Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 3161–3165

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and NK_1/NK_2 Binding Activities of a Series of Diacyl-Substituted 2-Arylpiperazines†

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Received 16 April 2002; accepted 24 July 2002

Abstract—The synthesis and binding affinity for hNK_1 and hNK_2 receptors of a series of diacyl substituted 2-aryl piperazines are described. SAR evaluation led to the racemic derivative **11g** as an apparent dual inhibitor. Chiral chromatographic separation of **11g** led to the observation that NK_1 activity was shown by one enantiomer (**13a**) and NK_2 activity was shown by the other enantiomer (**13b**). X-ray crystallographic analysis of the crystalline di-BOC derivative of the NK_2 active piperazine (**15**) showed that the 2*R* configuration was associated with NK_2 activity. Further derivatization indicated that dual NK_1/NK_2 activity could be built into the 2*R* series.

The family of neurokinins, or tachykinins, consists of three related neuropeptides (Substance P, neurokinin A, and neurokinin B) that possess the same *C*-terminal sequence. They interact to different degrees with three G-protein linked receptors, known as NK_1, NK_2, and NK_3, but each has selectivity for one of them. Substance P shows greatest affinity for NK_1, neurokinin A for NK_2, and neurokinin B for NK_3 receptors.[2]

Recent interest in dual NK_1/NK_2 antagonists has been based in part on the expectation that a drug showing these activities would affect favorably several undesirable elements of bronchial asthma. Substance P (NK_1 agonist) is known to be involved in the extravasation and consequent inflammation of the airways, and neurokinin A (NK_2 agonist) appears to be involved in bronchoconstriction and cough.[3,4] Because of this anticipated activity in asthma there have been numerous efforts to design potent dual NK_1/NK_2 antagonists that have resulted in a number of structural leads. These series were developed mainly from existing NK_1 or NK_2 antagonist leads as described in the review by Gerspacher and von Sprecher.[5] More recently, this approach is illustrated in work from, for instance, our laboratories[6,7] and from AstraZeneca.[8]

Our own work was based, in part, on the 1-phenyl ethylenediamine fragment present in the early Pfizer lead, CP-99,994, a potent NK_1 antagonist.[9] By incorporating this potential pharmacophore into a piperazine ring the general target **1** was designed, as shown in Figure 1.[10] The orientation of the two pairs of aryl rings in the two structures is very similar.

We chose the unsubstituted phenyl (**2a**) and the 3,4-dichlorophenyl (**2b**) piperazines as representative of structures present in several neurokinin antagonists. The key intermediates needed for these syntheses were

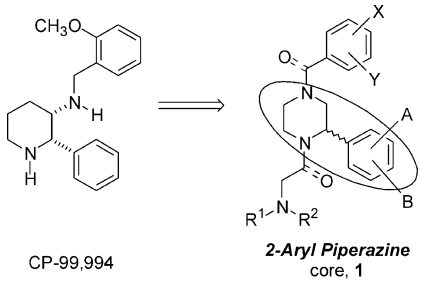

Figure 1. Design of potential piperazine antagonists of the neurokinins.

†See ref. 1

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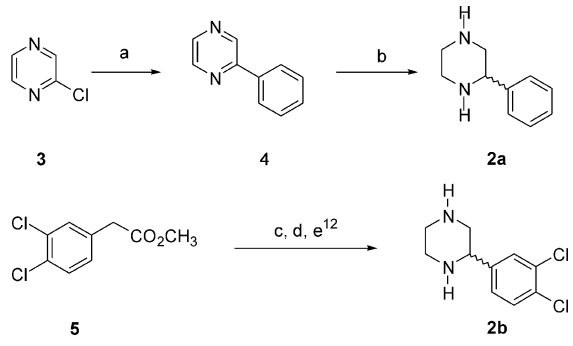
PII: S0960-894X(02)00645-5

the 2-arylpiperazines. Two different routes were used to prepare them (Scheme 1). Unsubstituted 2-phenylpiperazine **2a** was prepared from 2-chloropyrazine **3** by a nickel-catalyzed coupling with phenyl Grignard reagent[11] to produce 2-phenylpyrazine **4**. Catalytic reduction produced **2a** in about 70% overall yield. We anticipated that a similar route would not be suitable for preparing the 3,4-dichlorophenylpiperazine **2b** because of potential dechlorination in the catalytic reduction step. We chose to use the method described by Roderick et al.[12] starting from methyl 3,4-dichlorophenylacetate **5** to produce **2b** in three steps. The products (**2a**, **b**) were racemic and our early work was carried out with these racemates.

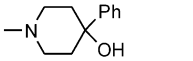
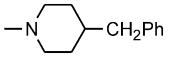
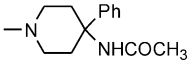
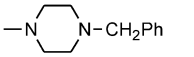
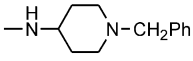
Our initial plan was to retain a basic nitrogen at the 4-position of the piperazine ring, to mimic the basic substituent at the 3-position in CP-99,994 (Fig. 1). Incorporation into **1** of substituted amines in which the substituents, R^1 and R^2, resemble those present in known NK_1 and/or NK_2 antagonists, led to the series of compounds shown in Table 1. The synthesis of these compounds is shown in Scheme 2. This involved selective alkylation of **2a** and **2b** at the less hindered 4-position to produce **6** followed by low-temperature acylation at the 1-position giving **7** and, finally, substitution of the bromine by a series of amines to yield the products **8a**–**j**. Some products (e.g., **8a**) showed a moderate level of NK_1 activity but no NK_2 activity at the highest level tested (1 μM). The use of 4-amino-1-benzylpiperidine produced **8i** and **8j**, which were the first targets to show a significant level of dual NK_1/NK_2 binding affinity. Retaining the 4-amino-1-benzylpiperidine unit allowed us to examine the effect of substitution at the 4-position of the piperazine.



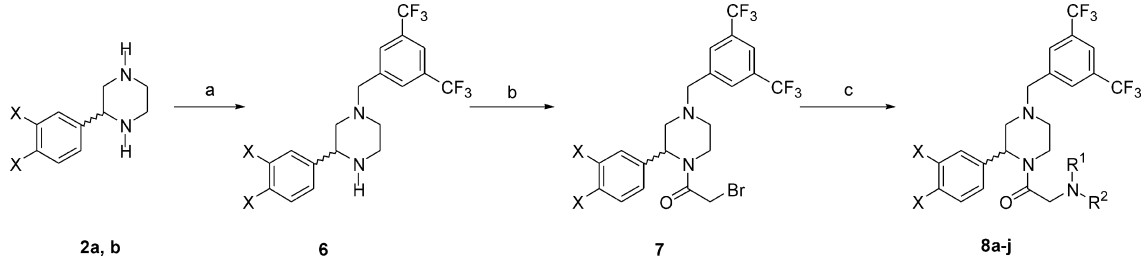
Scheme 1. (a) $(Ph_2PCH_2)_2NiCl_2$, PhMgBr, THF (95%); (b) $Pd(OAc)_2$, H_2, CH_3CO_2H, 50 psi, 4 h (75%); (c) NBS; (d) $H_2N–CH_2–CH_2–NH_2$; (e) LAH, Et_2O.

Synthesis of this series is outlined in Scheme 3. Initial protection of the more reactive 4-position of the 2-aryl piperazine with $(BOC)_2O$ led to intermediates **9a** and **9b**. This was followed by incorporation of the bromoacetyl unit and then the 4-amino-1-benzylpiperidine, as described before (Scheme 2) to produce intermediates

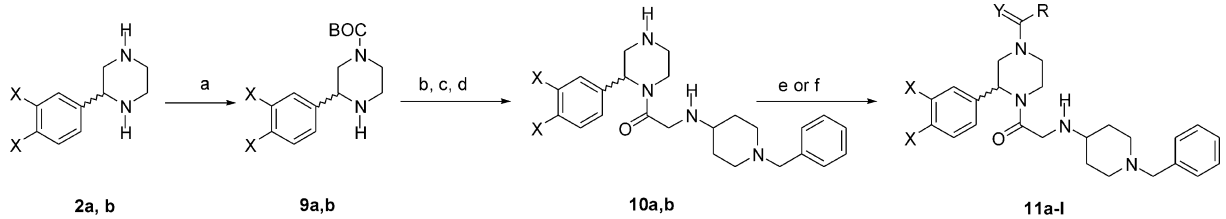
Table 1. Discovery of the initial leads

Compd	X	–NR^1R^2	NK_1[a] K_i (nM)	NK_2[a] K_i (nM)
8a	H		13	>1000
8b	Cl		32	>1000
8c	H		37	>1000
8d	Cl		110	>1000
8e	H		48	>1000
8f	Cl		148	>1000
8g	H		48	>1000
8h	Cl		28	>1000
8i	H		17	300
8j	Cl		30	175

[a]NK_1 and NK_2 Binding assays: Binding data are the average of two or three independent determinations. Receptor binding assays were performed on membrane preparations containing recombinant human NK_1 or NK_2 receptors in CHO cells. [^{3}H]Sar SP and [^{3}H]NKA were used as the ligands for the NK_1 and NK_2 receptor assays respectively, at the experimentally derived K_d values. K_i values were obtained using the Cheng and Prusoff equation.[13]



Scheme 2. (a) 3,5-$(CF_3)_2C_6H_3–CH_2Br$, Et_3N, CH_2Cl_2, −78 °C to rt; (b) $BrCH_2COBr$, Et_3N, CH_2Cl_2, −78 °C; $R^1R^2N–H$, Et_3N, CH_2Cl_2.



Scheme 3. (a) $(BOC)_2O$, MeOH, −78 °C; (b) $BrCH_2COBr$, $(i\text{-}Pr)_2NEt$, CH_2Cl_2; (c) 4-amino-1-benzylpiperidine; (d) 4 M HCl in dioxane; (e) R-CH_2–Br(Cl); (f) R-CO_2H, HOBT, Et_3N, DEC.

Table 2. Effect of substitution at the 4-position of the piperazine ring

Compd	X	Y	R	NK_1[a] K_i (nM)	NK_2[a] K_i (nM)
11a	H	O=	–CH_2–[3,5-$(CF_3)_2$–C_6H_3]	136	>1000
11b	H	H,H		135	>1000
11c	Cl	O=	–CH_2–[3,4,5–$(CH_3O)_3$–C_6H_2]	>1000	>1000
11d	Cl	O=	–[3,4,5–$(CH_3O)_3$–C_6H_2]	112	124
11e	Cl	O=	–[3,5–$(CF_3)_2$–C_6H_3]	5.3	17

10a and **10b** after removal of the BOC protecting group. Alkylation or acylation produced the series of compounds **11a–e** shown in Table 2. Very limited activity was seen with phenylacetyl derivatives **11a–c** whereas the 3,5-disubstituted benzoyl derivative **11e** appeared to show enhanced potency at both the NK_1 and NK_2 receptors.

We inferred from these results that diacylpiperazines, such as **11e**, constituted the best lead. We carried out an optimization of the acid group by synthesizing numerous aromatic amide derivatives of which a selection is shown in Table 3. This indicated that the 3,5-dimethylbenzoyl derivative **11g** gave optimum binding potency. The benzoyl derivative **11f** showed good NK_2 binding potency but lacked significant NK_1 activity confirming the need for the 3- and 5- substituents on the aromatic ring. However, highly polar substituents on the ring are not favored (e.g., **11k**).

Next, we investigated the influence of absolute stereochemistry on activity. Our initial assumption was that activity would reside in one enantiomer with the other being much less potent, or inactive. Compounds **11e** and **11g** were separated into their corresponding enantiomers (**12a**, **12b** and **13a**, **13b**) by chiral HPLC on a Daicel Chiralpak AD® column. Testing of the enantiomers showed that, contrary to our expectations, NK_1 activity resided in one enantiomer (**12a** and **13a**) and NK_2 activity resided in the other (**12b** and **13b**), as shown in Table 4. The absolute stereochemistry was determined as shown below. At this time we confirmed, using appropriate functional assays, that the racemates or enantiomers were antagonists, although their potency was rather low (Table 4).

In order to determine which enantiomer possessed which biological activity we resolved an early intermediate. The 2-arylpiperazines were the obvious choice because all subsequent reactions could be directed towards optimizing the desired enantiomer. 2-(3,4-Dichlorophenyl)piperazine **2b** was converted to its *N*-acetyl-L-leucine salt which was separated by fractional crystallization (Scheme 4).[14] In fact, both enantiomers, **14a** and **14b,** could be obtained pure using this salt without the need to use *N*-acetyl-D-leucine to obtain the second enantiomer. Conversion of the pure enantiomers to the same compounds, previously obtained by chiral HPLC (**13a** and **13b**), confirmed by bioassay which enantiomer bound to which neurokinin receptor.

To establish the absolute configuration of the NK_2 binding series the relevant piperazine enantiomer (**14b**) was converted to its crystalline di-BOC derivative **15** (Scheme 4) which was subjected to X-ray structure determination using the anomalous dispersion method.[20] Results showed that the NK_2-active enantiomer possessed the 2*R* configuration. Therefore, the NK_1 active series was produced from the 2*S* isomer. A stereo view of 15, with the methyl groups of the BOC group removed for clarity, is shown in Figure 2.

Evaluation of the data obtained on the pure enantiomeric products (Table 4) suggested that introducing NK_1 activity into the 2*R* series might be more feasible than attempting to build NK_2 activity into the 2*S* series. NK_2 activity in **12a** and **13a** is extremely weak (K_i > 200 nM) and would need to be increased almost two orders

Table 3. Modifications to the 4-aroyl substituent in **11** (X = Cl; Y = O)

Compd	R	NK_1 K_i (nM)	NK_2 K_i (nM)
11f	–C_6H_5	93	3.4
11g	–[3,5–$(CH_3)_2$–C_6H_3]	1.8	4.9
11h	–[3,5–F_2–C_6H_3]	23	22
11i	–[3,5–$(CH_3O)_2$–C_6H_3]	7.1	27
11j	–[3,5–Cl_2–C_6H_3]	7.7	14
11k	–[3,5–$(OH)_2$–C_6H_3]	>1000	>1000
11l	–[3,5–Br_2–C_6H_3]	3.2	14

Table 4. Separation of enantiomers of **11e** and **11g**

Compd	NK_1 K_i (nM)	NK_2 K_i (nM)	pA_2[a,c] NK_1	pA_2[b,c] NK_2
11e	5.3	17.2	7.1	
12a	1.8	270		
12b	48	8.3		
11g	1.8	4.9	6.5	6.5
13a	0.9	219	8.2	
13b	25	3.4		

[a]The functional NK_1 bioassay was performed in isolated guinea pig vas deferens (gpvd) induced to contract by electrical field stimulation (EFS). Inhibition of the SP-induced NK_1-mediated enhancement of the EFS-induced sympathetic neurogenic contractions of gpvd was used as a measure of antagonist activity.[15,16]

[b]The functional NK_2 bioassay was performed using NKA-induced airway contractions of isolated hamster trachea (ht). The inhibition of NKA-induced airway contraction was used as a measure of NK_2-receptor antagonist activity.[17,18]

[c]Antagonists were applied to the baths 30 min before the application of rising cumulative concentrations of SP (gpvd) or NKA (ht). Apparent pA_2 values were estimated from the magnitude of the antagonist-induced rightward parallel shifts in the agonist concentration–response curves, using the method of Furchgott[19] and assuming competitive kinetics and equilibrium conditions.

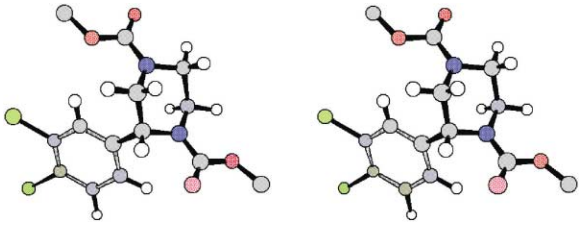
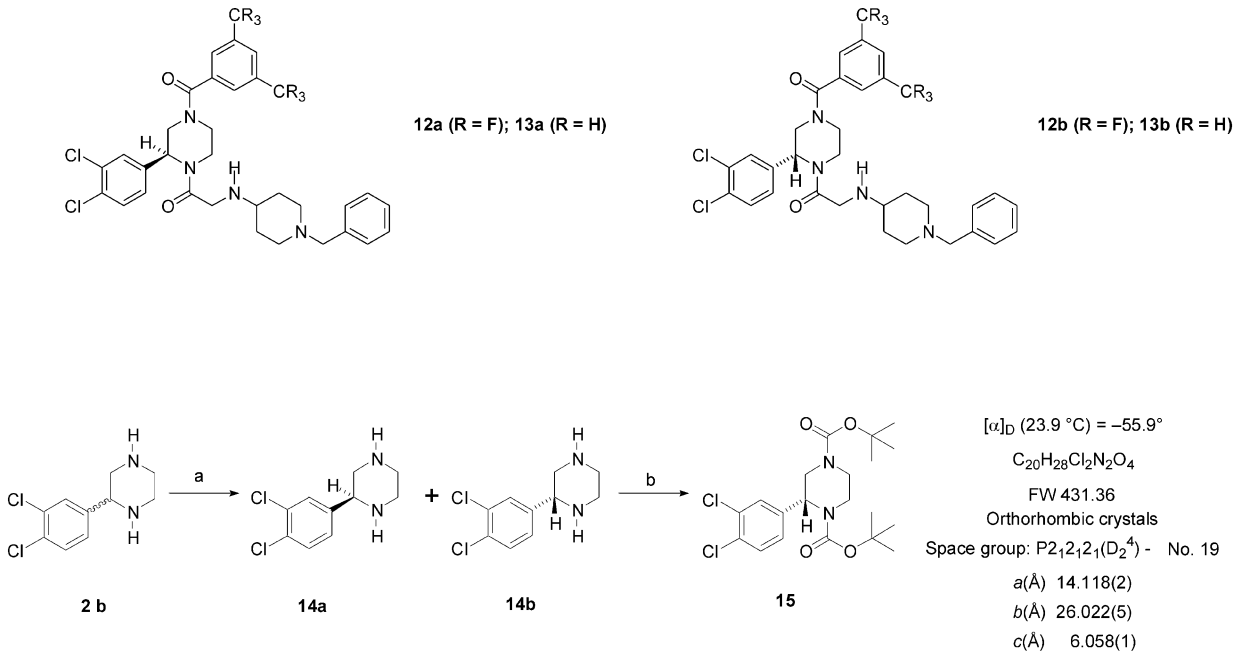
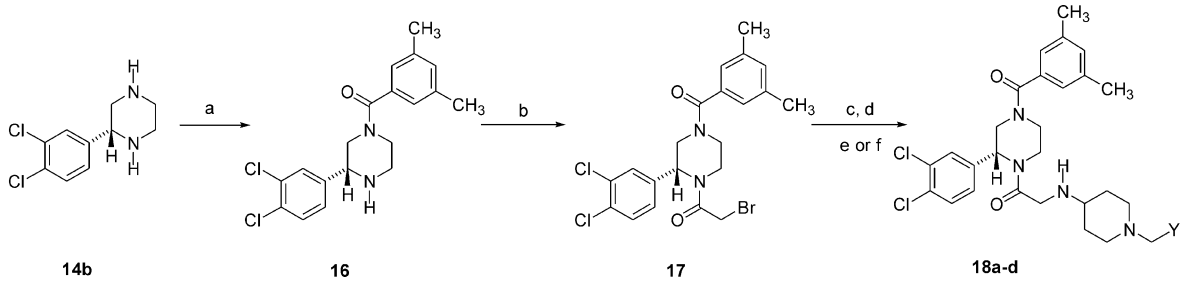


Figure 2. Stereo view of the 2*R*-piperazine unit (methyls of BOC groups removed for clarity).



Scheme 4. (a) *N*-Acetyl-L-leucine salt; recrystallize from MeOH/EtOAc, liberate free base, recrystallize free base from hexanes to >99% ee; (b) $(BOC)_2O$; MeOH; −78 °C to rt; recrystallize from EtOH.



Scheme 5. (a) 3,5-$(CH_3)_2C_6H_3$–CO_2H, HOBT, Et_3N, DEC, 0 °C; (b) $BrCH_2COBr$, $(i\text{-}Pr)_2NEt$, CH_2Cl_2, −78 °C; (c) 4-Amino-N^1-BOC-piperazine, $(i\text{-}Pr)_2NEt$, CH_2Cl_2, (d) 4 M HCl in dioxane; (e) Y-CH_2–Br, Et_3N, CH_2Cl_2; (f) Y-CHO, $NaBH_3CN$, AcOH, MeOH.

of magnitude to be useful. However, NK_1 activity in **12b** and **13b** is quite respectable ($K_i < 50$ nM) and only needs to be improved by about one order of magnitude to produce a useful dual antagonist. An additional requirement in each case, of course, is to retain binding to the original receptor. To test this hypothesis we chose to modify the group attached to the 4-aminopiperidine unit of **13b**. This was carried out as shown in Scheme 5 in which the more reactive 4-position of **14b** was coupled with 3,5-dimethylbenzoic acid giving **16** followed by low temperature acylation with bromoacetyl bromide to produce **17**. Displacement of the activated halogen in **17** with N^1-BOC-protected 4-aminopiperidine followed by deprotection produced the free amine. This amine was then alkylated either directly with alkyl halides or by reductive alkylation to produce a series of alkylated derivatives.

A selection of the substituents synthesized is shown in Table 5 (**18a**–**d**). The unsubstituted piperidine **19** showed very little NK_1 activity and even NK_2 activity was reduced compared to **13b**. While it was possible to retain NK_2 activity by appropriate substitution, only slight improvement in NK_1 binding activity was found (15 nM for **18a** compared to 25 nM for **13b**). However, when the piperidine nitrogen was acylated with protected amino-acid derivatives, followed by deprotection (Scheme 6), we found another slight increase in binding potency (11 nM for **20c**) in one compound (Table 6).

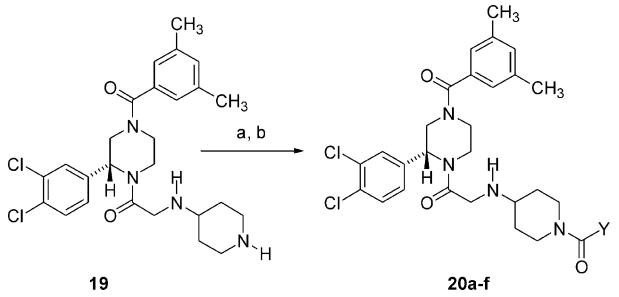
We have shown that, in the 2-(3,4-dichlorophenyl)piperazine series, NK_1 activity resides in the 2*S* enantiomer and NK_2 activity resides in the 2*R* enantiomer. Furthermore, dual NK_1/NK_2 antagonists can be produced in the 2*R* series with a substituted 4-aminopiperidine substituent. This template is being used as a basis to explore the potential of the diacylpiperazines as dual antagonists.

Table 5. Modification of the substituent on the piperidine nitrogen

Compd	Y	NK_1 K_i (nM)	NK_2 K_i (nM)
19	No substituent on N	215	20
18a	4-CH_3CONH–C_6H_4—	15	1.3
18b	2-Imidazolyl	92	3.0
18c	3-Pyrrolyl	35	1.3
18d	4-(2-CH_3CONH)–thiazolyl	52	0.9

Table 6. Amino acid derivatives in the 2*R* series

Compd	Y	NK_1 K_i (nM)	NK_2 K_i (nM)
20a	*S*-Phg	143	16
20b	*R*-Phg	42	1.1
20c	*S*-Phe	11	1.1
20d	*R*-Phe	21	3.2
20e	*S*-Tyr	32	7.0
20f	*S*-Trp	70	28



Scheme 6. (a) *N*-BOC-Z-CO_2H, HOBT, Et_3N, DEC; (b) 4 M HCl in dioxane.

Acknowledgements

The authors would like to thank Mr. W. Tom for developing the procedure described in reference 12, Mr. C. Rizzo and Mr. S. Tozzi for the in vitro functional assay data, Mr. Z. Zhan and Mr. C. Richard for the NK_1 and NK_2 binding data, and Dr. J. Kaminski and Ms. L. Weber for help with the X-ray structure representation.

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20. Compound **15**, mp 153–154 °C, $[\alpha]^{23.9} = -53.9°$ (3.54 mg in 2 mL MeOH), was crystallized from 95% EtOH. Crystal data: $C_{20}H_{28}Cl_2N_2O_4$, $M = 431.46$, orthorhombic, space group $P2_12_12_1$, $a = 14.118(2)$ Å, $b = 26.022(5)$ Å, $c = 6.058(1)$ Å, $V = 2226(1)$ Å^3, $Z = 4$, $D_{calcd} = 1.287$ g cm^{-3}, μ(Cu Kα radiation, $\lambda = 1.5418$ Å) = 28.9 cm^{-1}, crystal size: 0.03×0.06×0.60 mm. Intensity data (2640 non-equivalent reflections, $\theta_{max} = 75°$) were recorded on an Enraf-Nonius CAD4 diffractometer. The crystal structure was solved by direct methods. Full-matrix least-squares refinement of atomic positional and thermal parameters (anisotropic C, Cl, N, O, fixed H contributions) converged (max. shift:esd = 0.03) at $R = 0.045$ ($R_w = 0.058$) over 1446 reflections with $I > 2.0\sigma(I)$. Crystallographic data (excluding structure factors) have been deposited with the Cambridge Crystallographic Data Centre, deposition number CCDC 175702. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44–1223–336033; e-mail: deposit@ccdc.cam.ac.uk).