate moiety at N1, several other carbamate analogs were prepared and the compounds were tested as single diastereomers or ~1:1 mixtures of diastereomers (Table 2). The most potent of the analogs was the isobutyl carbamate **31** with an IC_{50} of 232 nM as a D1/D2 mixture. This analog was much preferred over the benzyl, butyl, and 2-ethyl hexyl analogs. To determine the effect of replacing the carbamate oxygen with nitrogen a series of ureas were prepared. Both mono- and disubstituted ureas produced a loss in potency. The D2 diastereomer **33b** of the hydroxy ethyl urea was much more potent than the D1 diastereomer (**33a**). Interestingly the direct nitrogen analog of **28** (compound **34**) was >40× less potent. We also investigated direct alkyl substituted analogs with the cyclopentyl methyl diastereomers **38** being the most potent at 854 nM. Increasing the bulk of the appended cycloalkane produced a negative effect on potency since analogs **39a**, **39b**, and **40** were in the single digit μM range.

The SAR of the tetrazole group was also investigated (Table 3). Replacing the methyl group with methyl and *tert*-butyl acetoxy groups **43** and **45** produced a large decrease in potency. The homologated methyl acetoxy group (**44**) retained some potency at 4.4 μM. Substitution with the hydroxy ethyl group produced analogs **46a** and **46b**, which displayed modest potency. The amide and acid functionalities **50** and **52** produced a large loss of potency, while the basic amine **51**, nitrile **53**, and cyclopropyl group **54** displayed moderate activities. None of the analogs were superior to the 2-methyl tetrazole moiety in analog **28**.

The discovery that one of the tetrazole diastereomers was much more potent prompted us to pursue an optically active synthesis. Preparation of all four stereoisomers of **28** (**55a–d**)[18] revealed that the C2-*R*-second eluting diastereomer (**55d**) was the most potent at 143 nM (Fig. 4). The stereochemistry at the benzylic position was not assigned. Analog **55d** was found to have low clearance following iv dosing in rats, however, the solubility of **55d** was extremely poor at <10 ng/mL.

In summary, we have utilized a tetrahydroquinoxaline core to produce potent CETP inhibitors. A key observation in this process was that a shortened headpiece is optimal for the tetrahydroquinoxaline core. The replacement of the methyl ester with the methyl tetrazole moiety was also an important advance for the program. The methyl tetrazole offered improved potency and potential metabolic stability. Efforts to improve on tetrazole substitution and N1 aniline substitution were not successful in producing superior compounds to **28**. The synthesis of all four stereoisomers **55a–d** revealed that compound **55d** was by far the most potent with an IC_{50} = 143 nM in a human plasma CETP assay. This compound, **55d**, displays a rat PK profile with low clearance, moderate volume of distribution, and low solubility.

Acknowledgment

The authors thank T.K. Pope and Gregory Poch for performing the PK analysis and Michelle Livingston for the solubility determination of **55d**.

References and notes

1. Vrecer, M.; Turk, S.; Drinovec, J.; Mrhar, A. *Int. J. Clin. Pharmacol. Ther.* **2003**, *41*, 567.
2. Brewer, H. B., Jr. *N. Engl. J. Med.* **2004**, *350*, 1491.
3. Nicholls, S. J.; Nissen, S. E. *Eur. Heart J.* **2005**, *26*, 853.
4. Ito, M. K. *Am. J. Manag. Care* **2002**, *8*, S315.
5. Inazu, A.; Brown, M. L.; Hesler, C. B.; Agellon, L. B.; Koizumi, J.; Takata, K.; Maruhama, Y.; Mabuchi, H.; Tall, A. R. *N. Engl. J. Med.* **1990**, *323*, 1234.
6. Clark, R. W.; Sutfin, T. A.; Ruggeri, R. B.; Willauer, A. T.; Sugarman, E. D.; Magnus-Aryitey, G.; Cosgrove, P. G.; Sand, T. M.; Wester, R. T.; Williams, J. A.; Perlman, M. E.; Bamberger, M. J. *Arterioscler. Thromb. Vasc. Biol.* **2004**, *24*, 490.

7. Shinkai, H.; Maeda, K.; Yamasaki, T.; Okamoto, H.; Uchida, I. *J. Med. Chem.* **2000**, *43*, 3566.
8. Cao, G.; Escribano, A. M.; Fernandez, M. C.; Fields, T. Gernert, D. L.; Cioffi, C. L.; Herr, R. J.; Mantlo, N. B.; Martin De La Nava, E. M.; Mateo, Herranz, A. I.; Mayhugh, D. R.; Wang, X., WO05037796, **2005**.
9. Sikorski, J. A. *J. Med. Chem.* **2006**, *49*, 1.
10. Gray, B. D.; Jeffs, P. W. *J. Chem. Soc., Chem. Commun.* **1987**, 1329.
11. Prepared from the esterification of commercially available 3,5-bis(trifluoromethyl) phenylacetic acid.
12. Aliphatic regio-substitution at N-1 versus N-4 was determined via NMR 1-D (one dimensional) NOESY (nuclear Overhauser enhancement spectroscopy) via soft shaped pulse excitation or 2-D gHMBC (gradient Heteronuclear Multiple Bond Correlation).
13. The structural assignment for **14** was based upon gHMBC NMR.
14. Plasma CETP (Cholesteryl Ester Transfer Protein) activity is determined with a radioactive assay that measures the transfer of ^{3}H-CE from HDL to LDL. Inhibitor, human plasma (which contributes CETP and LDL), ^{3}H-CE-HDL (diluted to 50 μg/mL cholesterol), and final DMSO concentration 4% (v/v) are incubated on a 96-well round-bottom polypropylene plate for 6 h at 37 °C. The reaction is stopped by adding 10 U of heparin in 0.3 M $MnCl_2$ (final concentration) to precipitate the LDL. Plates are centrifuged at 1000*g* (2500 cpm, GH 3.8 rotor, Beckman Allegra 6 centrifuge). Volume of supernatant equal to 2/3 of assay volume is removed for counting in a Topcount to quantitate amount of ^{3}H-CE-HDL remaining.
15. Herr, R. J. *Bioorg. Med. Chem.* **2002**, *10*, 3379.
16. Alkylation of the tetrazole moiety produced a general trend with the N2 isomer as major accompanied with minor amounts of the N1 isomer (5–15%). The regiochemistry of compound **27** was determined through gHMBC NMR.
17. Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.
18. These analogs were prepared as described in Scheme 4 utilizing optically active tetrahydroquinoxaline **6**. The *R* and *S* tetrahydroquinoxaline cores were prepared as described in Scheme 1 utilizing commercially available (*R*)-(−)-2-amino-1-butanol and (*S*)-(+)-2-amino-1-butanol.