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Exploring a pocket for polycycloaliphatic groups in the CXCR3 receptor with the aid of a modular synthetic strategy

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ABSTRACT

A CXCR3 pocket capable of accommodating polycycloaliphatics was explored using a modular synthetic strategy. The systematic studies reveal that the tricyclic 2-adamantane and bicyclic (iso)bornyl group are efficiently recognized by CXCR3.

CXCR3 is a G protein-coupled chemokine receptor involved in a variety of inflammatory and infectious diseases and in certain metastasis processes.[1,2] Several series of small CXCR3 antagonists have been developed to explore the associated physiological and clinical roles of CXCR3.[1–10] Of these, azaquinazolinone AMG487 (**1**, Fig. 1) is the most widely described in the public domain, while some of the highest affinities reside with the broad class of piperazinyl-piperidines **2** (e.g., **2a/b**: pIC_{50} = 9.5/9.7, respectively).[2,3,10] The diversity amongst reported CXCR3 antagonists is high and it remains a challenge to identify 'CXCR3-priviliged' molecular motives.[2]

Our attention was drawn to a few bicycloaliphatic groups that seem to be readily accommodated by CXCR3.[8,9] Archetypical is the unique (−)myrtenyl group, which was found in SAR studies on 1-aryl-3-piperidin-4-yl-ureas (e.g., **3**).[8] In general, several examples are known in which chemokine receptor antagonists pick up considerable affinity by binding to hydrophobic domains of the chemokine receptor.[11,12] Therefore, with the goal of contributing to the understanding of CXCR3–ligand binding, we decided to explore a hypothesized CXCR3 pocket for polycycloaliphatics through a modular synthetic approach.

Our design strategy involved equipping the benzyl-aminopiperidine part of piperazinyl-piperidine **2** with the polycycloaliphatic groups of choice (Fig. 2). The rationale for this strategy was twofold: (1) We envisioned a highly modular synthetic protocol in which a reductive amination using simple building blocks is key, paving the way for efficient identification and exploration of the pocket. (2) The reported picomolar-affinity of **2** may allow the removal of a substantial molecular portion without absolute loss of affinity.

In the synthesis of virtually all products, two or three subsequent reductive aminations were used to install the benzyl, polycycloaliphatic and methyl groups (Scheme 1). Routes A and B both start from a mono-protected dibasic core and carbonyl building blocks but the peripheral groups are installed in different order. Routes C and D are essentially the same as routes A and B but use amine-building blocks for the polycycloaliphatic unit. Last, route E adds an N–Me through an Eschweiler–Clark reductive amination. Almost all reductive aminations proceeded smoothly with functionalities such as double bonds, azides and pentafluorophenyl groups left intact. Many non-commercial building blocks were prepared by methods described in the literature (see Supplementary data).

A few building blocks and final compounds required alternative approaches (Scheme 2). 1-Adamantyl substituted pyridine **4** was benzylated to salt **5** followed by a swift reduction to **22**. Benzylation of **4** by 4-ClBnBr was preferred instead of by 4-Cl,2-F–BnBr. Sequential treatment of keto-ester **6** with 4-chloro-2-fluorobenzaldehyde and then 2-adamantaneamine gave enamine **31**. This enamine resisted $NaBH(OAc)_3$, but $NaCNBH_3$ in AcOH provided a separable mixture of diastereomeric esters **32** and **33**. The relative stereochemistry of these could not be unambiguously determined. Aniline **57** was obtained from azide **56** by a Zn-based reduction. Urea **37** was prepared by corresponding isocyanate-chemistry.

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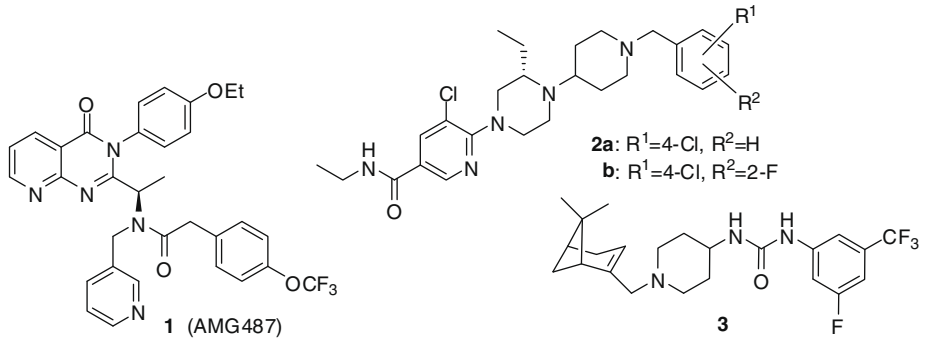


Figure 1. Selected CXCR3 antagonists.

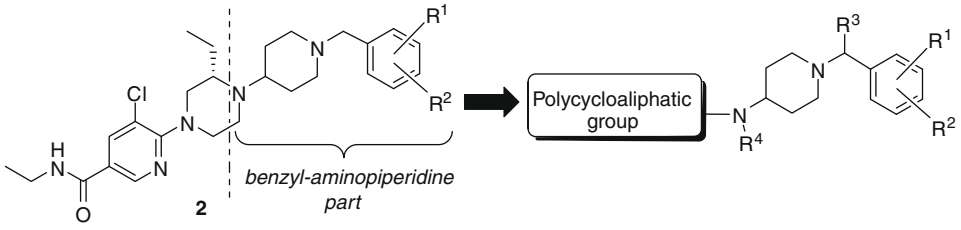
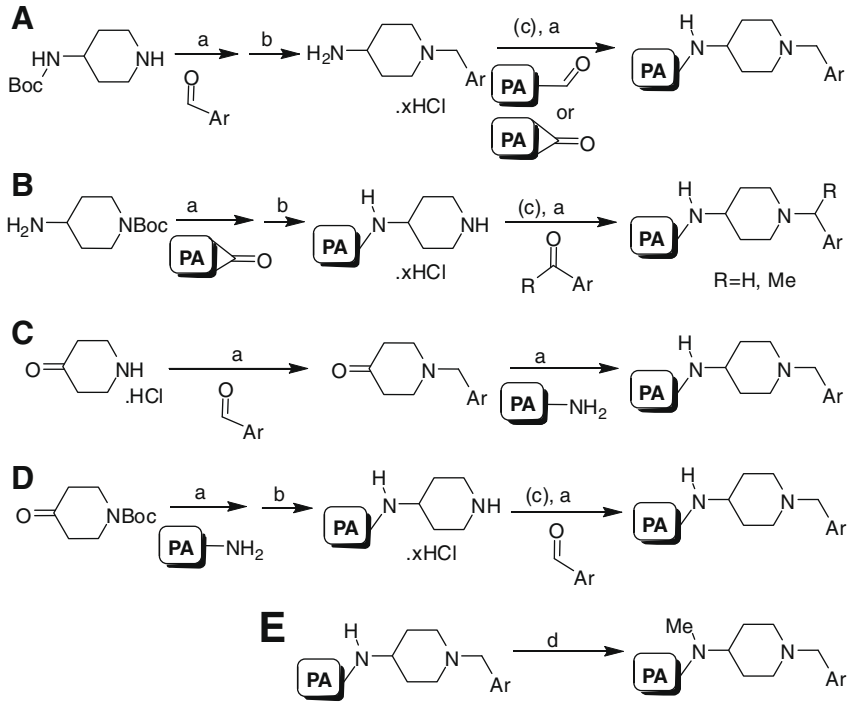


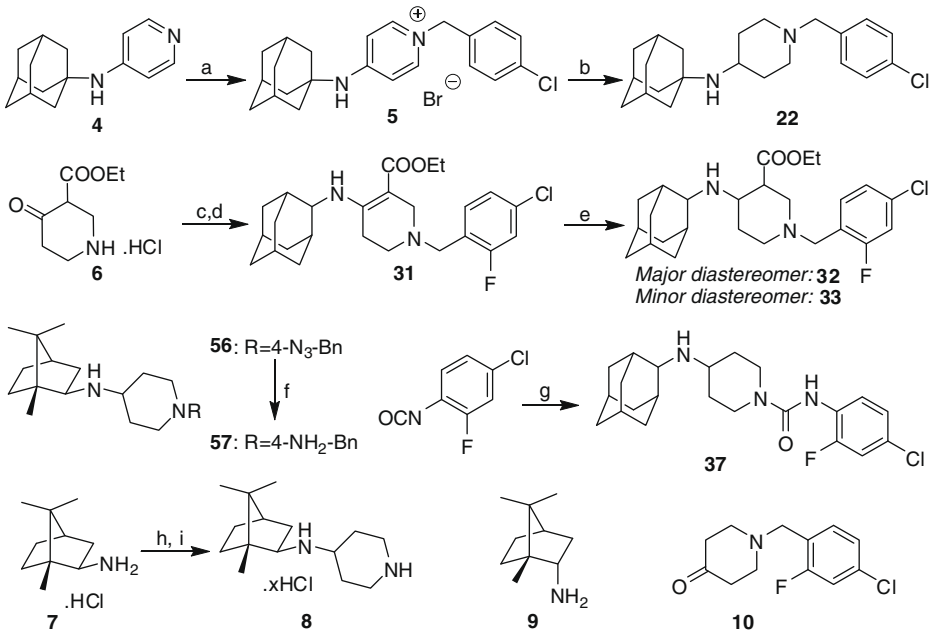
Figure 2. Design strategy.



Scheme 1. Modular synthetic routes A–E. Key: (a) $NaBH(OAc)_3$, (AcOH), (Et_3N), DCE, rt, 1–9 d. (b) HCl (2.0 M in dioxane), rt, 3–20 h. (c) 1 N aq NaOH, DCM, extraction. (d) HCOOH, 37% aq H_2CO, reflux, 2–4 h. **PA** = polycycloaliphatic group.

R-isobornylamine **7** was reductively alkylated on 20 g scale to *R*-*exo*-piperidine **8**. *exo*/*endo* Epimerization, that is, from an isobornyl to a bornyl unit, may occur during reductive aminations on **7** through positional isomerisation of the intermediate imine,[13] possibly compromising stereochemical integrity of final products. As an exemplary case, we inspected two epimers **38** (*exo*) and **60** (*endo*) (vide infra), obtained after reductive aminations on epimeric amines **7** and (commercial) **9** with ketone **10**. Extensive NMR analysis (see Supplementary data) showed that both were distinguishable, epimerically pure and of the expected stereochemical architecture.

All compounds were tested for their affinity for human CXCR3 (hCXCR3) by measuring the displacement of ^{125}I-CXCL10 binding to membranes of HEK293 cells expressing hCXCR3.[14,15] Here, the



Scheme 2. Synthesis of specific building blocks and final compounds. Key: (a) 4-ClPhCH$_2$Br, toluene, reflux, 4 h (84%). (b) NaBH$_4$, MeOH, rt to 80 °C, 3 h (37%). (c) 4-Cl,2-F–PhCHO, NaBH(OAc)$_3$, DCE, rt, 18 h (61%). (d) NaBH(OAc)$_3$, DIPEA, 2-adamantane-amine.HCl, AcOH, DCE, rt, 72 h (67%). (e) NaBH$_3$CN, AcOH, rt, 18 h (20% of **32**, 10% of **33**). (f) NH$_4$Cl, Zn(s), EtOH/H$_2$O, rt, 20 h (31%). (g) 4-(2-adamantylamino)-piperidine, DCM, rt, 18 h (36%). (h) 1-Boc-4-piperidinone, NaBH(OAc)$_3$, AcOH, DCE, rt, 1 d (66%). (i) HCl (2.0 M in dioxane), rt, 2 h (62%).

reference piperazinyl-piperidine **2a** gave pK_i = 8.5. Our initial SAR attempts left intact the 4-amino-piperidine core and mostly utilized the 2-F,4-Cl-benzyl group, as in **2b** (Fig. 2 and Table 1). Neither attachment of a diphenylpropyl- (**11**) or a tetrahydroisoquinolinyl-group (**12**), nor of monocyclic units such as pyrrolidinyl-, cyclooctenylmethyl-, cyclohexyl- and cyclohexylmethyl-groups (**13–16**) provided appreciable affinity. The same was true for the bicyclic (−)-myrtenyl group (**17**), which is in sharp contrast to the 1-aryl-3-piperidin-4-yl-urea series (**3**).[8] A tropine moiety had a detrimental effect on affinity (**18**). Gratifyingly, a tricyclic 2-adamantane group lifted the affinity to pK_i = 6.8 (**19**). Compound **19** was found to be an antagonist (pK_b = 6.1 ± 0.1, n = 3) as measured by its inhibitory effect on [^{3}H]-inositolphosphates levels after stimulation with 50 nM CXCL10 in HEK293T-hCXCR3/Gα_{qi5} cells.[14]

A small SAR exploration with other benzyl groups revealed that 4-Cl-substitution (**20**) is slightly less efficient than 4-Cl,2-F substitution yet still beneficial (**20** vs **21**) and that 2-adamantane substitution is superior over 1-substitution (**20** vs **22**). Reverting back to the 4-Cl,2-F-benzyl pattern, it was found that elongation of the *N*-adamantane distance decreased affinity (**23**, **24**). N-Methylation of **19** and **23** to **25** and **26**, respectively, consistently lowered affinity. Dimethyladamantane **27** was tolerated but to a lesser extent as **19**. Last, azaadamantane **28** had little affinity.

The 4-aminopiperidine-benzyl spacer was also varied (Table 2). Substitution on the benzylic CH$_2$ (**29**), methylene insertion adjacent to the piperidine (**30**) or introduction of an (un)saturated ester (**31**– **33**) led to more than a log unit drop in affinity. An inverse 4-amino-piperidine (**34**), 3-amino-piperidine (**35**), azepane (**36**) and urea (**37**) were also tested but none matched **19**. Therefore, we decided to retain the 4-amino-piperidine-benzyl unit throughout the remainder of our work.

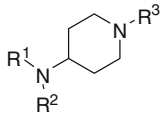
To further define the pocket, we sought to investigate in-depth an additional polycycloaliphatic group. This also provided an opportunity to overcome the high crystallinity and suboptimal solubility kinetics of many symmetrical adamantane-compounds. We selected the bicyclic isobornyl group, derived from camphor. When compared to **19**, *R*-isobornyl analogue **38** displayed somewhat lower affinity (pK_i = 6.4) and functional activity (pK_b = 5.7 ± 0.1, n = 3), but had satisfactorily reduced crystallinity. Interestingly, camphor itself is a key unit of an unrelated series of CXCR3 antagonists that was disclosed in a patent shortly after our switch to an isobornyl unit,[16] suggesting a more general applicability of camphor-like groups as CXCR3–ligand motives.[17]

We tested twenty-one *R*-isobornyl-compounds with chemically diverse benzyl groups (Table 3). Plain benzyl or heterocyclic groups (**39–42**) were ineffective. Of some fluorinated benzyl groups (**43–46**), the pentafluorobenzyl group (**46**) gave good affinity (pK_i = 6.3). Analogous to the 2-adamantane series, the 4-Cl contributes much more to the affinity of **38** than the 2-F (**47** vs **48**). Likewise, omission of 2-F from a 4-MeO,2-F derivative has no significant effect (**49** vs **50**). A MeO-scan (**50–52**) revealed the *para*-position as the preferred anchor for substitution and we thus introduced additional substituents on this position (**53–58**). Of these, the 4-CF$_3$O– (**53**) and 4-N$_3$ substituents (**56**) units gave sub-μM affinity.

In contrast to the achiral 2-adamantane system, the isobornyl group has multiple stereomers providing a subtle way to probe the 'polycycloaliphatic-pocket'. However, little effect of stereochemistry was observed between stereomers **38**, **60** and **62** (Table 4). Last, both N-methylation (**59**, **61**) and methylene insertion (**63**) led to a drop in affinity (Table 4), reinforcing the SAR similarity between the tricyclic 2-adamantane and bicyclic (iso)bornyl series.

Summarizing, with the aid of a highly modular synthetic approach we probed a hCXCR3 pocket able to efficiently accommodate specific bi- and tri-cycloaliphatics. A comparison of the best compounds to several monocyclic counterparts clearly puts forward the interaction of a polycycloaliphatic moiety with a complementary CXCR3 pocket as a straightforward yet very significant ligand binding element. Thereby, our results confirm that hydrophobic domains of chemokine receptors may provide efficient anchor points for small molecules. Our findings will be translated to future series from our lab and can generally be useful in the design of CXCR3 antagonists.

Table 1
Exploration of polycycloaliphatic group



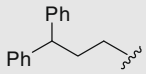
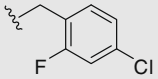
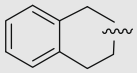
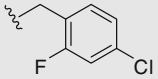
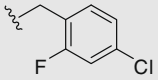
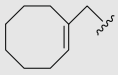
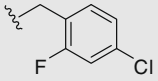
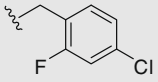
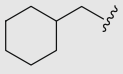
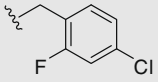
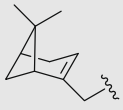
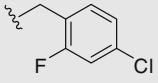
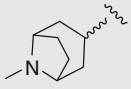
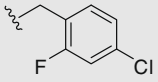
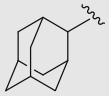
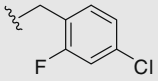
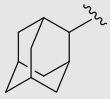
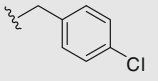
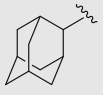
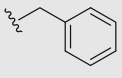
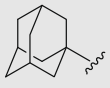
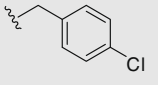
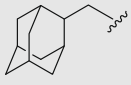
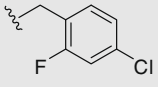
#	R^1	R^2	R^3	Route [a]	pK_i ± SEM[b]
2a	—	—	—	n.a.[c]	8.5 ± 0.2
11		H		*C*	<4
12		—		*C*	5.3 ± 0.0
13		—		*C*	<5
14		H		*A*	5.6 ± 0.1
15		H		*C*	5.3 ± 0.1
16		H		*C*	5.4 ± 0.0
17		H		*A*	5.4 ± 0.2
18		H		*A*[d]	<5
19		H		*A/C*	6.8 ± 0.2
20		H		*B*	6.4 ± 0.1
21		H		*B*	5.4 ± 0.1
22		H		n.a.[e]	6.0 ± 0.0
23		H		*A*	6.0 ± 0.1

Table 1 (*continued*)

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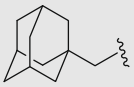
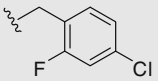
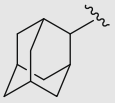
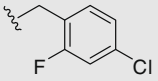
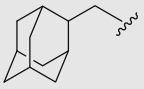
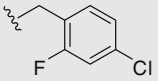
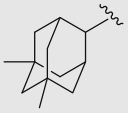
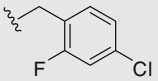
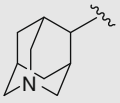
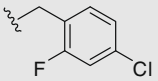
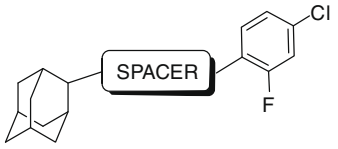
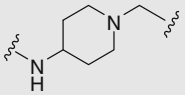
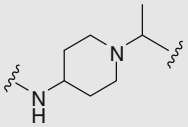
24		H		*A*[f]	6.3 ± 0.0
25		Me		*E*	5.9 ± 0.0
26		Me		*E*	5.6 ± 0.1
27		H		*A*	6.3 ± 0.1
28		H		*A*	5.1 ± 0.1

[a] Codes refer to Scheme 1.
[b] $n = 2–5$.
[c] See Ref. 10.
[d] Obtained as a ~6/1 mixture of diastereomers.
[e] See Scheme 2.
[f] Used as maleate salt.

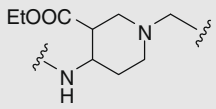
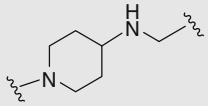
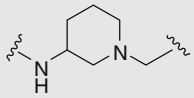
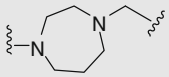
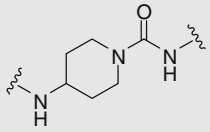
Table 2
Exploration of spacer



#	Spacer	Route[a]	pK_i ± SEM[b]
19		*A/C*	6.8 ± 0.2
29		*B*	5.3 ± 0.0
30		*B*[c]	5.4 ± 0.0
31		n.a.[d]	5.3 ± 0.2
32		n.a.[e]	5.3 ± 0.1

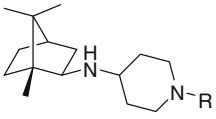
(*continued on next page*)

Table 2 (*continued*)

#	Spacer	Route[a]	pK_i ± SEM[b]
33		n.a.[f]	5.4 ± 0.1
34		*A*[g]	5.4 ± 0.0
35		*D*[h]	5.7 ± 0.1
36		*A*[g]	5.8 ± 0.1
37		n.a.[d]	<5

[a] Codes refer to Scheme 1.
[b] $n = 2–5$.
[c] Carried out with *tert*-butyl 4-(aminomethyl)piperidine-1-carboxylate instead of with *tert*-butyl 4-aminopiperidine-1-carboxylate.
[d] See Scheme 2.
[e] Major diastereomer, see Scheme 2.
[f] Minor diastereomer, see Scheme 2.
[g] Carried out with *tert*-butyl 4-aminopiperidine-1-carboxylate (for **34**) or with 10 equiv of homopiperazine (for **36**, omitting HCl treatment) instead of *tert*-butyl piperidin-4-ylcarbamate (see Scheme 1).
[h] Carried out with *N*–Boc-3-piperidinone instead of *N*–Boc-4-piperidinone.

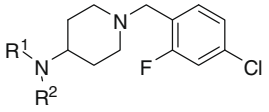
Table 3
Exploration of aromatic group in isobornyl series

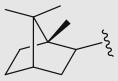
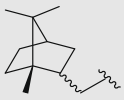


#	R	Route[a]	pK_i ± SEM[b]
38	4-Chloro-2-fluorobenzyl	*C/D*	6.4 ± 0.1
39	Benzyl	*D*	5.6 ± 0.1
40	2-Pyridylmethyl	*D*	5.1 ± 0.2
41	3-Pyridylmethyl	*D*	<5
42	2-Furylmethyl	*D*	5.2 ± 0.1
43	4-Fluoro-3-trifluoromethylbenzyl	*D*	5.6 ± 0.1
44	3-Fluoro-5-trifluoromethylbenzyl	*D*	5.4 ± 0.1
45	2,4-Difluorobenzyl	*D*	5.7 ± 0.1
46	2,3,4,5,6-Pentafluorobenzyl	*D*	6.3 ± 0.2
47	4-Chlorobenzyl	*D*	6.3 ± 0.1
48	2-Fluorobenzyl	*D*	5.2 ± 0.1
49	2-Fluoro-4-methoxybenzyl	*D*	5.8 ± 0.1
50	4-Methoxybenzyl	*D*	5.7 ± 0.1
51	3-Methoxybenzyl	*D*	5.1 ± 0.1
52	2-Methoxybenzyl	*D*	5.0 ± 0.1
53	4-Trifluoromethoxybenzyl	*D*	6.1 ± 0.1
54	4-(Methoxycarbonyl)benzyl	*D*	5.5 ± 0.1
55	4-Acetamidobenzyl	*D*	<5
56	4-Azidobenzyl	*D*	6.1 ± 0.0
57	4-Aminobenzyl	n.a.[c]	5.2 ± 0.2
58	4-(Dimethylamino)benzyl	*D*	5.4 ± 0.2

[a] Codes refer to Scheme 1.
[b] $n = 2–5$.
[c] See Scheme 2.

Table 4
Exploration of (iso)bornyl substituent



#	R^1	Stereochemistry	R^2	Route[a]	pK_i ± SEM[b]
38		*R-exo*	H	*C/D*	6.4 ± 0.1
59			Me	*E*	5.8 ± 0.1
60		*R-endo*	H	*C*	6.4 ± 0.2
61			Me	*E*	5.9 ± 0.1
62		*S-exo*	H	*C*	6.2 ± 0.2
63		2 Epimers[c]	H	*A*	5.7 ± 0.1

[a] Codes refer to Scheme 1.
[b] $n = 2–5$.
[c] Mixture of epimers (~2/1).

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Supplementary data

Syntheses of building blocks, activity curves, NMR spectra and optical rotations of selected compounds. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.02.093.

References and notes

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